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RESEARCH ARTICLE

Cancer Genetics and Epigenetics

Targeted DNA sequencing of high-grade serous ovarian carcinoma reveals association of TP53 mutations with platinum resistance when combined with gene expression

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Abstract

High-grade serous ovarian carcinoma (HGSC) is the most common subtype of ovarian cancer and is among the most fatal gynecological malignancies worldwide, due to late diagnosis at advanced stages and frequent therapy resistance. In 47 HGSC patients, we assessed somatic and germline genetic variability of a custom panel of 144 known or suspected HGSC-related genes by high-coverage targeted DNA sequencing to identify the genetic determinants associated with resistance to platinum-based therapy. In the germline, the most mutated genes were *DNAH14* (17%), *RAD51B* (17%), *CFTR* (13%), *BRCA1* (11%), and *RAD51* (11%). Somatically, the most mutated gene was *TP53* (98%), followed by *CSMD1/2/3* (19/19/36%), and *CFTR* (23%). Results were compared with those from whole exome sequencing of a similar set of 35 HGSC patients. Somatic variants in *TP53* were also validated using GENIE data of 1287 HGSC samples. Our approach showed increased prevalence of high impact somatic and germline mutations, especially those affecting splice sites of *TP53*, compared to validation datasets. Furthermore, nonsense *TP53* somatic mutations were negatively associated with patient survival. Elevated *TP53* transcript levels were associated with platinum resistance and presence of *TP53* missense mutations, while decreased *TP53* levels were found in tumors carrying mutations with predicted high impact, which was confirmed in The Cancer Genome Atlas data ($n = 260$). Targeted DNA sequencing of *TP53* combined with transcript quantification may contribute to the concept of precision oncology of HGSC. Future studies should explore targeting the p53 pathway based on specific mutation types and co-analyze the expression and mutational profiles of other key cancer genes.

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KEYWORDS
biomarkers, ovarian carcinoma, platinum resistance, TP53, treatment response

What's new?

Biomarkers for better stratification of patients with ovarian cancer into existing targeted therapy scenarios and clinical trials are still missing. In this analysis of somatic and germline genetic variability using a custom panel of 144 high-grade serous ovarian carcinoma-related genes, gene expression of highly somatically mutated *TP53* correlated with mutation type and resistance to platinum therapy. Combined analysis of somatic genetic background and *TP53* expression may thus be valuable in predicting therapy response. Furthermore, high-coverage custom-targeted sequencing was superior to whole-exome sequencing for identification of potentially clinically impactful *TP53* variants, both somatic and germline.

1 | INTRODUCTION

Ovarian cancer (OVC) is the eighth most frequent cause of cancer death in women globally.[1] Approximately 85%–90% of ovarian cancer originates in epithelial cells and is thus designated epithelial ovarian cancer (EOC). High mortality of this malignancy is mainly due to most EOC patients being diagnosed in advanced stages (International Federation of Gynecology and Obstetrics [FIGO] III or IV) when the 5-year survival rate reaches approximately 20%–45%[2,3] and treatment options are often limited.[4]

The predominant histological subtype of EOC is high-grade serous carcinoma (HGSC), with an aggressive phenotype associated with high mortality.[5,6] The most frequent molecular alterations in HGSC tumors are in the genes *TP53*, *BRCA1/2*, and genes relevant for the homologous recombination repair (HRR) deficiency (summarized in[7]). In *BRCA1/BRCA2/TP53* mutation non-carriers, pathogenic or likely pathogenic variants have been identified in known cancer genes such as *CHEK2*, *MUTYH*, *PMS2*, *RAD51C*,[8] *RAD51D*, *ATM*, *FANCM*, and *PALB2*.[9]

As standard therapy for EOC, a combination of cytoreductive surgery and adjuvant chemotherapy regimens using platinum derivatives and the taxane paclitaxel is recommended.[10] The development of molecular profiling allowed the identification of the roles of genetic variability in personalized therapy of EOC. Today, on the basis of *BRCA1/2* mutations and testing for genomic instability, poly (ADP-ribose) polymerase inhibitors (PARPi) represented by olaparib, rucaparib, niraparib, or anti-angiogenic agents such as bevacizumab, are incorporated into the therapy of recurrent EOC.[11,12] Most ongoing clinical trials focus on targeted approaches and combination of standard chemotherapy with immunotherapy, mainly durvalumab, and pembrolizumab with the selection of patients based on immune responsivity profiling, such as identification of high microsatellite instability (MSI-H), mismatch repair deficiency (dMMR) and HRR deficiency (HRD).[13]

Due to the high percentage of patients with recurrence, it is extremely important to explore molecular profiles influencing the response of patients to platinum (or taxane). Some molecular changes that accompany the emergence of therapy resistance have been identified, for example, higher prevalence of somatic HRD in the platinum-sensitive subgroup of HGSC.[14] Except for HRR pathway alterations, other DNA repair pathways may be affected through genetic variation in, for example, *ERCC2-6*, *DDB1*, *XPC*, *RFC1*, *RAD23B*, and *MNAT*.[15,16] Our recent whole-exome sequencing (WES) study of EOC compared germline and somatic profiles of EOC patients with differences in sensitivity to therapy. We showed that chemotherapy-resistant EOC have higher somatic mutational rates in *TP53* and lower in several Hippo pathway genes.[17] Studies providing insights into the mechanisms of platinum resistance of ovarian carcinoma revealed that the inactivation of several tumor suppressor genes (*RB1*, *NF1*, *RAD51B*, and *PTEN*), as well as amplification of *CCNE1* and overexpression of the *ABCB1* membrane transporter, contributed to platinum resistance in EOC.[18] Other genes associated with platinum resistance in ovarian cancer were previously found (*ESRP1*, *LDHA*, *DDX5*, and *HEXA*).[19] Furthermore, our previous gene expression profiling studies identified putative biomarkers of EOC prognosis to be associated with sensitivity to therapy including transmembrane transporters, for example, *ABCA7*, *ABCA10*, *ABCB1*, *ABCC1*, *ABCE1*, *ABCG2*, and *SLC16A14* and cell cycle regulating genes *PLK1*, *PRC1*, *NR1H4*, *KIF14*, and *CIT*.[20–22] However, the role of genetic variability of these putative biomarkers of EOC prognosis in chemoresistance is not well characterized.

In the present study, a targeted panel of 144 genes was sequenced in tumors and blood of 47 HGSC patients. The panel consisted of the most important EOC cancer drivers and their related genes and genes associated with EOC risk, prognosis, and resistance as mentioned above, including those identified in our previous studies. All selected genes and their functional classification are shown in Supplementary Table S1. We aimed to address germline and somatic genetic variability by the evaluation of small substitutions, insertions and deletions (indels). Results were compared with our previous findings by WES[17] and the main advantages and disadvantages of both approaches were described. Somatic genetic background and *TP53* gene expression profile in patients stratified by the platinum resistance status and overall survival (OS) was compared and significant associations were found.

