Clinical Cancer Research

Homologous Recombination DNA Repair Pathway Disruption and Retinoblastoma Protein Loss Are Associated with Exceptional Survival in High-Grade Serous Ovarian Cancer

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Abstract

Purpose: Women with epithelial ovarian cancer generally have a poor prognosis; however, a subset of patients has an unexpected dramatic and durable response to treatment. We sought to identify clinical, pathological, and molecular determinants of exceptional survival in women with high-grade serous cancer (HGSC), a disease associated with the majority of ovarian cancer deaths.

Experimental Design: We evaluated the histories of 2,283 ovarian cancer patients and, after applying stringent clinical and pathological selection criteria, identified 96 with HGSC that represented significant outliers in terms of treatment response and overall survival. Patient samples were characterized immunohistochemically and by genome sequencing.

Results: Different patterns of clinical response were seen: long progression-free survival (Long-PFS), multiple objective responses to chemotherapy (Multiple Responder), and/or greater than 10-year overall survival (Long-Term Survivors). Pathogenic germline and somatic mutations in genes involved in homologous recombination (HR) repair were enriched in all three groups relative to a population-based series. However, 29% of 10-year survivors lacked an identifiable HR pathway alteration, and tumors from these patients had increased Ki-67 staining. $CD8^+$ tumor-infiltrating lymphocytes were more commonly present in Long-Term Survivors. RB1 loss was associated with long progression-free and overall survival. HR deficiency and RB1 loss were correlated, and co-occurrence was significantly associated with prolonged survival.

Conclusions: There was diversity in the clinical trajectory of exceptional survivors associated with multiple molecular determinants of exceptional outcome in HGSC patients. Concurrent HR deficiency and RB1 loss were associated with favorable outcomes, suggesting that co-occurrence of specific mutations might mediate durable responses in such patients. *Clin Cancer Res; 24(3); 569–80. ©2017 AACR.*

See related commentary by Peng and Mills, p. 508

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Translational Relevance

By considering high-grade serous ovarian cancer patients who deviate from a common pattern of initial response, subsequent relapse, and the progressive development of chemotherapy resistance, it may be possible to identify tumor or host factors that could be modified in patients with a more typical disease trajectory. The variation seen in the clinical pattern of response and recurrence seen in the patient cohort analyzed here suggests multiple factors may influence long-term patient survival. Therefore, consideration should be given to analyzing specific clinical subgroups of long-term survivors to maximize the ability to discern causative factors. Patients who have residual disease following surgery and yet have no disease relapse following adjuvant chemotherapy are of particular interest. That these patients are enriched for loss of RB1 expression and homologous recombination repair pathway mutations indicates that unusually long survival may arise from chance co-occurrence of mutations that render tumors highly sensitive to chemotherapy.

Introduction

Variation in survival and extreme responses to treatment in cancer patients provides valuable opportunities to investigate biological determinants of therapeutic response and outcome. Exceptional survivors have recently received increased attention (1) because they may suggest novel therapeutic approaches to apply to more typical cancer patients.

Approximately three quarters of ovarian cancer deaths are associated with high-grade serous cancer (HGSC), which is typically diagnosed at an advanced stage (2). Although fewer than half of all patients with HGSC will be alive at five years, some survive much longer (3, 4). Residual disease following primary surgery is a significant negative prognostic factor for advanced-stage epithelial ovarian cancer (5, 6), but optimal debulking does not fully explain unusually good clinical outcomes, because approximately half of all long-term (10 years) survivors have residual disease following debulking surgery (4).

Inactivation of the homologous recombination (HR) DNA repair pathway is particularly important in influencing response to platinum-based therapy in HGSC (7). Mutations in *BRCA1/2* (8–10), or genomic changes associated with HR-pathway inactivation (11), are also predictive of response to poly(ADP-ribose) polymerase (PARP) inhibitors. Higher response rates to chemotherapy in patients with germline *BRCA1/2* mutations are reflected in increased 5-year survival (12, 13). However, survival advantage diminishes thereafter, and fewer patients with *BRCA1* mutations are alive at 10 years compared with those with a *BRCA2* mutation or noncarriers (14). It is unclear whether differences in response and survival in mutation-positive women are associated with the type or position of mutations in *BRCA1/2*, or additional genomic changes in the tumors (12, 13).

Other prognostic factors in HGSC include molecular subtype defined by gene expression profiles (15, 16) and *CCNE1* (cyclin E1) amplification (17). *CCNE1* amplification is mutually exclusive with *BRCA1/2* mutation and thus is a biomarker of HR-intact tumors that are less likely to respond to platinum-based chemotherapy (16, 18, 19). Finally, the presence of $CD8^+$ tumor-infiltrating lymphocytes is strongly associated with improved survival (20).

In this first detailed clinical and molecular characterization of exceptional response in HGSC, we studied three partially overlapping patient outlier groups, namely, those with progression-free survival of >36 months despite having residual disease following debulking surgery, patients with three or more complete responses to chemotherapy, and/or those who survived more than 10 years. Our findings highlight differences in clinical trajectories of HGSC patients with unusually favorable outcomes, and show that in addition to mutations in the HR pathway, high Ki-67, high CD8 and co-occurrence of *RB1* and HR pathway mutations are associated with long-term survival.

Materials and Methods

Patients

Patients were identified in the population-based Australian Ovarian Cancer Study (AOCS, $n = 1{,}776$) and the Gynaecological Oncology Biobank at Westmead Hospital, Sydney (GynBiobank, $n = 507$; Supplementary Fig. S1). This project was conducted with Human Research Ethics Committees approval. AOCS and GynBiobank were approved by Human Research Ethics Committees at all participating centres, and written informed consent was obtained from all participants. Patient identifiers (ID) used in this article were randomly generated for the purpose of publication. Median follow-up was 9.95 years (95% confidence interval, 9.7–10.2 years) from diagnosis.

Cases were selected according to the following inclusion criteria: (i) histologically confirmed serous ovarian, fallopian or peritoneal carcinoma; (ii) International Federation of Gynecology and Obstetrics (FIGO) stage IIIC or IV disease; (iii) high grade (grade 2 or grade 3) at diagnosis; (iv) primary treatment incorporating a platinum-based agent; and (v) an exceptionally good clinical outcome, defined by a long progression-free interval (Long-PFS), multiple complete treatment responses (Multiple Responder) and/or long survival (Long-Term Survivors). Long-PFS was defined as >36 months progression-free survival from diagnosis and restricted to cases with macroscopic residual disease after primary cytoreductive surgery. Multiple Responders had complete responses (assessed by CA125 criteria, ref. 21) to three

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or more lines of platinum-based chemotherapy. Response to first line was only included in patients with residual disease after surgery. Long-Term Survivors had an overall survival period of at least 10 years (120 months) after histological diagnosis. Collectively, these three patient subgroups are referred to as Exceptional Responders. Statistical considerations for the selection of cutoff points to identify outliers in treatment response and overall survival are detailed below.

