Influence of p53 Isoform Expression on Survival in High-Grade Serous Ovarian Cancers

Katharina Bischof1,2, Stian Knappskog3,4, Sigrun M. Hjelle4,7, Ingunn Stefansson5,6, Kathrine Woie3, Helga B. Salvesen1,7, Bjorn T. Gjertsen1,7 & Line Bjorge1,2

High-grade serous ovarian carcinoma (HGSOC) is characterised by alterations in the p53 pathway. The expression levels of p53 isoforms have been shown to be associated with patient survival in several cancers. This study examined the predictive and prognostic effects of the expression levels of TP53 pre-mRNA splicing isoforms and TP53 mutations in tumour tissues in 40 chemotherapy responders and 29 non-responders with HGSOC. The mRNA expression levels from total p53, and total Δ133p53, p53β, p53γ isoforms were determined by RT-qPCR, and TP53 mutation status by targeted massive parallel sequencing. The results from these analyses were correlated with the clinical outcome parameters. No differential expression of p53 isoforms could be detected between the chemosensitive and chemoresistant subgroups. In a multivariate Cox regression model, high levels of total Δ133p53 were found to be an independent prognosticator for improved overall survival (HR = 0.422, p = 0.018, 95% CI: 0.207–0.861) and reached borderline significance for progression-free survival (HR = 0.569, p = 0.061, 95% CI: 0.315–1.027). TP53 mutations resulting in loss of function or located at known hotspots were predictive of tumour characteristics and disease progression. These findings suggest that total Δ133p53 mRNA can be a biomarker for survival in HGSOC.

Mutations in the TP53 gene are early and almost ubiquitous events in the genesis of high-grade serous ovarian carcinoma (HGSOC)1–4. Although various classes of mutations occur throughout the coding region of the TP53 gene in human cancers, there is a clear enrichment of missense or nonsense single base substitutions affecting the DNA-binding domain of the protein5. Since large-scale sequencing data for TP53 mutations became available, the predictive and prognostic roles of defined subcategories of TP53 mutations have been extensively investigated. Alterations that result in a loss of function (LOF) or oncogenic mutations conferring to tumour-promoting abilities have been shown to be associated with chemoresistance and worse survival in ovarian cancers6–9. Small molecule therapies targeting mutated p53 proteins in cancer cells are under development for the treatment of ovarian cancer and may lead to TP53 mutational guided therapy10,11.

In humans, the TP53 gene encodes RNA that is edited by pre-mRNA splicing, yielding at least 12 protein isoforms expressed to various degrees in different tissues and under various physiological conditions12 (Fig. 1). Emerging evidence has linked the deregulated expression of these isoforms to cancer13–18. The amino-terminally truncated Δ133p53 isoform is the product of alternative promoter usage and has been shown to exert dominant-negative functions toward canonical p53 in colon cancer cell line models18. In addition, Δ133p53 has been shown to inhibit replicative senescence and to promote cellular proliferation19. The biological role of the carboxy-terminally truncated p53β and p53γ isoforms, both products of alternative splicing of intron 918–20, is still being debated. However, these isoforms have been shown to enhance p53’s tumour-suppressive transcriptional activity of Bax and p2121,22.