2 | MATERIALS AND METHODS

This section provides only a brief description of methods. For more details and additional references, see the expanded Supplementary Methods.

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2.1 | Patient samples

Blood and primary tumor tissue samples were obtained from 47 patients diagnosed with HGSC at the University Hospital Kralovske Vinohrady and University Hospital Motol (Prague, Czech Republic), University Hospital in Pilsen (Pilsen, Czech Republic), and the University Hospital in Hradec Kralove (Hradec Kralove, Czech Republic). The tissue samples collected during surgery (resection) were histopathologically verified, immediately fresh frozen and stored at −80°C until further processing.

The following personal and clinicopathological data were retrieved from the patients' medical records: patient's age at diagnosis, tumor grade, FIGO stage, histological type, adjuvant chemotherapy regimens, presence of peritoneal metastases, and residuum after surgery. OS was defined as the interval from the date of surgery to the date of death or last follow-up. Response status was determined as the interval elapsed between the date of the last dose of platinum-based chemotherapy and the date of relapse, progression, death, or last follow-up (based on the platinum-free interval, PFI).[23] All collected clinicopathological data for patients enrolled in the present study are summarized in Supplementary Table S2.

2.2 | Isolation of nucleic acids and quantity/quality determination

DNA from peripheral blood lymphocytes was isolated and stored according to the published procedure.[24] DNA and RNA from tumor tissue was isolated and all nucleic acids were quantified as published previously.[22] See Supplementary Methods for a detailed description.

2.3 | Next-generation DNA sequencing

2.3.1 | Gene panel design for targeted sequencing

A comprehensive list of genes with potential impact on EOC resistance was compiled based on the results of our previous studies and further expanded by literature search.[15–22,25] In total, 144 genes were included in the final panel (Supplementary Table S1).

2.3.2 | Library preparation, sequencing, data analysis

Libraries were prepared using SureSelect XT HS2 DNA Library Preparation Kit (Agilent Technologies, Inc.) and sequenced on the Illumina NovaSeq 6000 platform (Illumina, Inc.) in 150 bp paired-end mode. Analysis and filtering of sequencing data was done according to our previous work[26] with updated tools (see Supplementary Table S3 for list of tools including version numbers).

2.4 | Quantitative Real-Time PCR (qPCR)

To determine the relative gene expression of *TP53* transcripts, cDNA was synthesized from 0.5 μg of total RNA samples using RevertAid First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania). Quality of cDNA was checked by PCR as described previously.[27] The qPCR study design adhered to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments Guidelines (MIQE[28]). Samples were analyzed in duplicates; those with a standard deviation larger than 0.5 Ct were reanalyzed. *PPIA*, *UBC*, and *YWHAZ* genes were used as reference genes for the normalization of results based on their stability in ovarian tissue assessed previously.[21] The $2^{-\Delta Ct}$ method was used for relative quantification of gene expression, and the $2^{-\Delta\Delta Ct}$ method was used for fold change (FC) calculation[29] in groups divided by difference in sensitivity to therapy.

2.5 | Statistical analysis of variants and clinical data

Associations between categorical values such as genotypes and clinical data were analyzed using the Pearson chi-square or two-sided Fisher's exact test. For the comparison of continuous variables such as age and gene expression, the Kruskal–Wallis test was used (when comparing only two groups, the Mann–Whitney *U* test and Wilcoxon rank sum test). The tested clinical variables were as follows: platinum resistance based on the 6-month PFI cut-off,[23] menopausal status (pre- vs. post-menopausal), stage, grade, and presence of residuum after surgical tumor removal. For the evaluation of germline variants, Fisher's exact test with the Monte-Carlo max(T) permutation test was used. The survival functions were computed by the Kaplan–Meier method. Cut-offs defined by quartiles were tested and the "optimal cut-off" was defined as the highest statistical significance by the log-rank test. Correction of p-values was performed using the Benjamini-Hochberg procedure (false discovery rate—FDR).

2.6 | External datasets

From a previously published cohort of EOC patients,[17] only HGSC samples ($n = 35$, Supplementary Table S2) were used for comparisons of targeted sequencing results of the present study with those of WES.

For validation of somatic variants in *TP53*, we utilized the American Association for Cancer Research (AACR) Genomics Evidence Neoplasia Information Exchange (GENIE) 13.0 (January 2023) dataset,[30] composed of tumor panel sequencing data from various panels in multiple major cancer centers, reduced to HGSC samples comparable with the study cohort ($n = 1287$).

For validation of expression levels of *TP53* in relation to its mutation status, we used the RNAseq gene expression and DNAseq mutation data of the Genomic Data Commons (GDC) The Cancer Genome Atlas (TCGA)-OV cohort, downloaded from the University of

California Santa Cruz Xenabrowser portal (https://xenabrowser.net), which were then filtered to only primary ovarian tumors with both data types available ($n = 260$). The filtered cohort was deemed sufficiently similar to our study cohort (1 patient with Stage I, 16 with Stage II, 200 with Stage III, 43 with Stage IV; 32 with Grade 2, 228 with Grade 3) for the purpose, despite the database not containing a detailed description of subtypes, therefore not allowing for accurate selection of only HGSC samples.

3 | RESULTS

3.1 | Patients' characteristics

Relevant clinical data of ovarian HGSC patients included in the study are shown in Supplementary Table S2. The mean patients' age at diagnosis was 59.3 ± 10.0 years. All tumor samples were histologically classified as HGSC. The majority were at Stage III (94%) and Grade 3 (90%). Twenty-two patients were treated with neoadjuvant chemotherapy including paclitaxel and platinum derivatives followed by surgery and adjuvant therapy. The rest of the patients ($n = 25$) were treated only with adjuvant chemotherapy. Adjuvant regimens combined paclitaxel with carboplatin in all patients. Patients with PFI ≤6 months ($n = 18$) were considered resistant and patients with PFI >6 months ($n = 29$) sensitive to therapy. The mean PFI was ~3 months for resistant and ~21 months for sensitive patients participating in the study.

3.2 | Sequencing coverage

For normal samples, the mean coverage of the targeted regions (1.3 Mb) was 166 ± 67 (median 155), with 91 ± 8% (median 94%) of bases covered at least 50× and 70 ± 22% (median 78%) at least 100×. For tumor samples, the mean coverage was 538 ± 122 (median 536), with 94 ± 5% (median 95%) of bases covered at least 150× and 76 ± 14% (median 80%) at least 300×. A detailed description of sequencing metrics for each sample is provided in Supplementary Table S4.