All eligible Exceptional Responder cases ($n = 112$; Supplementary Table S1) underwent pathology review to confirm diagnosis and histological subtype and to determine histological grade according to standardized criteria (22). Review was completed by expert gynecological pathologists using a complete set of hematoxylin and eosin (H&E)–stained diagnostic slides or H&E-stained slides from 1 to 3 representative diagnostic block/s selected by the original reporting pathologist. Previous analysis performed by the AOCS has shown the two review methods are comparable (13). We were not able to obtain slides for review from 4 patients.

High histopathological grade, *TP53* mutation, abnormal p53 staining, and WT1 positivity characterize HGSC, whereas low-grade serous carcinoma (LGSC) are typically *TP53* wild-type and harbor *ras* pathway mutations (16, 23–27). We applied these molecular criteria to the initial cohort of 112 patients: 8 cases (7%) that had features consistent with *ras* pathway-activated LGSC were excluded from further analyses (Supplementary Table S1). We excluded a further 8 cases for which biospecimens were not available for research, resulting in 96 patients for which blood, tissue, and/or tissue microarray cores were available. All 96 cases of Exceptional Responders were confirmed HGSC based on pathological features, presence of *TP53* mutation, abnormal p53 expression, and/or WT1 expression (Supplementary Table S1).

Comparison control cohorts were obtained from the same patient populations as the Exceptional Responder groups. Cases were matched for stage (FIGO IIIC/IV) and histology (serous) and included all cases, except those that met the Exceptional Responder criteria. For comparison of patient characteristics (Table 1), we analyzed the clinical data from the AOCS serous cohort (2002–2006, $n = 710$, i.e., 785 cases excluding cases that met Exceptional Responder criteria).

Clinical definitions

Progression-free survival (PFS) was defined as the time interval between the date of histological diagnosis and the date of disease progression, determined by CA125 serum levels or imaging according to Gynecological Cancer Intergroup criteria (21), clinical deterioration or death. Overall survival (OS) was defined as the interval between diagnosis and death from any cause, or date of last follow-up for women who were alive. Response to post-relapse lines of treatment was assessed using routine serum CA125 measurements based on Gynecological Cancer Intergroup CA125 criteria (21), and a complete response was defined as normalization of CA125 levels maintained for $\geq$28 days.

Statistical analyses

The Long-PFS cutoff of >36 months was based on analysis of progression-free interval for a large cohort of consecutively recruited cases with advanced stage (IIIC/IV), serous ovarian cancer (AOCS, $n = 782$, median PFS, 12.75 months, recruited 2002–2006). The cutoff point was determined using conditional survival analysis to define the probability of remaining progression-free, having not progressed at specified time points, based on the approach given by Harshman and colleagues (28). This was determined by calculating the probability of being progression free for a further 6 months given that patients have not progressed at 6, 12, 18, 24, 30, 36, 42, 48 to 84 months, respectively. For patients not progressing at or post-36 months, this probability of approximately 80% was constant and 10% higher than those patients not progressing at times <36 months. A similar cutoff point was found using a second method calculating two standard deviations beyond the median (>30.6 months) in the same unselected cohort. The more conservative cutoff of >36 months was used to define Long-PFS in all analyses.

Conditional survival analysis in patients with stage IIIC/IV serous carcinoma (AOCS, $n = 785$) showed that $\geq$84 months OS was consistent with exceptional survival. We also calculated the median OS for the same cohort and found two standard deviations beyond the median to be 118.2 months (median OS, 36.6; SD, 40.81). Both methods supported a conservative cutoff point of >10 years for Long-Term Survivors.

For molecular studies, differences in proportions between groups were assessed by χ^2 test and corresponding 95% confidence interval. Correlations between molecular alterations were estimated by Pearson correlation. The Kaplan–Meier product limit method was used to estimate and plot PFS and OS probabilities and the corresponding time-to-event were compared between groups using the log-rank test. All comparisons were two-sided, and a 5% level of significance was used to declare a statistical difference. The reverse Kaplan–Meier method (29) was used to quantify follow-up time. Statistical analyses were performed using IBM SPSS (version 23) and Stata 10.0 (StataCorp LP).

Molecular analyses

Germline *BRCA1* and *BRCA2* mutation status was available for 89 of 96 Exceptional Responder HGSC patients, through their clinical record and/or previous mutational analyses (13, 30). For cases with tumor samples available (82 of 96 Exceptional Responders), we assessed tumor DNA for pathogenic mutations in 32 genes associated with ovarian cancer, HR and DNA repair (Supplementary Table S3), including *TP53*, *BRCA1*, and *BRCA2*. Sequence analysis of the 32 genes was performed using two types of sequence data: (i) 66 cases were sequenced using the 32-gene panel targeted DNA sequencing approach (see Supplementary Methods for additional details), and (ii) 19 cases had previously undergone comprehensive whole-genome sequencing of primary tumor DNA and matched germline (lymphocyte) DNA (30). The same selection criteria were applied to cases sequenced using either method. Three patients were sequenced by both approaches and showed concordance between pathogenic mutations detected.

BRCA1 and *RAD51C* promoter methylation was assessed by methylation-sensitive high-resolution melting analyses. CD8, RB1, p16, Ki-67, WT1, and p53 protein expression was determined by immunohistochemistry.

Further experimental methods are provided in Supplementary Data.