1Centre for Cancer Biomarkers CCBio, Department of Clinical Science, University of Bergen, 5020, Bergen, Norway. 2Department of Gynecology and Obstetrics, Haukeland University Hospital, 5021, Bergen, Norway. 3Department of Oncology, Haukeland University Hospital, 5021, Bergen, Norway. 4Section of Oncology, Department of Clinical Science, University of Bergen, 5020, Bergen, Norway. 5Department of Pathology, Haukeland University Hospital, 5021, Bergen, Norway. 6Centre for Cancer Biomarkers CCBio, Department of Clinical Medicine, Section for Pathology, University of Bergen, 5020, Bergen, Norway. 7Department of Internal Medicine, Haematology Section, Haukeland University Hospital, 5021, Bergen, Norway. Helga B. Salvesen is deceased. Correspondence and requests for materials should be addressed to L.B. (email: line.bjorge@uib.no)
Figure 1. (A) Overview of FIGO disease stages represented by the series for this study and illustration of sample collection and extraction of DNA and RNA. (B) Structure of the human TP53 gene comprising 11 exons. P1 = proximal promoter encoding full-length p53, P2 = internal promoter resulting in Δ133p53 product. Alternative splicing sites (^). Primer location is indicated by coloured arrows and is indexed on the lower left. (C) Provides a more detailed schematic illustration of the distinct functional and structural domains of the 393 amino acid long p53 protein. TA1 and TA2 form the transactivation domain, followed by the proline rich domain (PD) and the DNA binding domain, where the three most frequently found point mutations in high-grade serous gynaecological cancers are indicated. The tetramerization domain (TET) and basic region (BR) form the C-terminus. (D) Illustrates the exon composition of canonical p53 and relevant p53 isoforms. Abbreviations: Transactivation domain (TA), DNA binding domain (DBD), C-terminal oligomerization domain (OD). The N-terminally truncated isoform Δ133p53 is a product of the regulation of an internal promoter in intron 4 (P2). The C-terminally altered p53^β and p53^γ isoforms have alternative sequences after amino acid 332. Molecular weight indicated in kilodalton (kd) on the right side.
was found to be a predictor of PFS (univariate analysis \( p = 0.007 \); multivariate analysis [hazard ratio \( \beta = 2.317 \), \( p = 0.033 \), 95% CI: 1.071–5.012]). The results of the univariate analysis showed disease stage to be significantly associated with overall survival (OS), \( p = 0.002 \), and the results of the multivariate model showed borderline significance (hazard ratio = 2.299, \( p = 0.051 \), 95% CI: 0.998–5.297). The presence of residual disease after surgery also had an effect on PFS, as seen in the univariate risk model (\( p = 0.032 \)), and OS, as seen in the univariate analysis (\( p = 0.017 \) and the multivariate analysis (hazard ratio = 3.735, \( p = 0.041 \), 95% CI: 1.054–13.240). Other clinicopathological variables, such as patient age and tumour grade, had no significant effect on the survival parameters.

### mRNA levels of p53 isoforms in HGSOC.

The levels of the total p53 mRNA were quantified, and breakpoint-specific qPCR assays were applied to detect the amino-terminal truncated total Δ40p53 and total Δ133p53 isoforms, and the carboxy-terminal truncated p53β and p53γ isoforms. Thus, the levels of the p53 transcripts containing each of the breakpoints, but not the combined mRNA species (e.g. Δ133p53β and Δ133p53γ) could be quantified. We identified the p53 isoforms total Δ133p53 and the splice isoforms p53β and p53γ in all but one of the samples (68 of 69); however, the expression levels of the total Δ40p53 mRNA could not be traced in any of the analysed samples. The landscape of the p53 isoforms was dominated by total Δ133p53 isoforms, which

| Clinical parameters | Poor response Mean (range) | Good response Mean (range) |
|---------------------|-----------------------------|-----------------------------|
| Age at diagnosis, in years | 59 (31–75) | 61 (44–77) |
| Time to progression, in months | 3 (1–6) | 42 (17–137) |
| FIGO stage | grade 2 8 (28%) Grade 3 21 (72%) | 7 (17%) 33 (83%) |
| Residual disease ≤1 cm | 16 (67%) Complete — | 12 (38%) 16 (50%) |
| Residual disease >1 cm | 8 (33%) | 4 (12%) |

Table 1. Distribution of clinicopathologic characteristics for 69 patients within subgroups of high grade FIGO stages ≥ IIIC enriched for either good response; ≥17 months progression free survival after completed primary treatment (surgery followed by adjuvant chemotherapy) or poor response with disease progression within 6 months after completed primary treatment.
represented up to 50.6% of the total p53 mRNA; p53β and p53γ accounted for a maximum of 3.3% and 2.5% of the total pool of p53 isoforms, respectively (Fig. 2A). The p53γ isoform showed the largest variability between the lowest and the highest expressing samples (108-fold). The differences between the highest and the lowest expressing samples for the p53β and total Δ133p53 isoforms were 86-fold and 54-fold, respectively.