3.3 | Germline variability in HGSC patients

In general, 9263 germline variants were found in the set of 47 HGSC patients (mean per patient 2295.2 ± 88.4 and median 2292; min. 2131–max. 2493). A total of 7329 variants passed filters (see Supplementary Methods) and were in Hardy–Weinberg Equilibrium (HWE) $p > .001$. The presence of rare and deleterious variants was assessed and 80 of these variants were identified. Pathogenic variants are likely to have a causative clinical effect and thus we decided to concentrate only on these variants since common variants are unlikely to result in phenotype changes.[31] When focusing only on highly impactful variants, the top mutated genes were *DNAH14* ($n = 9$ patients), *RAD51B* ($n = 8$ of which 7 had splicing variants), and *BRCA1* ($n = 5$).

3.3.1 | Germline genetic profiles of HGSC patients divided by platinum resistance

To further explore the associations of germline genetic profiles of HGSC patients with their phenotype features, we compared patients resistant and sensitive to chemotherapy, based on the 6-month PFI cut-off. For an oncoplot comparing all types of rare variants in the top 20 genes between the two groups of patients, see Figure 1A. First, we analyzed rare pathogenic variants (total number of variants was 80 in 41 patients). However, due to the scarcity of variants and low numbers of patients after in subgroups based on their phenotype, we were not able to compare their mutation profiles. Survival analyses using the full cohort did not show any statistically significant results.

Second, individual variants, as well as haplotypes, associating with response to therapy were identified. For this purpose, we calculated associations of all polymorphic variants with resistance or sensitivity of the patients (Figure 1B, Supplementary Table S5). Three variants were below the significance threshold ($p < .001$): Intronic variants rs4682097 and rs5851826 (tagging also rs5003791, rs147052896, rs9809384, rs9809404, and rs9860819) in membrane transporter *SLC9C1* on chromosome 3 and rs1190110 (tagging rs9671923, rs17723597 and rs35176149; *SLC35F4*—membrane transporter) on chromosome 14. We performed haplotype analysis of these two genes; however, no haplotype block was formed after the removal of tagged variants ($R^2 > .8$) in linkage disequilibrium analysis.

3.3.2 | Comparison of rare pathogenic germline genetic variants with whole exome sequencing

In order to examine the utility of targeted sequencing in the detection of pathogenic rare and hard-to-identify variants, we compared the sequencing results obtained from panel sequencing with our previous results obtained by WES. The superiority of the higher-coverage targeted approach was demonstrated by vastly more detected variants when compared to WES (Figure 1C). From the 80 rare and deleterious variants, only 17 were seen by WES. Notably, the extra variants were considered pathogenic and mostly affected splicing (located in exon-intron boundaries) or transcription (stop loss/gain).

3.4 | Somatic variability in HGSC tumors

3.4.1 | Overview of somatic variants

All patients carried at least two somatic mutations of any type (average 10.4 ± 4.9 per patient, median 9), with at least one of the variants being coding with predicted high or moderate impact (average 3.4 ± 1.7 per patient, median 4) (Figure 2A,C). The top mutated gene was

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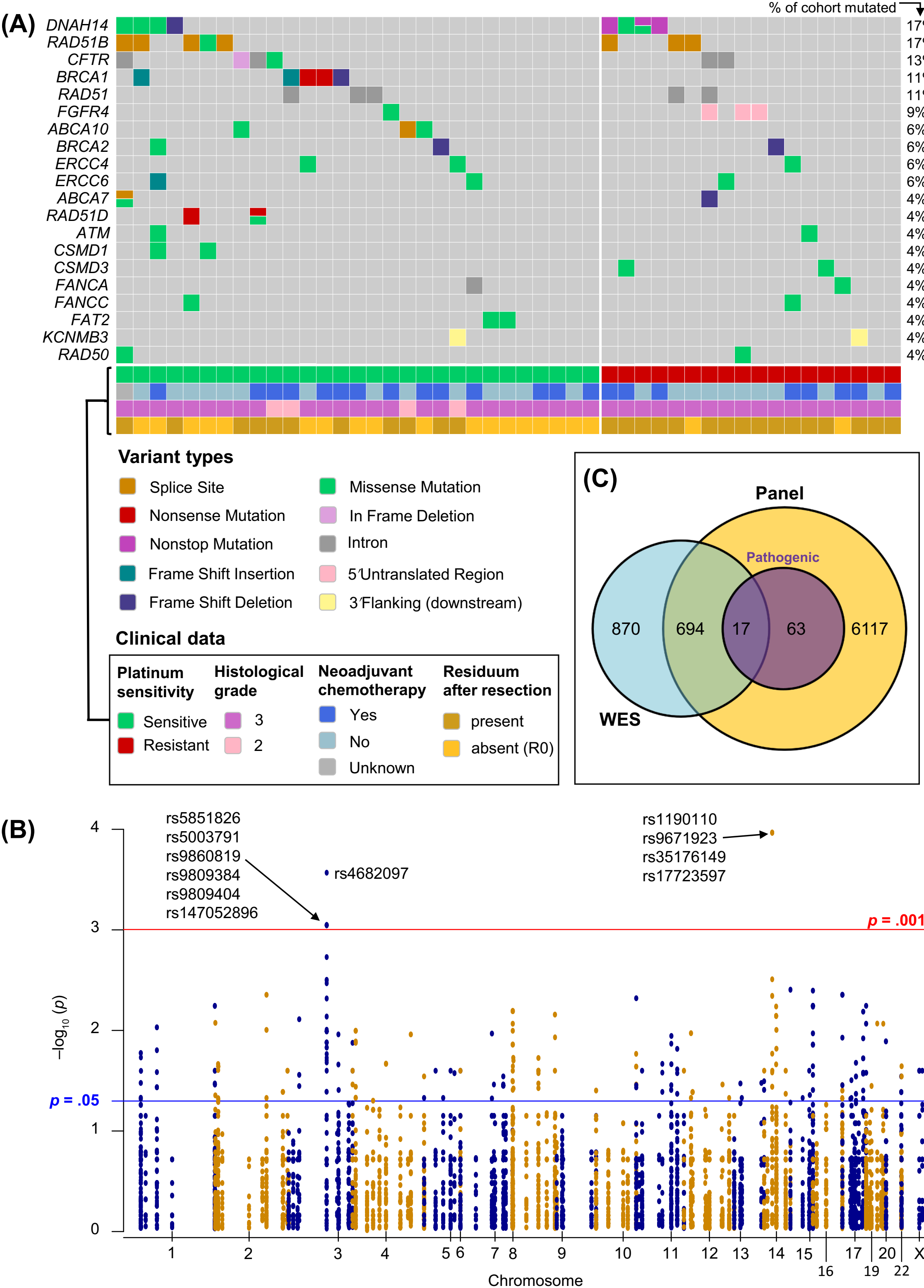

FIGURE 1 (A) Oncoplot of the top 20 genes with rare germline variants in HGSC patients ($n = 47$). Color coding represents types of functional impact. The percentage of mutations presented in our group of patients is shown together with the type of mutation. (B) Associations of germline variants with resistance of HGSC patients to platinum-based chemotherapy. Patients were divided into groups based on resistance or sensitivity according to their PFI. *P*-values are adjusted according to empirical *p*-value (pointwise), or lower-*p*-value permutation count (EMP1). (C) Overlap of rare pathogenic germline variants (dark purple) in HGSC patients detected by the targeted panel (yellow) and by whole-exome sequencing (WES, blue).