Results

Distinct clinical patterns of exceptional survival

We considered several objective clinical, surgical and histopathologic criteria to define HGSC patient subsets with a

Table 1. Clinical characteristics by patient subgroups

Characteristics	Long-PFS ($n = 73$) *n* (%)	*P*	Multiple Responders[d] ($n = 21$) *n* (%)	*P*	Long-Term Survivors[e] ($n = 43$) *n* (%)	*P*	Control[f] ($n = 710$) *n* (%)
Age at diagnosis, y							
Median	59	0.30[a]	56	0.04[a]	59	0.58[a]	62
Range	29–77		39–75		40–77		24–80
Primary site							
Ovary	58 (79)	0.01[b]	14 (67)	0.17[b]	31 (72)	0.05[b]	537 (76)
Peritoneum	9 (12)		5 (24)		8 (19)		154 (22)
Fallopian tube	6 (8)		2 (10)		4 (9)		19 (3)
FIGO stage							
IIIC	62 (85)	0.80[b]	18 (86)	0.81[b]	34 (79)	0.42[b]	595 (84)
IV	11 (15)		3 (14)		9 (21)		115 (16)
Residual disease[g]							
Nil	0 (0)	<0.001[b]	2 (10)	0.03[b]	0 (0)	0.006[b]	146 (21)
≤1 cm	35 (48)		14 (67)		18 (42)		222 (31)
>1 cm	24 (33)		4 (19)		15 (35)		197 (28)
Size unknown	12 (16)		1 (5)		9 (21)		86 (12)
Not resected	2 (3)		0 (0)		1 (2)		15 (2)
Not known	0 (0)		0 (0)		0 (0)		44 (6)
Neoadjuvant therapy							
Yes	1 (1)	<0.001[b]	3 (14)	0.66[b]	0 (0)	0.002[b]	128 (18)
No	72 (99)		18 (86)		43 (100)		582[k] (82)
Primary platinum therapy							
Yes	73 (100)	0.16[b]	21 (100)	0.45[b]	43 (100)	0.28[b]	691[k] (97)
No	0 (0)		0 (0)		0 (0)		19 (3)
Primary treatment details[h]							
Platinum/taxane	61 (84)	0.06[b]	18 (86)	0.35[b]	34 (79)	0.35[b]	561 (79)
Platinum only	4 (5)		0 (0)		3 (7)		74 (10)
Platinum/taxane/other[i]	4 (5)		2 (10)		4 (9)		45 (6)
Platinum/other[j]	4 (5)		1 (5)		2 (5)		11[k] (2)
No treatment	0 (0)		0 (0)		0 (0)		19 (3)
Current status							
Alive with no progression	41 (56)	<0.001[b]	0 (0)	0.44[b]	29 (67)	<0.001[b]	28 (4)
Alive with progression	8 (11)		0 (0)		7 (16)		24 (3)
Dead	24 (33)		21 (100)		7 (16)		658 (93)
Progression-free survival from diagnosis (months)							
Median	161.9	<0.001[c]	18.2	0.32[c]	Undefined	<0.001[c]	11.9
Range	36.7–247.5		9.60–37.0		15.6–247.5		0–149.8
Overall survival from diagnosis (months)							
Median	Undefined	<0.001[c]	70.2	0.004[c]	Undefined	<0.001[c]	33.1
Range	36.7–247.5		43.2–122.8		120.8–247.5		0–268.6
>10 years overall survival from diagnosis	40 (55)		1 (5)		43 (100)		33 (5)
Overall grade							
1	0 (0)	0.10[b]	0 (0)	0.56[b]	0 (0)	0.08[b]	33 (6)
2	13 (19)		4 (22)		13 (31)		109 (20)
3	57 (81)		14 (78)		29 (69)		416 (75)
Not known/neoadjuvant[l]	3		3		1		152

NOTE: Exceptional Responder patient subgroups were compared with unselected patients with advanced stage (stage IIIC/IV) serous ovarian cancer (control). Statistical comparisons were calculated as follows:

[a]Mann–Whitney test.

[b]χ^2 test.

[c]Log-rank test.

[d]Includes one case that meets the criteria for Long-PFS, Multiple Responder and Long-Term Survivor.

[e]Includes 40 cases that meet the criteria for Long-PFS and Long-Term Survivor.

[f]Unselected serous ovarian cancer cohort excluding cases that meet criteria for Long-PFS, Multiple Responder or Long-Term Survivor ($n = 75$).

[g]Patients who had residual disease after primary cytoreductive surgery but the size was not recorded were classified as "Size unknown," and patients for whom it was not recorded whether or not they had residual disease were classified as "Not known."

[h]Intraperitoneal chemotherapy received (number of patients indicated in brackets): Long-PFS (1), Multiple Responder (1), Control (6).

[i]Other chemotherapy received in addition to platinum/taxane: Long-PFS: gemcitabine (3), topetecan (1): Multiple Responder: gemcitabine (1), liposomal doxorubicin (1); Long-Term Survivor: gemcitabine (3), topetecan (1); Control: gemcitabine (18), liposomal doxorubicin (18), topetecan (6), bevacizumab (1), etoposide (1), and doxorubicin (1).

[j]Other chemotherapy received in addition to platinum: Long-PFS: gemcitabine (2), cyclophosphamide (1), not known (1): Multiple Responder: cyclophosphamide (1); Long-Term Survivor: cyclophosphamide (1), not known (1). Control: gemcitabine (4), topotecan (3), cyclophosphamide (1), not known (3).

[k]Includes 3 patients where primary chemotherapy details are not known.

[l]Patients who received neoadjuvant chemotherapy or for whom grade was not known were excluded from overall grade analysis.

favorable outcome, collectively termed Exceptional Responders. HGSC diagnosis was based on histopathology, presence of *TP53* mutation, abnormal p53 expression, and WT1 expression (ref. 25; Supplementary Table S1).

To identify clinical and biological dependencies, we first identified patients where a favorable outcome could be associated with response to primary platinum-based chemotherapy. We identified 73 patients with an unusually prolonged PFS of >36 months (Long-PFS). This time point was based on conditional survival analysis, and a similar cutoff was found using a second method, calculating two standard deviations beyond the median (>30.6 months; Materials and Methods). We took into consideration that response to primary treatment is the combined effect of debulking surgery and chemotherapy. Therefore, to characterize patients with unambiguously chemoresponsive tumors, we restricted analysis to cases with residual disease after surgery, including 26 women who were nonoptimally debulked (>1 cm residual disease or tumor not resected), and yet had a rapid and sustained fall in serum CA125 following chemotherapy (CA125 normalization >36 months; Fig. 1A and Supplementary Fig. S2).

We also identified 21 patients with multiple responses to chemotherapy. Acquired chemoresistance is common in HGSC, and many patients experience diminished responses to each line of treatment (31). Within AOCS, less than 3% of stage IIIC/IV patients (21/785) had three or more complete responses to platinum-based chemotherapy, providing a threshold for selection of patients whose tumors appeared to have limited capacity for developing resistance (Fig. 1B and Supplementary Fig. S2).