Correlation between levels of p53 isoforms and total p53. The mRNA levels of the total Δ133p53 isoforms and the p53β and p53γ isoforms were all highly significantly correlated (p53β vs. p53γ R = 0.896, p < 0.001; p53β vs. total Δ133p53 R = 0.494, p < 0.001; p53γ vs. total Δ133p53 γ R = 0.459, p < 0.001; Fig. 2B). The expression levels of the individual isoforms were found to be independent of the total p53 levels (p > 0.25 for all of the comparisons; Fig. 2C).

Relative expression of p53 isoforms to total p53 associated with TP53 mutation status. The levels of total p53 were significantly lower in the TP53 wild-type tumours (n = 5) than in the mutated specimens (n = 26; p = 0.036) (Fig. 3A). While the absolute mRNA levels of the p53 isoforms were not correlated with

### Table 2. Established, clinical prognosticators for survival in univariate and multivariate Cox regression analysis in 69 patients with HGSOC. Abbrevations: HR = Hazard ratio. CI = Confidence interval.

|                  | Progression-free survival Multivariate | Overall survival Multivariate |
|------------------|----------------------------------------|------------------------------|
|                  | Univariate P-value | HR (95% CI) | P-value | Univariate P-value | HR (95% CI) | P-value |
| Age at diagnosis, in years | 0.557 | 0.994 (0.958–1.032) | 0.77 | 0.257 | 0.976 (0.934–1.021) | 0.292 |
| Disease stage III vs. IV | 0.007 | 2.317 (1.071–5.012) | 0.033 | 0.002 | 2.299 (0.998–5.297) | 0.051 |
| Tumor grade II vs. III | 0.65 | 0.843 (0.411–1.731) | 0.643 | 0.956 | 1.135 (0.456–2.826) | 0.785 |
| Residual disease 0 vs > 0 | 0.032 | 1.810 (0.830–3.950) | 0.136 | 0.017 | 3.735 (1.054–13.240) | 0.041 |

Figure 2. mRNA expression of p53 isoforms in tumour samples illustrated as (A). Histogram displaying fractions of p53 isoforms to total p53 mRNA in individual specimens. (B) Expression of p53 isoforms between each other; p53γ versus p53β, p53β versus Δ133p53, p53γ versus Δ133p53. (C) Expression levels of total p53, together with individual p53β, Δ133p53 and p53γ isoforms.
the TP53 mutation status, the relative expression levels of the p53 isoforms as a fraction of the total p53 levels were higher in the wild-type specimens compared to the mutated specimens (total $\Delta 133p53$ relative to total p53 $[p = 0.036]$, $p53\beta$ relative to total p53 $[p = 0.006]$, $p53\gamma$ relative to total p53 $[p = 0.016]$; Fig. 3B–D).

**Prognostic effect of p53 isoforms.** In further analyses, the levels of each isoform and the relative level, as a fraction of the total p53 levels, were used for comparisons with the clinical parameters. In the univariate OS analysis, the relative expression of total $\Delta 133p53$ was weakly associated with the prognosis (logrank $p = 0.173$; Fig. 4A). This association was further examined in a Cox proportional-hazards survival model. Known clinical prognostic factors for ovarian cancer patients (age at diagnosis grouped by median, tumour stage and presence of residual tumour) were introduced as binary variables, together with the relative $\Delta 133p53$ expression grouped by median ($\Delta 133p53$ ratio high vs. low). The relative expression of total $\Delta 133p53$ isoforms to total p53 was found to be associated with longer OS (hazard ratio $= 0.422$, $p = 0.018$, 95% CI: 0.207–0.861 Table 3). For the other isoforms, no prognostic role could be established in the univariate analyses (Fig. S1) or the multivariate analyses.