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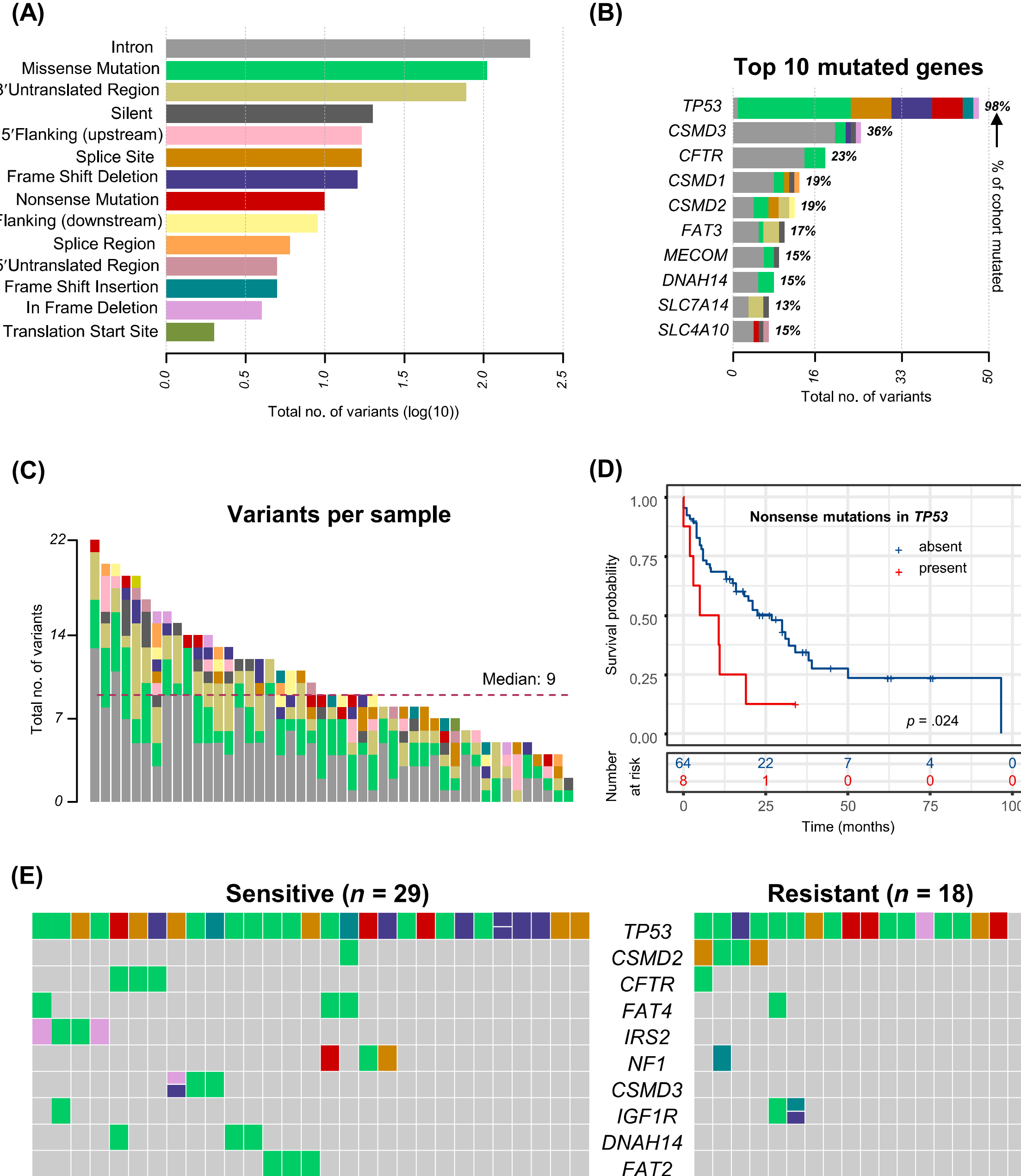

FIGURE 2 Distribution of somatic variants in HGSC patients ($n = 47$). All color coding is according to categories in Figure 3A. (A) Number of variants found (x-axis, logarithmic scale) according to classification. (B) Top 10 mutated genes by the number of variants (x-axis). The percentage of patients harboring variants in the genes are shown next to their respective bars. (C) Numbers of variants per sample (y-axis). (D) Platinum-free interval of patients harboring nonsense mutations in *TP53* versus wild-type patients. (E) Top 10 most mutated genes (high or moderate impact variant only) compared between platinum-sensitive and platinum-resistant patients.

TP53 (98% of the cohort), almost exclusively with high or moderate impact variants (Figure 2B).

3.4.2 | Somatic genetic profiles of HGSC patients divided by platinum resistance

Due to *TP53* being mutated in the vast majority of samples and other genes being rarely mutated, differential mutation analysis of individual genes was problematic. *DNAH14* was significantly more mutated in sensitive patients (7 out of 29) than resistant (0 out of 18; $p = .034$; not significant after FDR adjustment). When considering all variants with high or moderate functional impact (Figure 2E), no differentially mutated genes could be found. However, comparison of types of mutations separately revealed significantly shorter PFI of HGSC patients with presence of nonsense *TP53* mutations ($p = .017$, Figure 2D).