Lastly, we identified 43 Long-Term Survivors (OS > 10 years). Of these, 29 (67%) remained progression free, and 14 (33%) had one or multiple periods of relapse and treatment-induced remission (Fig. 1C and Supplementary Fig. S2). This clinical variability illustrates that there are diverse ways leading to extended survival. Collectively, 96 HGSC patients were identified as Exceptional Responders, with partial overlap between the groups (Fig. 1D).

Clinical characteristics of Exceptional Responders

Clinical characteristics of the HGSC exceptional response groups are summarized in Table 1. Compared with unselected serous ovarian cancer patients, Multiple Responders were younger, while Long-PFS and Long-Term Survivors were a similar age at diagnosis. Patients with Long-PFS were less likely to have primary peritoneal cancer, and all three Exceptional Responder groups were less likely to have had neoadjuvant chemotherapy. Additional clinical descriptors and longitudinal CA125 profiles are provided in the Supplementary Data.

The majority of Long-PFS patients (41/73; 56%) were alive with no progression at time of analysis (Table 1). Indeed, many Long-PFS became Long-Term Survivors (40/73), although this was not always the case, with some patients having an extended progression-free interval but lack of chemoresponse upon recurrence (e.g., patient 15051; Supplementary Fig. S2). Conversely, Long-Term Survivors did not necessarily have extended PFS or sustained responses to therapy (e.g., patient 5693; Supplementary Fig. S2). Almost no Multiple Responders became Long-Term Survivors, with only one case being in both groups (Fig. 1D).

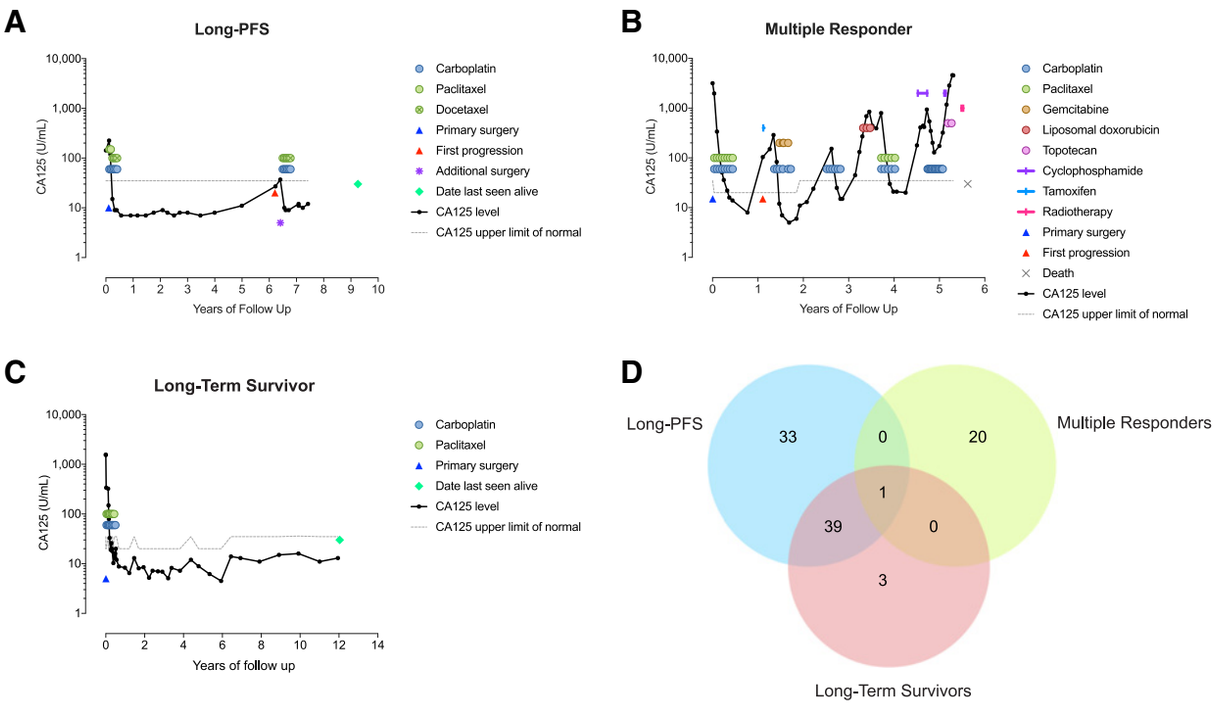

Figure 1.

Clinical patterns of Exceptional Responders. Plots of CA125 levels of patients representing the three exceptional responder subgroups: **A,** Long-PFS; **B,** Multiple Responders; **C,** Long-Term Survivors. Black lines, CA125 levels on a log scale; dotted gray lines, upper limit of normal for each CA125 measurement. The upper limit of normal can vary depending on the type of CA125 assay performed. Colored circles and rectangles represent administration of different lines of treatment as indicated. Also indicated is the time of primary surgery (blue triangle), first progression (red triangle), death (gray cross), or date last seen alive (green diamond). **D,** Venn diagram indicates number of patients in each clinical response category and any overlap between groups.

TP53 mutations in Exceptional Responders are typical of HGSC

TP53 is almost invariably mutated in HGSC (16, 24). We considered whether distinct *TP53* mutations were associated with exceptional survival. Tumor tissue was available for DNA analysis in 82 of the 96 included cases. *TP53* mutations were identified in 98% (80/82; Fig. 2A and Supplementary Table S4). Most *TP53* mutations were missense (64%, 51/80) and the distribution and classes of *TP53* mutations were similar to those previously reported (16) in HGSC ($P = 0.99$, χ^2 test; Supplementary Fig. S3A and S3B). The frequency of mutation type was not different between exceptional responder subgroups. Eleven TP53 residues were recurrently mutated in Exceptional Responders (i.e., mutated in more than one case; Supplementary Table S4), representing 39% of cases. Patients with these *TP53* mutations in an unselected HGSC cohort (16) from The Cancer Genome Atlas (TCGA; $n = 67/265$) failed to demonstrate a significant survival advantage (median OS, 49.24 months, compared with 44.51 months for the remainder of the cohort; $n = 198$; $P = 0.45$; Supplementary Fig. S3C).