**p53 isoforms as predictive marker for sensitivity to chemotherapy.** No significant difference was observed in the expression levels of the isoforms between the two predefined groups of chemoresistant and chemosensitive patients ($\Delta 133p53$ $p = 0.135$; $p53\beta$ $p = 0.961$; $p53\gamma$ $p = 0.496$; Mann-Whitney rank test). PFS was regarded as a surrogate marker for the efficacy of first-line chemotherapy and chemosensitivity. Higher than median levels of $\Delta 133p53$ were not associated with significantly altered time to progression, based on the results of the univariate survival analysis using the Kaplan-Meier estimator (logrank $p = 0.216$; see Fig. 4B). In the multivariate survival analyses, the expression of $\Delta 133p53$ grouped by median ($\Delta 133p53$ high vs. low) reached borderline significance for PFS (hazard ratio $= 0.569$, $p = 0.061$, 95% CI: 0.315–1.027 Table 3). For the other isoforms, the results of the univariate analyses (Fig. S2) and the multivariate analyses showed no association between the absolute or relative expression and survival.

**Associations between p53 isoform levels and clinicopathological parameters.** No significant differences in the p53 isoform expression were observed between the completely resected patients and the patients...
for whom complete resection could not be achieved. In addition, a comparison of the moderate and low differen-
tiated cases showed no differences in the p53 isoform levels. The expression of the p53\(\gamma\) isoform was associated
with age when grouped by median (\(p=0.044\)); however, the expression of the other isoforms showed no associ-
ation with patient age. The levels of the p53\(\beta\) isoform relative to the total p53 were significantly higher expressed
in the FIGO stage III vs. IV (\(p=0.05\)) specimens.

Clinical relevance of TP53 mutation status. The combined data for the TP53 mutation status and the
p53 isoform expression were available for 31 patients. The main reason for this limited number of cases was lack
of access to germline DNA (needed to distinguish somatic and germline variants detected in tumour). The known
TP53 hotspot mutations, such as R248W, R175H and R273C, were among the mutations identified (see Table 4).
The cases were categorised as follows: i) a group showing the LOF mutations or the above-mentioned hotspots
(n = 9), ii) a TP53 wild-type group (n = 5) and iii) a group consisting of unclassified TP53 mutations (n = 17).
The LOF and hotspot mutations were more commonly seen in the FIGO stage IV tumours (\(p=0.003\)) and a low
differentiation grade (\(p=0.024\)).

The possible correlation of the TP53 mutation status with PFS as a surrogate for response to chemotherapy
was tested. The patients carrying tumours with the three groups of mutations showed a significantly different
response to chemotherapy (\(p=0.029\), with the unclassified mutations revealing the longest PFS when compared
to the wild-type and the LOF/hotspot patients. Similar results were found with the univariate survival analysis

|                         | Progression free survival | Overall survival |
|-------------------------|---------------------------|-----------------|
|                         | HR (95% CI)    | P-value | HR (95% CI)    | P-value |
| Age at diagnosis; in years | 0.993 (0.956–1.031) | 0.710   | 0.975 (0.931–1.021) | 0.274   |
| Tumor stage IIIC vs. IV | 2.694 (1.259–5.765) | 0.011   | 2.357 (1.092–5.088) | 0.029   |
| Residual disease; in cm 0 vs. >0 | 1.928 (0.911–4.077) | 0.086   | 5.254 (1.481–18.642) | 0.010   |
| \(\Delta133p53\) High vs. Low | 0.569 (0.315–1.027) | 0.061   | 0.422 (0.207–0.861) | 0.018   |
| High vs. Low            |                           |          |                |          |