Next, we compared mutational rates in OVC-specific driver genes ("OVC drivers"; $n = 22$) and a set of candidate OVC therapy resistance genes ("OVC resistance genes"; $n = 13$) as listed in Figure 3A (sources in Supplementary Table S6). No set was differentially

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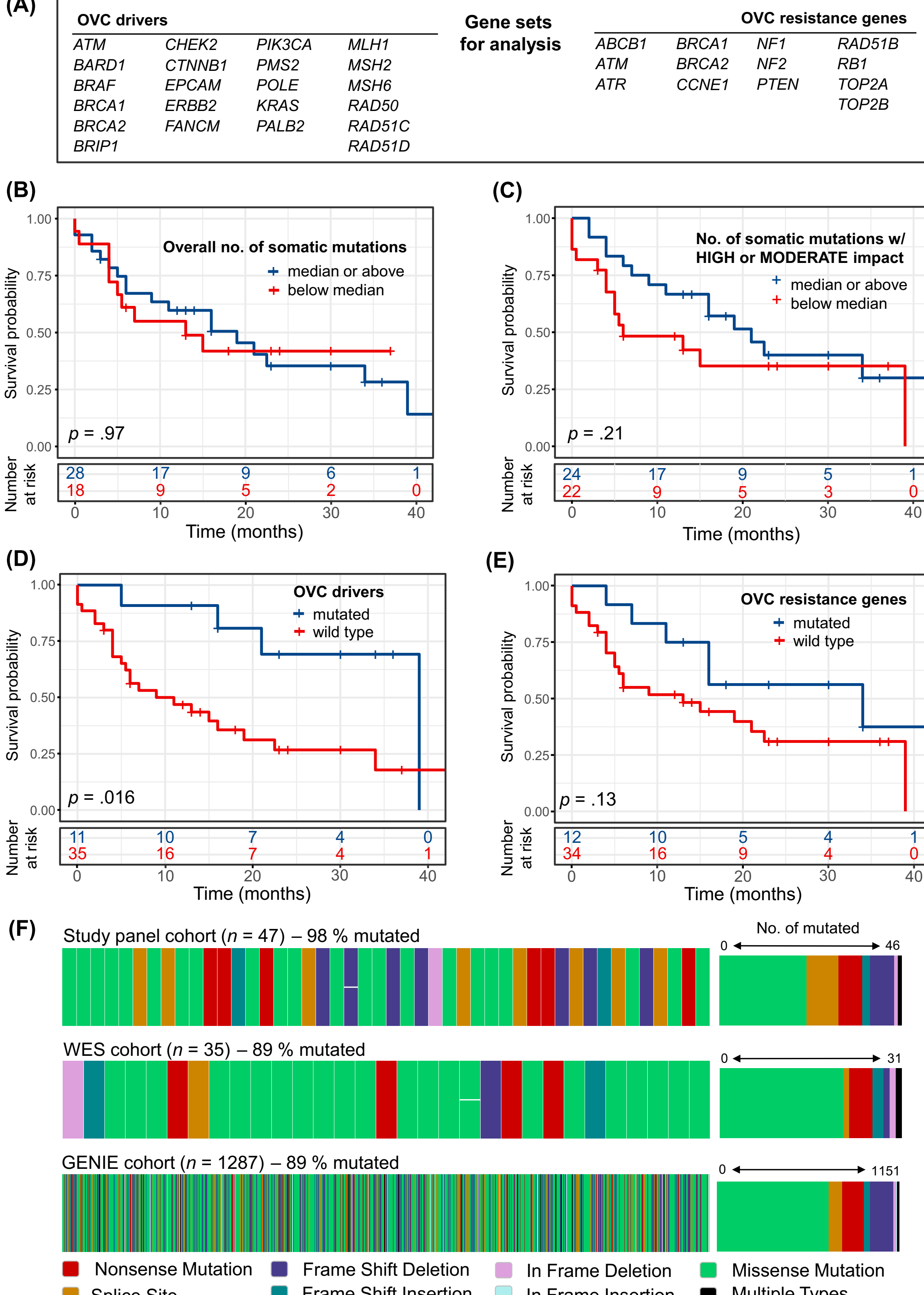

FIGURE 3 (A) Gene sets used for survival analysis (see Supplementary Table S5 for sources). (B–E) Platinum-free interval of patients divided by the median number of somatic mutations (B), median of somatic mutations with high or moderate protein impact (C), mutation status of the OVC drivers (D) and OVC resistance genes (E). Unadjusted *p*-values by the Log rank test. (F) Comparison of somatic variants with high and moderate impact in *TP53* between the study data, our previous WES data[17] and the AACR GENIE panel sequencing data.[30] Types of variants per patient and the overall proportion per cohort.

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mutated when considering all types of variants, but both were more frequently mutated in sensitive patients when counting only variants with high or moderate impact ($p = .033$ for OVC drivers, $p = .017$ for therapy resistance genes; both $p = .033$ after FDR adjustment). However, this difference was also mirrored in the whole dataset, where sensitive patients had significantly more mutations with high or moderate impact (mean 3.90 vs. 2.56, $p = .007$; unpaired t-test), but not statistically significantly more mutations overall (mean 11.31 vs. 8.94, $p = .110$).

To see whether these associations between mutational rate and platinum sensitivity also correspond to survival, we compared the survival of patients split by the median number of mutations (below vs. above or equal) and also by the high/moderate impact mutation status of the gene sets. Indeed, while there was no difference in the overall number of mutations (Figure 3B), patients with high/moderate impact variants had slightly longer survival than those without, although not statistically significantly (Figure 3C). However, the difference was much more pronounced for the OVC drivers ($p = .016$; Figure 3D), and only slightly more for the OVC resistance genes ($p = .13$; Figure 3E).

3.4.3 | Comparison of somatic variants in TP53 with WES and GENIE data

We compared somatic *TP53* mutation rates (high or moderate impact only) in our panel with already published data. Firstly, we compared present data with our results on a HGSC cohort studied previously by WES[17] ($n = 35$), with an overlap of 6 patients. The *TP53* mutation rate was slightly lower (89%) in the WES data, especially due to the lack of splice site variants (8/47 [17%] for panel compared to 1/35 [3%] for WES) (Supplementary Figure S1A). No splicing variants were detected in the overlapping 6 patients by either method.

To see if the splice site variants were specific to our panel sequencing method or to our cohort, we compared their frequency with that in the GENIE dataset ($n = 1287$).[30] Here, only 88 (7%) HGSC patients carried splice site mutations, which was also significantly lower than our cohort ($p = .02$, Fisher's exact test) (Figure 3F). Many of the variants found in the study cohort were also among the most frequent in the much larger GENIE cohort; however, we practically did not detect any variants outside the main functional domains (except for one splice site variant), where especially frameshift deletions and nonsense variants can be apparently quite common, although individual variants themselves are usually discrete (typically 1 patient in 1287) (Supplementary Figure S1B).

3.5 | TP53 gene expression and its association with clinical data and mutation status in TP53

Firstly, in our cohort of HGSC patients with available RNA ($n = 43$), we measured the mRNA levels of *TP53* transcripts using specific fluorescent assays targeting different locations of the *TP53* gene, as specified in Supplementary Figure S2. Levels of *TP53*-1 and *TP53*-2 mRNA were successfully detected in HGSC tumors unlike the *TP53*-3 transcript, where the expression was below the limit of detection. The mRNA levels of *TP53*-1 and *TP53*-2 correlated highly significantly with each other (Spearman's rho test; $r^2 > .8$, $p = .001$). We therefore considered *TP53*-1 as the main mRNA transcript and compared its relative expression in sensitive HGSC tumors ($n = 28$) with that in resistant HGSC tumor tissues ($n = 15$). The mRNA levels of *TP53*-1 were significantly higher in HGSC resistant patients in comparison to sensitive ones ($p = .022$).