HR and DNA-repair pathway mutations

Germline *BRCA1/2* mutation status was obtained for 89/96 Exceptional Responders through clinical records and/or mutational assessment (Materials and Methods). While a higher proportion of Long-Term Survivors (9/39, 23.1%, $P = 0.34$), Long-PFS (18/67, 26.9%, $P = 0.05$) and Multiple Responders (4/20, 20%, $P = 0.73$) had germline *BRCA1/2* mutations compared with an unselected HGSC cohort (ref. 13; 98/574, 17%; Fig. 2B; Supplementary Table S6), the majority were not mutation carriers. Somatic inactivation of HR DNA repair is also important in influencing response to platinum-based therapy in HGSC (16). Comprehensive mutation analysis of *BRCA1*, *BRCA2*, and other HR DNA repair genes (Supplementary Table S3) was performed on tumor samples from 82 of 96 Exceptional Responders, as well as *BRCA1* and *RAD51C* promoter methylation profiling. Taking into consideration only truncating mutations (nonsense, frameshift, and splice site) and missense mutations previously reported to be pathogenic, 10 genes were identified as mutated (*BRCA1*, *BRCA2*, *RAD51C*, *BRIP1*, *CDK12*, *PTEN*, *ATR*, *CHEK1*, *CHEK2*, and *RAD51D*; Fig. 2A and Supplementary Table S5). Overall, 73% (60/82) of Exceptional Responders had evidence of HR pathway disruption, compared with 41% (128/316, $P < 0.001$, χ^2 test) in the TCGA HGSC cohort (ref. 16; Fig. 2C). The TCGA study previously reported (16) HR pathway disruption in up to 51% of cases. However, the TCGA samples underwent whole-exome sequencing, and additional putative mechanisms of HR inactivation were reported, including (i) mutations in additional Fanconi anemia genes that were not included on our panel (*FANCA*, *FANCC*, *FANCI*, *FANCL*, *FANCE*, *FANCG*, and *FANCM*), (ii) *EMSY* amplification, and (iii) missense variants of unknown or uncertain significance. For direct comparison with Exceptional Responders in this study, we only considered truncating mutations and pathogenic missense mutations in the genes in our panel (Supplementary Table S3). A greater proportion of Long-PFS patients with bulky residual disease (>1 cm) had HR defects compared with those with minimal residual disease ($\leq$1 cm; 79% vs. 61%; $P = 0.14$), and the highest proportion of HR defects were found in Multiple Responders (88%, $P < 0.001$; Fig. 2C; Supplementary Table S6), with a predominance of *BRCA1* mutations (59%), reflecting the role of HR deficiency in chemosensitivity.

RB1 loss is enriched in Long-PFS and Long-Term Survivors

A subset of 19 Exceptional Responders had whole-genome sequencing as part of our International Cancer Genome Consortium study (30), allowing us to investigate whether HR-pathway mutations were associated with other genomic aberrations. We previously observed that gene breakage results in inactivation of tumor suppressors in HGSC, including *RB1* (30). Inactivation of *RB1* was frequent in Exceptional Responders, particularly in Long-PFS patients with 6 of 11 cases (54.5%) affected by *RB1* gene breakage or homozygous deletion (Fig. 3A). To explore this observation further, and because *RB1* loss by gene breakage is not apparent in targeted sequence data, we validated RB1 protein detection by immunohistochemistry (Fig. 3B and Supplementary Fig. S4) and then assessed expression in a cohort of 313 patients, including 91 Exceptional Responders (Supplementary Fig. S1). RB1 loss was significantly more frequent in Long-PFS (35%, $P < 0.001$) and Long-Term Survivors (33%, $P = 0.001$) compared with unselected HGSC (13%; Fig. 3C and Supplementary Table S6). RB1 loss was enriched in Long-PFS patients who were non-optimally debulked compared with those with <1 cm residual disease (48% versus 27.5%; Fig. 3C, $P = 0.09$). Furthermore, in an independent analysis of unselected HGSC patients ($N = 1{,}083$), low *RB1* mRNA expression was associated with increased overall survival (Fig. 3D, $P = 0.014$).

Homogeneous p16 expression pattern is frequently used as a marker of RB1 loss. We found a significant correlation between RB1 loss and homogeneous p16 staining in Long-PFS ($P = 0.001$), Long-Term Survivors ($P = 0.001$) and control patients ($P = 0.04$; Pearson correlation); however, p16 staining alone was not enriched in Exceptional Responders (Fig. 4A). RB1 loss and homogeneous p16 were not correlated in Multiple Responders ($P = 0.116$; Pearson correlation).

Association between HR deficiency and RB1 loss

We identified enrichment for co-occurrence of RB1 loss and HR deficiency in Long-PFS (20/58, 34.5%) and Long-Term Survivor (11/33, 36.4%) groups compared with control HGSC (13/75, 17.3%) and Multiple Responders (3/17, 17.6%). HR deficiency and RB1 loss were highly correlated in Long-PFS ($P = 0.008$), Long-Term Survivors ($P = 0.014$), less so in controls ($P = 0.039$) and not in Multiple Responders ($P = 0.486$; Pearson correlation). Kaplan–Meier survival analysis of patients with HR-defective tumors demonstrated a significant survival advantage associated with concurrent RB1 loss in Exceptional Responders (Fig. 3E; $P = 0.03$).

Proliferative and immune cell markers reveal a heterogeneous pattern within the cohort

Immune cell infiltration, particularly in tumor epithelium, has been associated with favorable outcomes in multiple HGSC datasets (20). We scored $CD8^+$ lymphocytes on tissue microarrays comprising Exceptional Responders and controls (Supplementary Fig. S1 and Supplementary Table S7). Both intraepithelial and stromal CD8 scores were significantly higher in Long-PFS and Long-Term Survivors, but not Multiple Responders, compared with controls (Fig. 4A–C and Supplementary Table S6). Elevated levels of $CD8^+$ lymphocytes were associated with *BRCA1* mutations (Supplementary Fig. S5A and S5B), as previously observed (32–34), but not with other predicted HR-inactivating alterations, although sample sizes were small for these other groups. $CD8^+$ tumor infiltrates were also elevated in HR-intact tumors compared