Table 3. Prognostic and predictive impact of the p53 splice variant \(\Delta133p53\) for 69 women with HGSOC in
multivariate Cox regression analysis. \(\Delta133p53\)ratio denominates mRNA expression of \(\Delta133p53\) as a fraction of
the total p53 levels.
p53γ in most patients. This is in line with previous findings for uterine serous carcinomas26. While the p53 types15,29. in breast cancer cell lines and has been associated with impaired survival in patients with breast carcinomas16,17. general p53 transcription but rather have their own biological importance. This controlled side-production of p53 isoforms was associated with prolonged OS and PFS in multivariate survival analyses. However, the p53 isoform levels were not found to be significantly differentially distributed when the chemoresistant and chemosensitive cohorts were compared. These results are in line with a previous study that identified the Δ133p53 isoforms as favourable prognostic factors in TP53 mutated, advanced serous ovarian cancers24. In contrast, Δ133p53 has been shown to promote invasion and cancer cell stemness in breast cancer cell lines and has been associated with impaired survival in patients with breast carcinomas16,17. The exact roles of Δ133p53 isoforms and the means by which they become tissue-specific therefore remain to be elucidated. Furthermore, the higher relative expression of p53β to total p53 was associated with FIGO stage IIIC; thus, it could be hypothesised that p53β plays a role in the metastatic potential. The p53γ isoform is missing the transactivation and tetramerization domains that seem necessary for enabling the transcriptional competence of p53α, and the higher expression has been associated with tumour traits and favourable survival in other cancer types12,29. The present data also revealed that the mRNA level of Δ133p53 was much higher than the levels of p53α and p53γ in most patients. This is in line with previous findings for uterine serous carcinomas28. While the p53β and p53γ isoform may still be significant, one may speculate that the low levels of these mRNAs compared to the total levels of p53 mRNA may limit their prognostic influence. We found expression levels of p53 isoforms to be highly correlated with each other. One possible explanation for this relationship is the presence of simultaneous alterations on both the amino- and carboxy-terminus of the protein. However, the isoform expression was not directly proportional to the total levels of p53 mRNA. It is therefore likely that isoforms are not merely a by-product of general p53 transcription but rather have their own biological importance. This controlled side-production of

| Mutation       | AA-change |Responder to Carboplatin n (%) | Non-Responder to carboplatin n (%) |
|----------------|-----------|-----------------------------|----------------------------------|
| 7577545 T > C | M246V∗    | 1 (4.8%)                    | 0                                |
| 7577579 G > C | R248W∗    | 1 (4.8%)                    | 1 (7.1%)                         |
| 7578406 C > T | R175H∗    | 0                           | 1 (7.1%)                         |
| 7574002 CG > C| FrameshR342∗| 1 (4.8%)                  | 0                                |
| 7578271 T > C| H193R∗    | 1 (4.8%)                    | 1 (7.1%)                         |
| 7578190 T > C| Y220C∗    | 0                           | 1 (7.1%)                         |
| 7577141 G > T | G266V∗    | 1 (4.8%)                    | 0                                |
| 7577536 T > A | R249W∗    | 1 (4.8%)                    | 0                                |
| 7577121 G > A | R273C∗    | 0                           | 1 (7.1%)                         |
| 7577106 G > A | P278S∗    | 1 (4.8%)                    | 0                                |
| 7575707 C > T | M237T∗    | 1 (4.8%)                    | 0                                |
| 7577548 C > T | G245S∗    | 1 (4.8%)                    | 1 (7.1%)                         |
| 7575727 T > C| N235S∗    | 0                           | 1 (7.1%)                         |
| 7577509 C > A | E258Xnonsense∗ | 0                        | 1 (7.1%)                         |
| 7578535 T > C| K132R∗    | 1 (4.8%)                    | 0                                |
| 7572990 CT > C| FrameshR373∗| 0                        | 1 (7.1%)                         |
| 7574017 C > A | R337L∗    | 1 (4.8%)                    | 0                                |
| 7578527 A > C | C135G∗    | 1 (4.8%)                    | 0                                |
| 7578210 T > C| R213R     | 1 (4.8%)                    | 0                                |
| 7579472 G > C | P72R∗     | 2 (9.5%)                    | 3 (21.4%)                        |
| 7578271 T > A | H193L∗    | 1 (4.8%)                    | 0                                |
| 7577556 C > G | C242S*    | 1 (4.8%)                    | 0                                |
| 7577121 GCAC > G| DeletionR273∗| 0                          | 1 (7.1%)                         |
| 7577122 C > G| V2723∗    | 0                           | 1 (7.1%)                         |
| 7578529 A > C | F134C*    | 1 (4.8%)                    | 0                                |
| 7578442 T > G | Y163S*    | 1 (4.8%)                    | 0                                |
| 7578442 T > A | K120Xnonsense* | 1 (4.8%)                  | 0                                |

Table 4. Overview over mutations detected in our cohort. ∗Mutation has been reported earlier in the IARC archive39, 39, 39. defined as hotspot or LOF mutation. Abbrevations: AA amino acid.