Subsequently, the expression of TP53 transcripts was also measured in 35 HGSC samples from the WES cohort (overlap of 6 patients). The expression profile was similar to the first cohort with the highest levels of *TP53*-1, followed by *TP53*-2 and with no detectable expression of *TP53*-3. In the testing cohort, *TP53*-1 and *TP53*-2 were also highly correlated (Spearman's rho test; $r^2 > .8$, $p = .001$). While the difference in relative expression of *TP53*-1 between sensitive and resistant patients was not as significant as in the original cohort ($p = .281$) in the combined cohort of 73 samples, *TP53*-1 was found to be even more significantly upregulated in resistant HGSC patients ($p = .013$) than before.

Then, we compared relative *TP53*-1 and *TP53*-2 transcript expression levels between patients stratified by the predicted effect of *TP53* mutations. Patients with high effect *TP53* mutations (frameshift insertions and deletions, nonsense [stop gain], splice site, and start lost [transcription start site]) had significantly lower levels of both transcripts in their tumors than the rest. In contrast, patients with moderate effect (missense) mutations in *TP53* had significantly higher levels of both transcripts than the rest. This relationship was observed for both the original study set (panel set, Figure 4A,B) and for the combined set (Figure 4C,D). We also observed this trend, but even more strong, in the validation TCGA data of primary tumors with high grade ($n = 260$, Figure 4E).

Finally, we compared mRNA *TP53* transcript expression with the clinical data of HGSC patients in our examined cohort, such as age, stage, progression of disease, therapeutic response, and survival estimated as PFI. In concordance with relative expression findings, we also found a suggestive association of *TP53*-1 and *TP53*-2 transcript levels with the resistance status of HGSC patients. Particularly, resistant patients had significantly higher levels of *TP53*-1 and *TP53*-2 (Table 1; the Mann–Whitney test; $p = .014$ and $p = .047$, respectively). This finding was confirmed in the whole set of 73 patients (panel and WES cohorts combined) as shown in Table 1. Our data suggest that overall TP53 transcript level is better reflected by *TP53*-1 but the difference between *TP53*-1 and *TP53*-2 results is not dramatic. Higher levels of the *TP53*-1 transcript, but not *TP53*-2, were found in patients with the presence of residuum after surgery ($p = .037$) in the original study cohort, but the result was non-significant for both transcripts in the expanded cohort (Table 1), which points to the finding likely being a chance result.

Association between mRNA expression of *TP53* and survival of the examined cohort of HGSC patients was also performed. Patients with higher than median *TP53*-1 and *TP53*-2 expression had worse survival (Breslow test: $p = .038$ and $p = .015$; Log rank test: $p = .081$ and $p = .031$, respectively; Supplementary Figure S3).

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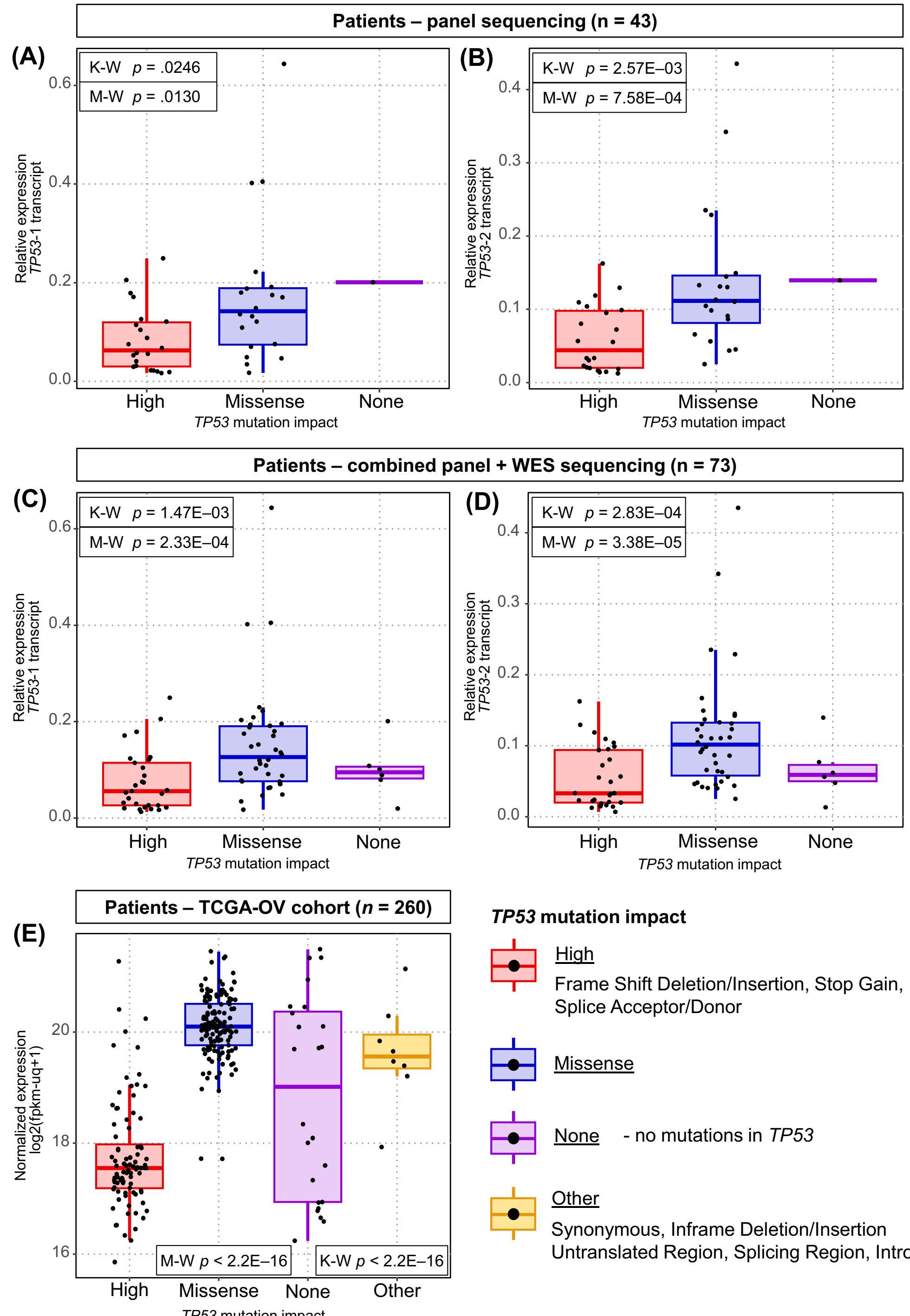