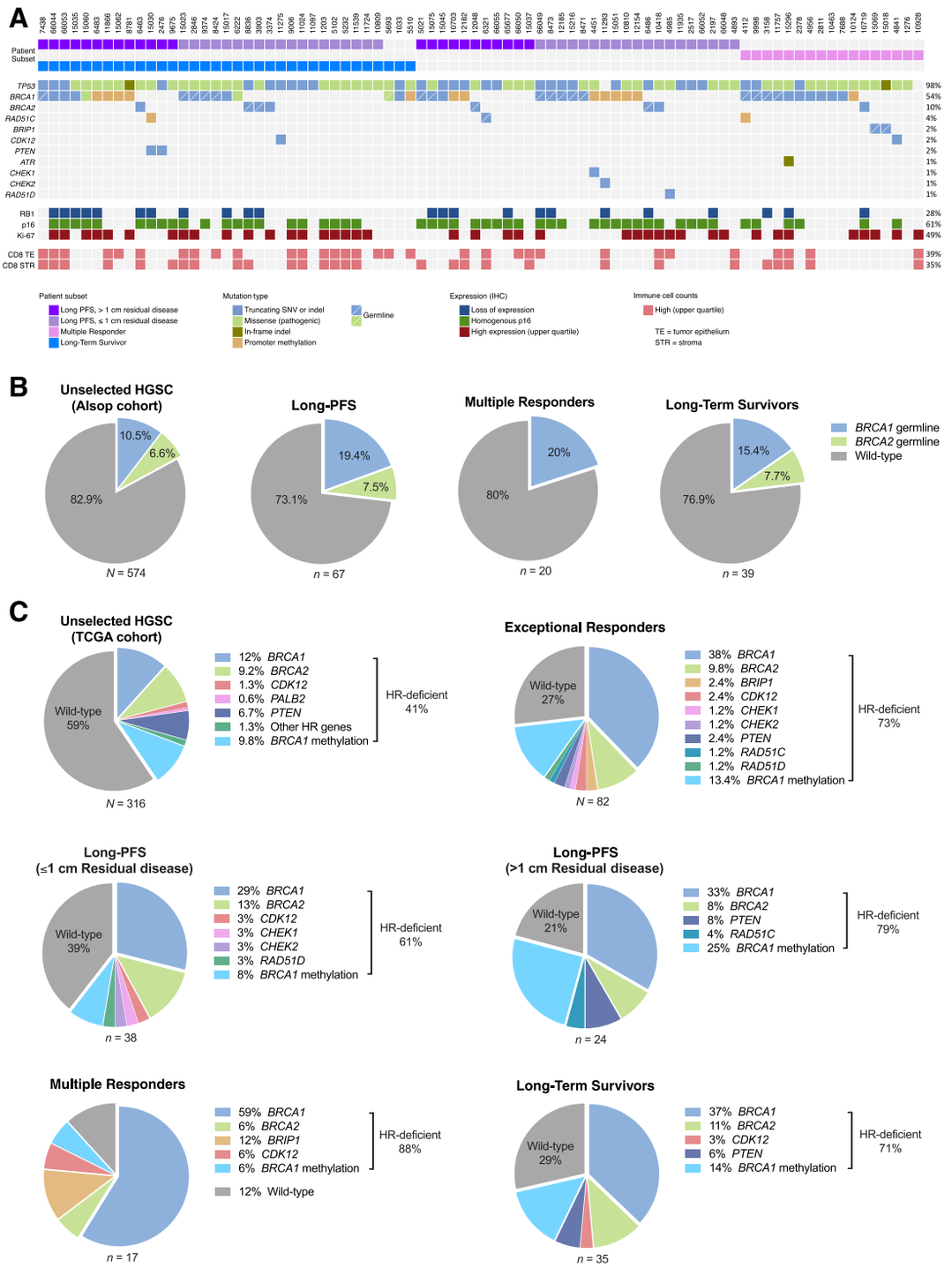

Figure 2.

HR pathway gene alterations in Exceptional Responders. **A,** Pathogenic alterations in HR and DNA repair genes detected by next-generation sequencing of tumor DNA from 82 Exceptional Responder patients. Patients are grouped by clinical subgroups, and mutated genes are listed in descending order from most to least frequently altered across the entire Exceptional Responder cohort. In addition, tissue microarrays of formalin-fixed paraffin-embedded HGSC specimens were stained with antibodies and assessed for RB1 protein loss, high Ki-67 expression, homogeneous p16 expression, and $CD8^+$ lymphocytes in tumor epithelium (TE) and stroma (STR). **B,** Prevalence of *BRCA1* and *BRCA2* germline mutations in unselected HGSC Alsop cohort (13) and each clinical subgroup. **C,** Proportion of patients affected by either germline or somatic HR pathway gene alterations in unselected HGSC TCGA cohort (16) and each clinical response group as indicated. One gene mutation is counted for samples with more than one change, ranking mutations in *BRCA1, BRCA2*, followed by other germline, somatic and promoter methylation events respectively. In the unselected HGSC cohort, the "other HR genes" altered were *ATM* (1), *FANCD2* (1), *MSH6* (1), and *RAD51* (1). The number of patients is indicated in parentheses.