(logrank p = 0.002; Fig. 4C). When introducing a combined biomarker panel including the TP53 mutation classes and levels of p53 isoforms into a Kaplan-Meier univariate model, we did not observe an enhanced discrimination between the patient groups (data not shown).

Discussion

The ability to predict response to first-line chemotherapy in HGSOC is currently limited. Better biomarkers are needed to optimise the therapeutic regimen for the patients. There are a limited number of studies assessing the prognostic relevance of the p53 network, including the aberrant expression of p53 isoforms reported in various human malignancies13–17,23–26,29,30. By quantification of the mRNA levels of selected p53 isoforms in HGSOC tumour tissues, from patients carefully selected based on chemotherapy responses, we showed that the over-expression of the total Δ133p53 isoforms was associated with prolonged OS and PFS in multivariate survival analyses. However, the p53 isoform levels were not found to be significantly differentially distributed when the chemoresistant and chemosensitive cohorts were compared. These results are in line with a previous study that identified the Δ133p53 isoforms (Δ133p53α,β,γ) as favourable prognostic factors in TP53 mutated, advanced serous ovarian cancers28. In contrast, Δ133p53 has been shown to promote invasion and cancer cell stemness in breast cancer cell lines and has been associated with impaired survival in patients with breast carcinomas16,17. The exact roles of Δ133p53 isoforms and the means by which they become tissue-specific therefore remain to be elucidated. Furthermore, the higher relative expression of p53β to total p53 was associated with FIGO stage IIIC; thus, it could be hypothesised that p53β plays a role in the metastatic potential. The p53γ isoform is missing the transactivation and tetramerization domains that seem necessary for enabling the transcriptional competence of p53α, and the higher expression has been associated with tumour traits and favourable survival in other cancer types12,29. The present data also revealed that the mRNA level of Δ133p53 was much higher than the levels of p53β and p53γ in most patients. This is in line with previous findings for uterine serous carcinomas28. While the p53β and p53γ isoform may still be significant, one may speculate that the low levels of these mRNAs compared to the total levels of p53 mRNA may limit their prognostic influence. We found expression levels of p53 isoforms to be highly correlated with each other. One possible explanation for this relationship is the presence of simultaneous alterations on both the amino- and carboxy-terminus of the protein. However, the isoform expression was not directly proportional to the total levels of p53 mRNA. It is therefore likely that isoforms are not merely a by-product of general p53 transcription but rather have their own biological importance. This controlled side-production of

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isoforms by the splice machinery may reduce the wild-type transcript effect. Additionally, some of the cases analysed may possess a more general, inherent splice chaos.

The TP53 mutation status had predictive significance when the TP53 mutations were categorised into (i) a group with LOF mutations and common hotspots (R273, R248, R175) that was compared with the survival of patients bearing cancers (ii) with the TP53 wild-type tumours or (iii) other, unclassified TP53 defects. There are conflicting reports on the predictive value of different TP53 mutation types in HGSOC. Similar to the findings in this study, other studies have also found the PFS in patients with TP53 wild-type tumours inferior to that of patients with non-canonical TP53 mutations. Because TP53 mutations are an almost universal observation in HGSOC, the observed wild-type status could be questioned. It is possible that TP53 is inactivated by alternative mechanisms in the patients with no detected TP53 mutation.

A correlation was found between TP53 LOF and hotspot mutations and the FIGO stage IV tumours. This is in line with previous studies revealing differential effects of the various TP53 mutations. The TP53 mutations seem to have a differential oncogenic capacity, depending on the mutation site and resulting downstream effect. The univariate analyses of the combined biomarker panels for the TP53 mutation classes and the expression of p53 isoforms did not enhance the significance of the prognostic information. This might have been the result of the small number of patients analysed in each group (n = 5–8; data not shown).

The clinical assessment of the TP53 mutation status are regularly performed by immuno-histochemical staining. This standard diagnostic approach is based on the accumulation of p53 protein in a majority of TP53 mutated cases. This accumulation of p53 protein is seen as the result of an increased protein stability of mutant p53. Here, we found that the TP53 mutated specimens also had higher levels of total p53 mRNA, and this might have contributed to the resulting protein accumulation.