FIGURE 4 Differences in expression of *TP53* mRNA in the panel set ($n = 43$) and in the combined set of HGSC patients ($n = 73$), based on *TP53* mutation status. High impact mutations are associated with lower *TP53* expression and missense mutations with higher TP53 expression. Relative expression (determined by qPCR) of transcripts *TP53*-1 and *TP53*-2 shows the same trend in the panel set (A and B, respectively) and in the set expanded with the validation WES cohort (C and D, respectively). The same but stronger trend can be also seen in validation TCGA-OV data (E), which were obtained via RNA sequencing (log2 normalized transcript abundance, FPKM-UQ). K-W—Kruskal–Wallis test for all groups. M-W—Mann–Whitney *U* test between the "High" and "Missense" groups. *P*-values are unadjusted.

TABLE 1 Significant associations of the relative *TP53*-1 and *TP53*-2 transcript levels with clinical data of HGSC patients.

	Resistant patients		Presence of residuum	
Cohort[a]	Panel ($n = 43$)	Combined ($n = 73$)	Panel ($n = 43$)	Combined ($n = 73$)
TP53-1	Higher expression, $p = .013$[b]	Higher expression, $p = .011$[b]	Higher expression, $p = .037$[b]	Non-significant, $p = .150$[b]
TP53-2	Higher expression, $p = .047$[b]	Higher expression, $p = .049$[b]	Non-significant, $p = .394$[b]	Non-significant, $p = .574$[b]

[a]Panel—original study cohort ($n = 43$); Combined—original study + WES cohort ($n = 73$).
[b]Evaluated by the Mann–Whitney test.

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4 | DISCUSSION

Ovarian cancer has dismal prognosis due to the late diagnosis and necessity to treat patients in advanced stages, together with a high risk of multidrug resistance. Despite the boom of tumor profiling, biomarkers for better patient stratification into existing targeted therapy scenarios and clinical trials are still missing. Therefore, we aimed to compare the value of whole exome and targeted gene panel sequencing for the prediction of platinum resistance status and prognosis of HGSC patients. Furthermore, we complemented our findings with intratumoral transcript levels of the main HGSC candidate gene *TP53*, which improved the prognostic value.

Somatic mutation analysis revealed at least two somatic mutations of any type in each examined patient. The most prevalent types, aside from intronic variants, were missense and 3′ untranslated region (UTR) alterations. Unsurprisingly, the top mutated gene was *TP53* with mutation rate of 98%, which is in line with mutation rates previously documented in the HGSC subtype.[18,22,32,33] Due practically the cohort being mutated in *TP53*, we stratified groups by the type of mutation and we found that carriers of *TP53* nonsense mutations had significantly worse survival than patients with wild type or other mutation types. The majority of identified *TP53* mutations were missense mutations, known to lead to the loss of the tumor suppressive function of p53 and the gain of new oncogenic functions,[33] making p53 an attractive therapeutic target, specifically for HGSC. WEE1 inhibitor AZD1775, targeting the p53 pathway, has shown an improved efficacy when combined with carboplatin in *TP53*-mutated EOC tumors regardless of the mutation type.[34] Other approaches focused on compounds capable of restoring the original p53 conformation, for example, the combination of the APR-246 compound with carboplatin and pegylated liposomal doxorubicin, which was effective in patients with relapsed platinum-sensitive HGSC[35] and further options are currently under discussion.[36] Nevertheless, the association of *TP53* mutations with HGSC resistance remains controversial. First, the presence of any *TP53* mutation was associated with platinum sensitivity in a previous study using the BROCA sequencing platform[37] in HGSC patients. Another study revealed significantly more prevalent gain-of-function *TP53* mutations in 64 HGSC platinum-sensitive patients using panel sequencing based on 26 oncodriver genes.[38] Discordantly, a study using the TCGA dataset of 264 HGSC patients revealed higher rates of platinum resistance in patients with oncomorphic *TP53* mutations (P151S, Y163C, R175H, L194R, Y220C, R248Q, R248W, R273C, R273H, R273L, R282W) compared with mutations falling into other categories.[39] Similarly, in our previous WES study of 50 EOC patients of various subtypes, platinum-resistant patients had a significantly higher somatic *TP53* mutational rate compared to sensitive patients.[17] In another study, gain-of-function *TP53* mutations were more frequent in platinum-resistant HGSC patients.[40] Finally, another WES-based study of 60 EOC patients did not reveal any association of *TP53* mutations with platinum sensitivity.[41] The above results together with our data suggest that the *TP53* mutation type may be more important than just mutation status (wild-type vs. mutated), and additional factors, for example, transcript levels or epigenetics may be in play as well.

To provide functional insight, we determined intratumoral expression levels of three *TP53* transcript probes. One of them, located at the 3′UTR, was expressed below the limit of quantification in all samples, and the other two (5′UTR and transcript center) highly correlated together. Thus, the following results represent both probes. A significantly higher *TP53* expression was found in platinum-resistant compared to sensitive patients. Simultaneously, patients with missense *TP53* mutations had higher transcript levels than the rest, while patients with nonsense mutations had lower transcript levels, a trend we also observed to be very strong in validation TCGA data of 260 samples. Only a few studies compared mutation and expression profiles of *TP53*. Our results are in concordance with the study of Cole et al., in which missense *TP53* mutations were found in high p53 protein expressing HGSC tumors, and low p53 levels were seen in tumors with other mutation types in a cohort of 72 HGSC patients.[42] Furthermore, a study comparing *TP53* isoforms in 31 HGSC patients identified significantly lower levels of total *TP53* transcript in *TP53* wild-type tumors than in mutated ones.[43] Nevertheless, a recent study using RNA-sequencing of 39 HGSC patients did not observe a significant difference in the *TP53* transcript expression between good and poor HGSC responders on the basis of progression-free interval to platinum-based therapy.[44] Comparison of ovarian cancer cell lines, p53-null (SKOV-3), *TP53* wild-type (A2780), and mutant $TP53^{R248}$ (OVCAR-3), showed the highest p53 protein expression for $TP53^{R248}$ cells in vitro.[45] Furthermore, cisplatin-resistant A2780 cells with wild-type *TP53* have reduced p53 and downstream signaling and avoid apoptosis compared to p53 mutated cells.[46] These results support our findings of higher *TP53* expression in patients with missense mutations and underline the importance of the assessment of *TP53* gene expression in context with specific mutation types in each patient.