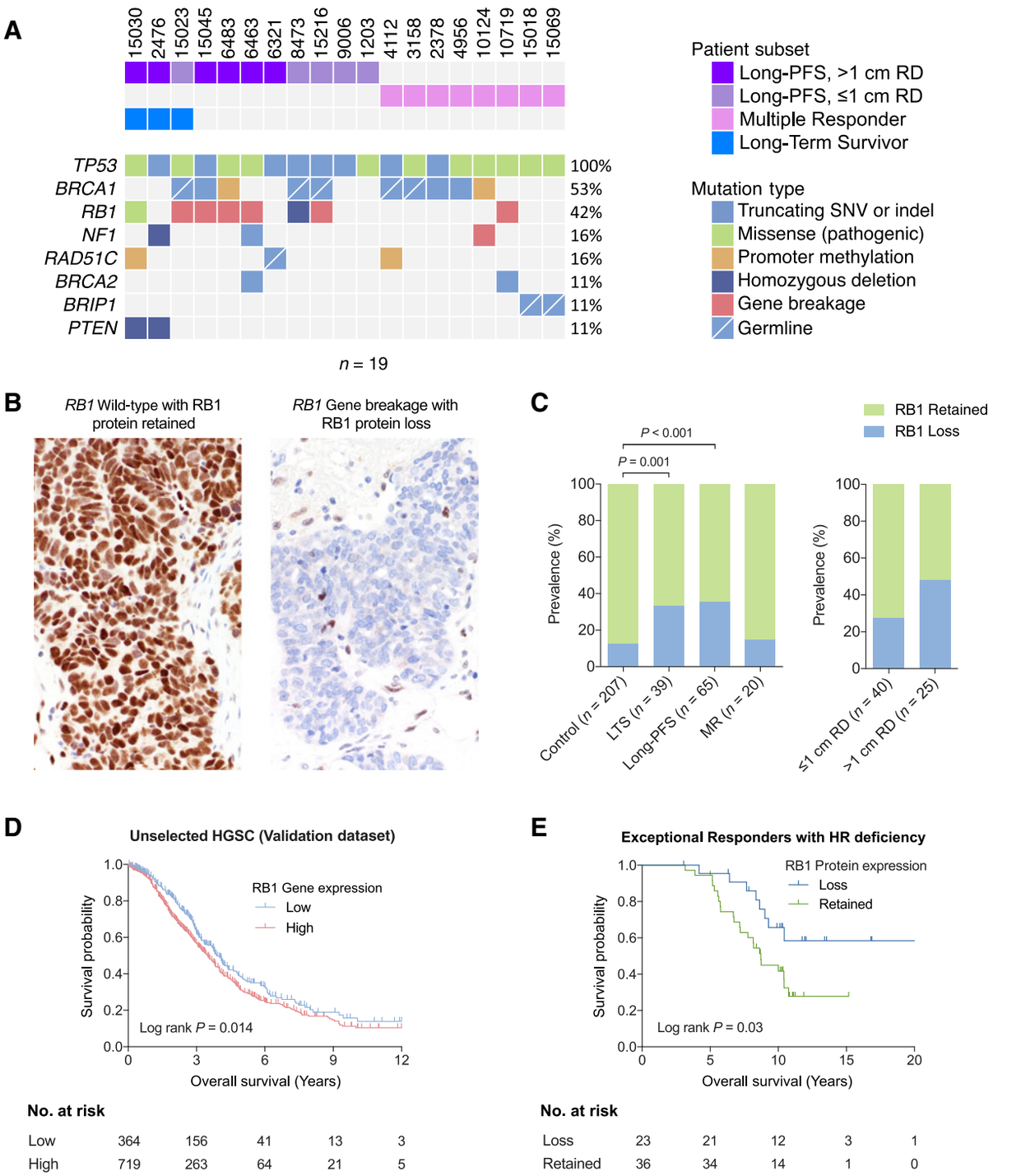

Figure 3.

RB1 loss and clinical associations in HGSC patients. **A,** Inactivating structural variants and mutations detected in tumor suppressor genes by whole-genome sequencing in 19 Exceptional Responders (30). **B,** Representative immunohistochemical staining patterns are shown for tumors in which RB1 protein expression was retained or lost. **C,** Proportion of tumors with RB1 loss in each clinical subgroup compared with control (unselected HGSC; left) and in Long-PFS patients with >1 cm residual disease (RD) compared with those with ≤1 cm (right; χ^2 *P* value reported). **D,** In a meta-analysis of 1,083 HGSC patients using publicly available gene expression array data sets (ref. 43; http://kmplot.com), patients with low tumor *RB1* mRNA expression were associated with significantly better overall survival (Affymetrix Probeset ID 203132_at; automatic cutoff). **E,** Kaplan–Meier estimates of overall survival for Exceptional Responders with HR-defective tumors, by RB1 protein expression.

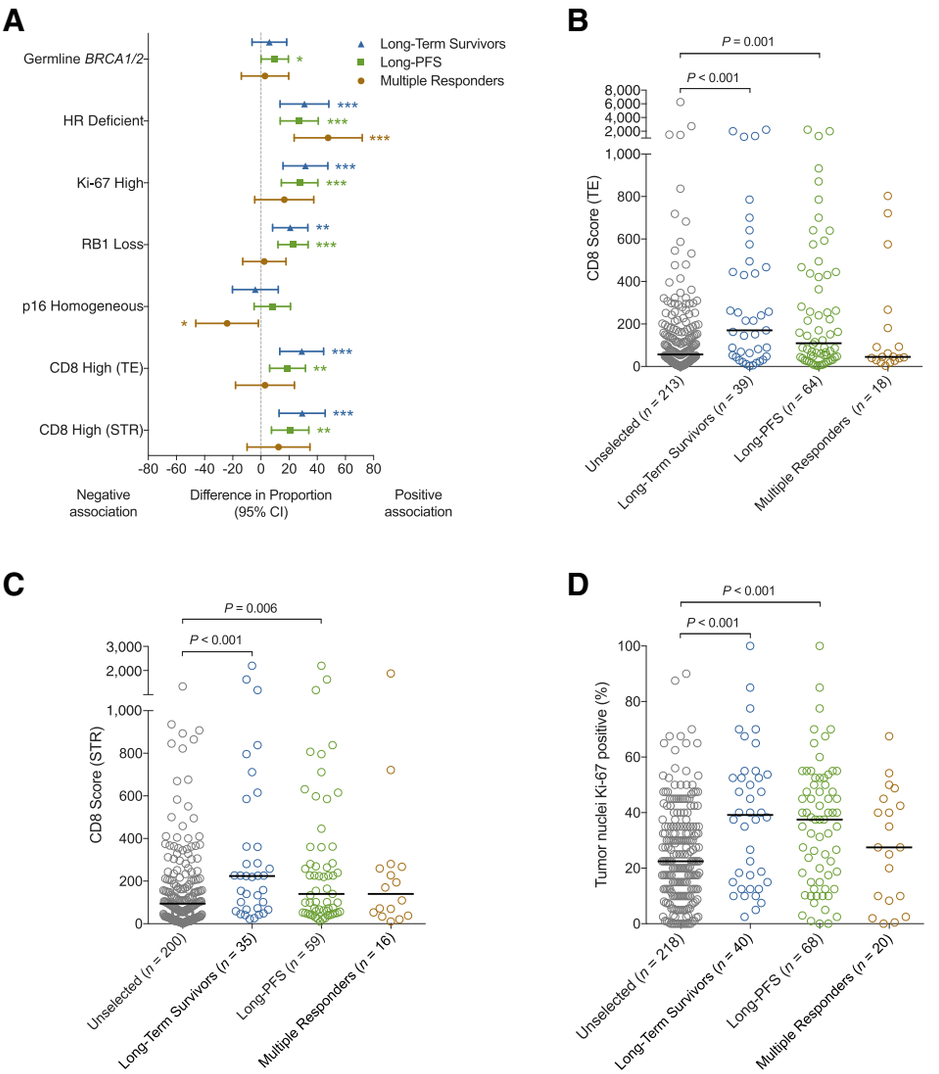

Figure 4.