Conversely, we found five patients to be TP53 wild-type. While, there is an ongoing debate whether TP53 mutations are ubiquitous in HGSOC, it is noteworthy that large sequencing efforts, such as TCGA, have also found a fraction of HGSOC to be TP53 wild-type. As such we believe our findings regarding mutational status, represent the true biology of our cases.

The small number of cases resulting from the rigorous exclusion of possible confounders must be regarded as limitation of this study. In addition, the expression of the total Δ40p53 isoform was not detected in the current experiments although this isoform has been detected in comparable series. Although the current experimental setup enabled the quantitative detection of mRNAs containing the specific breakpoints for the different p53 isoforms, we were unable to reveal the combined breakpoints leading to alterations on both the carboxy-terminus and the amino-terminus (total Δ133p53 includes the pool of Δ133p53α, Δ133p53β and Δ133p53γ isoforms). Currently, such mRNAs can be detected only qualitatively and, at best, semi-quantitatively. It is possible that isoform subtypes may possess an even better prognostic impact in HGSOC and other cancer types.

The ability to predict a good response to first-line chemotherapy in HGSOC is a crucial milestone in the implementation of personalised medicine. The term ‘BRCAness’ was introduced to describe ovarian or breast tumours that share molecular features and drug sensitivity with BRCA-mutated tumours. ‘BRCAness’ is associated with the response to PARP inhibitors, but no other molecular biomarkers play a role in the treatment stratification for HGSOC. The findings of this study point to the Δ133p53 isoform as a potential biomarker, pending further validation.

Methods

Patient characteristics. The original cohort of 493 individuals represents a consecutive collection of all of the patients diagnosed with and treated for EOC at Haukeland University Hospital, Bergen, Norway, during the period August 2001–June 2013. From this cohort, 40 patients with a good response to standard combination chemotherapy (‘responders’, defined as time to recurrence ≥17 months, n = 40) and 29 patients with a poor response to treatment (‘non-responders’, defined as progressive disease ≤6 months, n = 29) were selected to participate in the study. Advanced HGSOC was defined as FIGO stage IIIC or IV, high-grade (World Health Organization [WHO] Grade 2/3) serous histology and ovarian, primary peritoneal or fallopian origin. The patient demographic data and tumour traits, such as FIGO stage, as well as phenotypic characteristics, such as date of diagnosis and surgical treatment, level of complete cytoreduction and follow-up (at a mean of 41 months, range 2–144 months) were available for analysis (see Table 1).

All of the patients included in the study were treated with primary cytoreductive surgery and clinically staged according to the FIGO 2014 criteria. Surgical debulking was followed by platinum-based chemotherapy. Patients who were offered different treatment protocols, such as neoadjuvant chemotherapy and anti-angiogenic treatment, and patients who had concomitant cancers were excluded to avoid confounders. PFS was defined as the time interval, in months, between the date of the termination of adjuvant platinum-containing chemotherapy to the date of recurrence or last follow-up. OS was defined as the time interval, in months, from the date of the primary surgery to the last follow-up date or death from disease. Two patients were excluded from this part of the analysis because of missing follow-up data.

The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethical committee (Regional Committees for Medical and Health Research Ethics Vest [REK], University of Bergen, Norway; id: 2018/72). The biological material and phenotypic data that formed the basis of this project are part of the Bergen Gynecologic Cancer Biobank, Women’s Clinic, Haukeland University Hospital, Bergen, Norway (REK id: 2014/1907 and 2015/548). In accordance with the local ethics committee’s guidelines, written informed consent was prospectively obtained from all of the women before the collection of fresh frozen tumour tissue, blood samples and clinicopathologic parameters was initiated.
Tumour tissue. After collection at the time of primary diagnosis, the tissue specimens were immediately frozen in liquid nitrogen, and the clinical data were annotated. If there were multiple eligible primary biopsies, the ovarian mass was the preferred tumour site for analysis. The tumour content of the fresh frozen specimens was assessed in ethanol-fixed and hematoxylin- and eosin- stained sections. While the minimum cut-off for inclusion was set at 50%, the tumour purity was more than 80% in a majority of the included tissue samples (n = 39). The histopathological analysis was performed at the Haukeland University Hospital Department of Pathology. The specimens were fixed in buffered formaldehyde, embedded in paraffin and further processed in the laboratory before standard histological sections were made. Pathologists trained in gynaecologic oncology performed the diagnostic assessments.