Results of the present study suggest that mutations in specific ovarian oncodriver genes are associated with significantly longer survival of HGSC patients, while the overall mutation load based on all high and moderate functional impact mutations has no prognostic value, despite the panel consisting of mostly known or candidate cancer-relevant genes.

Additionally, the presence of germline and/or somatic alterations in the HRR pathway of DNA repair was highly predictive of platinum sensitivity and overall survival in our recent study based on WES in 50 ovarian cancer patients.[17] Improved overall survival in serous and non-serous ovarian carcinoma patients was associated with HRR mutations in the previous study,[47] and a higher prevalence of HRR deficiency in the platinum-sensitive HGSC subgroup was found in the Asian population.[14] High frequencies of *CSMD3* or *FAT3* alterations identified by us also comply with data on 489 samples from the TCGA dataset.[16]

Finally, yet importantly, the analysis of germline profile identified the most frequent deleterious variants in *DNAH14*, *RAD51B*, and *BRCA1*. *BRCA1/2* pathogenic germline variants predispose women to ovarian carcinoma.[48] Among HRR genes, we identified germline *RAD51B* variants in 17% of HGSC patients. Seven of eight patients carried splicing variants with a potentially high impact on the function of the RAD51B protein. Six patients carried the polymorphism

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rs751355274 (G>A/G>C/G>T) and one deletion rs1486045768 (delGA), while one had the coding variant rs34594234 (A>G). Although germline *RAD51C* and *RAD51D* mutations are more frequent in EOC cases than *RAD51B*,[49] the inactivation of *RAD51B* in HGSC contributes to platinum resistance.[18] Our findings underpin the notion that due to high prevalence of pathogenic variants, *RAD51B* should be considered an additional gene for clinical testing of hereditary ovarian cancer. More importantly, three intronic variants ("modifier" impact), rs4682097 (G>A,C,T) and rs5851826 (delA), in the *SLC9C1* membrane transporter gene and rs1190110 in *SLC35F4* significantly associated with HGSC platinum resistance (odds ratios 0.18, 5.33, and 5.69, respectively). *SLC9C1* rs4682097 is a common polymorphism (minor allele frequency in the European population, MAF = 0.39), and rs5851826 is a deletion delA with MAF = 0.45. In general, three human plasmalemmal proteins that are SLC9 family members are involved in human pathophysiology.[50] Similarly, rs1190110 (C>T) in *SLC35F4* is a common variant with MAF = 0.39 in the European population. Clinical significance of the above-mentioned variants in *SLC9C1* or *SLC35F4* is unknown.

The study has several benefits and limitations. A comparison of germline profiles obtained with targeted panel sequencing and WES shows that the former delivers considerably more variants, especially when located in exon-intron boundaries (affecting splicing) or at the ends of transcripts (stop loss/gain). These regions are less efficiently covered by WES capture probes than exon centers. Custom targeted sequencing with higher coverage, especially one focused on exon edges, is superior in calling these variants compared to a more general WES analysis, which is to be expected. Thus, our findings demonstrate known benefits of targeted sequencing, especially considering *TP53* for the prediction of therapy response in HGSC patients. This is further supported by the finding in somatic variants, where we also detected substantially more splicing variants in *TP53* with the targeted sequencing approach than using WES on a similar cohort of patients. However, a similar difference was also seen between our targeted approach and the GENIE cohort, which is also based on targeted sequencing. Our cohort therefore may not be representative of a more general population, and differences in panel design and bioinformatic approach likely also play a role. The GENIE dataset is generally clinically focused and therefore uses less sensitive and more stringent variant calling pipelines.

A modest sample size appears as the main study limitation. However, all samples were of the same EOC subtype—HGSC. The cohort was highly homogenous, with advanced stage and grade, well documented PFI, and the same ethnicity (Slavic Caucasian), a combination of qualities that is currently underrepresented in published data. In addition, the combination of high-coverage sequencing data with *TP53* gene expression, stringent raw data filtering, and detailed follow-up could be considered benefits of our study. Furthermore, analysis of our previously published data from WES confirmed most findings. Another limitation could be the selection of the 144 genes for the study, which was based on literature search for known or potentially relevant OVC genes, and our previous results. This list should not be considered exhaustive and should be expected to evolve in the future as the field matures further.

In conclusion, our study shows the benefit of combined analysis of germline and somatic profiles of HGSC patients with different platinum sensitivity and pinpoints the advantages of targeted gene panel over WES. Germline profile revealed, in addition to known *BRCA1* alterations, *DNAH14* and *RAD51B* as the other highly mutated genes. Somatic profiles of HGSC patients confirmed a high rate of *TP53* mutations and revealed an association of nonsense *TP53* mutations with patient survival. *TP53* transcript levels were associated with platinum resistance and *TP53* missense mutations. We show that targeted somatic and germline profiling combined with transcript expression may contribute to the concept of precision oncology of HGSC. Future analyses should include intersections between the expression and mutational profile of other crucial genes and explore targeting the p53 pathway based on specific mutation types.

AUTHOR CONTRIBUTIONS

Petr Holý: Formal Analysis; Methodology (lead); Software (lead); Validation; Visualization (lead); Writing—Original Draft (lead); Writing—Review and Editing (lead). **Viktor Hlaváč**: Formal Analysis (lead); Methodology; Software; Visualization; Writing—Original Draft; Writing—Review and Editing. **Karolína Šeborová**: Data Curation; Formal Analysis; Investigation; Visualization; Writing—Review and Editing. **Tereza Tesařová**: Data Curation; Investigation; Writing—Review and Editing. **Simona Šůsová**, **Mohammad Al Obeed Allah**: Investigation. **Lukáš Rob**, **Martin Hruda**, **Jiří Bouda**, **Alena Bartáková**, **Marcela Mrhalová**, **Kateřina Kopečková**, **Jiří Špaček**, **Iva Sedláková**: Data Curation; Resources; Writing—Review and Editing. **Pavel Souček**: Conceptualization; Writing—Original Draft; Writing—Review and Editing. **Radka Václavíková**: Conceptualization (lead); Funding Acquisition; Project Administration; Supervision; Writing—Original Draft; Writing—Review and Editing. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

DNA-seq data (BAM) mapped to the reference sequence (GRCh38) were submitted to The National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the BioProject ID: PRJNA955019 (targeted panel) and PRJNA814851 (WES). The

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data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Informed consent was obtained from all participants included in the study. All procedures performed in this study followed the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The experimental protocol and its ethics were approved by the Institutional Review Boards of the National Institute of Public Health, Prague, Czech Republic (approval reference no. NT-14056-3), University Hospital Kralovske Vinohrady approval reference no. EK-VP/2s/0/2018, University Hospital in Pilsen (approval reference no. 16-29013A) and University Hospital in Hradec Kralove (approval reference no. 201806 S20P).

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