Characterization of germline *BRCA1/2* mutation, HR deficiency, immune cell infiltration and proliferative capacity of tumors in Exceptional Responders. **A,** Proportions of each molecular alteration in clinical subgroups compared with control groups. Germline *BRCA1/2* and somatic HR pathway mutation rates were compared with those reported previously in HGSC cohorts (13, 16). The values for Ki-67 (upper quartile), RB1 (loss), p16 (homogeneous staining), and CD8 (upper quartile) are relative to unselected HGSC. Differences in proportions between groups were assessed by χ^2 test. *P* values are represented by asterisks: *, < 0.05; **, < 0.01; ***, < 0.001. Further details are listed in Supplementary Table S6. **B,** Density (in cells/mm^2) of CD8$^+$ lymphocytes in tumor epithelium (TE) and **C,** stroma (STR). **D,** Quantification of Ki-67 expression in tumor nuclei of different patient subgroups, detected by immunohistochemical staining. In scatter plots the horizontal lines indicate the median score. *P* values are shown for the subgroups that had significant differences to the median score of unselected HGSC (Mann–Whitney test).

with the control group ($P = 0.009$; Supplementary Fig. S5A), indicating that presumably HR-competent tumors can also engender immune responses.

We also evaluated proliferation status, reasoning that highly proliferative tumors may be more susceptible to chemotherapy. Ki-67 was significantly higher in Long-PFS and Long-Term Survivor groups ($P < 0.001$ and $P < 0.001$, respectively; Fig. 4A and D; Supplementary Tables S6 and S7). Ki-67 scores were highest in HR-proficient tumors (Supplementary Fig. S5C), potentially indicating a mechanism of sensitivity that is distinct from HR deficiency.

Discussion

The potential to stratify cancer care by molecular phenotype has focused attention on cancer patients with unusually favorable outcomes, including those with exceptional responses to systemic therapy (35). Previous analyses of cancer registry data (3) and clinical parameters (4) for factors that influence 10-year survival in ovarian cancer patients have highlighted the importance of tumor grade, histotype and optimal surgical cytoreduction. Therefore, any molecular analysis of long-term survival must also control for clinical and histopathologic factors associated with survival (36). Given the heterogeneity of ovarian cancer (2), we focused on the dominant ovarian cancer histologic subtype, HGSC, and provide the first detailed molecular characterization of patients with over 10-year survival and those with exceptional responses to chemotherapy. We compared exceptional cases with those representing the spectrum of responses seen in HGSC rather than just platinum-resistant cases, to avoid reidentifying determinants of resistance (17).

HGSC has the highest frequency of HR disruption of any cancer type, through somatic and germline mutations in *BRCA1/2* and their protein partners, and this is a major determinant of the unusually high rates of response to platinum-based therapy compared with other solid cancers (7, 16). Accordingly, we found an increased frequency of pathogenic mutations in HR proteins in our series relative to unselected HGSC (16). *BRCA1/2* germline mutations are not, however, sufficient to impart long-term survival; indeed, many *BRCA1/2* carriers have average or even poor

responses to therapy (13), suggesting that co-occurrence of other factors are needed to obtain durable responses to therapy and/or long-term survival. We found that RB1 loss, in conjunction with HR deficiency, was associated with particularly long survival, which is consistent with a previous analysis of RB1 expression in a large series of HGSC (37). The highest frequency of RB1 loss was seen in nonoptimally debulked Long-PFS patients, indicating an association with extreme chemosensitivity.

RB1 loss and gene disruption has been associated specifically with *BRCA1*-mutated basal-like breast cancers, which are molecularly similar to HGSC (38). Whether these are associated with response to platinum-based therapy, which is becoming more common in basal-like breast cancer, requires investigation. It is notable that loss of RB1 expression is seen in only a minority of lung adenocarcinoma patients, but is strongly associated with improved survival following cisplatin-based therapy (39). Further studies are warranted to determine whether RB1 loss is predictive for platin, taxane, or combined sensitivity in HGSC.

We identified a subset of patients in all three clinical groups where no HR pathway disruption was detected by mutation or methylation profiling. The TCGA analysis included additional putative HR variants, and it is possible that the proportion of HR-deficient Exceptional Responders in our study may be an underestimate. Whether the HR pathway is disrupted via untested mechanisms may be revealed by an analysis of genomic scarring (40). However, the observation of increased Ki-67 immunoreactivity in this group suggests they may be molecularly distinct and represent an opportunity to develop novel therapeutic interventions.

In addition to RB1 loss, we found higher densities of $CD8^+$ lymphocytes in Long-PFS and Long-Term Survivors compared with unselected cases. Intriguingly, this association did not apply to Multiple Responders, despite recent evidence that T-cell infiltrates can inhibit chemoresistance mediated by stromal fibroblasts in ovarian cancer (41). Instead, Multiple Responders were highly enriched for mutations involving the HR pathway. These patients resemble the cyclic pattern of relapse and repeated response to cisplatin seen in a *BRCA1* mouse model of breast cancer (42). In that model, a large deletion of *BRCA1* precludes the development of resistance by intragenic reversion. By extension, tumors of Multiple Responders may have an impaired ability to restore HR pathway activity despite the strong selective pressure of repeated platinum exposure. Few Multiple Responders became Long-Term Survivors, suggesting that while deregulation of the HR pathway can increase sensitivity to chemotherapy, it is not sufficient for a complete response leading to prolonged, 10-year survival. Cooperating factors, such as those identified in this study including immune cell infiltration, RB loss, and proliferation rate, appear to be required to confer long-term survival. One limitation in the classification of Multiple Responders is our reliance on CA125 criteria of response rather than radiological data. Serial imaging data were not available, so stringent CA125 criteria requiring normalization of CA125 were applied (Materials and Methods).

In summary, our findings suggest that distinct clinical patterns of exceptional response in HGSC are imparted by the interplay between HR pathway disruption, the chance co-occurrence of other mutations such as RB1, and immune factors. The substantial clinical, molecular, and immunological heterogeneity we observed, even within this highly selected cohort, indicates that large numbers of HGSC patients will be required to untangle these interactions. Given the rarity of Exceptional Responders, this will be possible only by international collaboration among investigators, patients, and patient advocates to identify these unusual individuals.

Disclosure of Potential Conflicts of Interest

K. Alsop reports receiving other commercial research support from Astra Zeneca. Y.C. Leung is a consultant/advisory board member for Royal Australian and New Zealand College of Obstetricians and Gynaecologists. P.J. Beale reports receiving speakers bureau honoraria from and is a consultant/advisory board member for AstraZeneca, Ipsen, and Roche. A. deFazio reports receiving commercial research grants from AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

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