Nucleic acid isolation and cDNA synthesis. DNA was isolated from the fresh frozen samples by tissue digestion at 65 °C in a lysis buffer containing NaCl, EDTA 0.5 M pH8.5, TrisHCl pH8, sodium dodecyl sulphate (SDS) 5%, protease K 20 mg/ml and H2O. Following overnight incubation, standard ethanol precipitation with sodium perchlorate and isopropanol was performed. DNA quantity was determined using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. RNA quantification was performed using a NanoDrop M-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). A total of 500 ng RNA was added to 20 μl reaction mix, and single-strand cDNA was synthesised using the Transcriptor Reverse Transcriptase system (Roche, Basel, Switzerland) according to the manufacturer’s protocol.

Quantitative real-time PCR. Quantitative real-time PCRs (qPCR) were performed using specific primers and hydrolysis probes (see Table S1) targeting total TP53 or the different isoforms of TP53, as was previously described36. Importantly, this experimental setup enabled the quantitative detection of the mRNA production of p53 isoforms; however, it did not allow for discrimination between the full-length molecules and their respective isoforms exhibiting alterations on both the carboxy-terminus and the amino-terminus. This important limitation is caused by the limited amplicon length used for the real-time qPCR. The mRNAs of the detected and quantified TP53 were the total p53, total Δ133p53, p53β and p53γ isoforms.

TP53 sequencing and mutation calling. The targeted massive parallel sequencing of the tumour DNA generated data on the TP53 mutation status. The fragmentation of 1,000 ng dsDNA was achieved using the Covaris® M220 Focused-ultrasonicator™ (Covaris, Woburn, MA, USA). The library preparation was performed using the Agilent SureSelectXT reagent kit (Agilent Technologies, Santa Clara, CA, USA), and the individual samples were run on a MiSeq instrument (Illumina, San Diego, CA, USA). This design included +/− 10 nucleotides at exon-intron borders, to cover potential splice site mutations. The TP53 data was extracted from a sequencing effort applying baits targeting 360 genes as previously described37. Preliminary mutation calling was performed using the MiSeq Reporter (MSR) software, and the raw mutation calling output was revised by the application of post-processing filters. All of the suspected TP53 mutations were validated by the manual inspection of the sequencing reads using the Integrative Genomics Viewer38.

Statistical analyses. The Shapiro-Wilk test was performed to assess the normality assumption. On the basis of the non-normal distributed expression levels of total p53, total Δ40p53, total Δ133p53, p53β and p53γ isoforms, we calculated the Spearman’s rank-order correlation for those variables. The Mann-Whitney U- and Kruskal-Wallis tests were applied to investigate the associations among the continuous variables (age and isoform expression levels) and the categorical variables (age grouped by median, disease stage, histological grade, presence of complete cytoreduction and chemoresistance as well as TP53 mutation status). Fisher’s exact test and Pearson’s chi-squared test were used for comparisons of the categorical variables (patient age grouped by median, presence of complete cytoreduction, chemosensitivity and mutation status). The univariate survival analysis was performed by the Kaplan-Meier method, and subsets of patients (divided by median absolute or relative to total p53 expression of isoforms) were compared using the log-rank test. Multivariate survival analyses were performed using the Cox proportional-hazards regression model in a one-step fashion. Important clinical predictors of survival, such as age, FIGO stage and resection status, were added as categorical covariates. In the Cox proportional-hazards model, the absolute expression of the p53 isoforms and the isoform expression relative to the total p53 were tested as predictors. All of the p-values were reported as two-sided, and p-values < 0.05 were considered significant. The statistical analyses were performed using the SPSS 22.0 software package (SPSS Inc., Chicago, IL, United States of America).

Data Availability
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author Contributions
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Additional Information
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