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Original Article

Investigation of selective glucocorticoid receptor modulation in high-grade serous ovarian cancer PDX models

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ABSTRACT

Objective: In ovarian cancer (OvCa), tumor cell high glucocorticoid receptor (GR) has been associated with poor patient prognosis. In vitro, GR activation inhibits chemotherapy-induced OvCa cell death in association with transcriptional upregulation of genes encoding anti-apoptotic proteins. A recent randomized phase II study demonstrated improvement in progression-free survival (PFS) for heavily pre-treated OvCa patients randomized to receive therapy with a selective GR modulator (SGRM) plus chemotherapy compared to chemotherapy alone. We hypothesized that SGRM therapy would improve carboplatin response in OvCa patient-derived xenograft (PDX).
Methods: Six high-grade serous (HGS) OvCa PDX models expressing GR mRNA (*NR3C1*) and protein were treated with chemotherapy +/− SGRM. Tumor size was measured longitudinally by peritoneal transcutaneous ultrasonography.
Results: One of the 6 GR-positive PDX models showed a significant improvement in PFS with the addition of a SGRM. Interestingly, the single model with an improved PFS was least carboplatin sensitive. Possible explanations for the modest SGRM activity include the high carboplatin sensitivity of 5 of the PDX tumors and the potential that SGRMs activate the tumor invasive immune cells in patients (absent from immunocompromised mice). The level of tumor GR protein expression alone appears insufficient for predicting SGRM response.
Conclusion: The significant improvement in PFS shown in 1 of the 6 models after treatment with a SGRM plus chemotherapy underscores the need to determine predictive biomarkers for SGRM therapy in HGS OvCa and to better identify patient subgroups that are most likely to benefit from adding GR modulation to chemotherapy.

Keywords: Ovarian Cancer; Patient-Derived Xenografts; Glucocorticoid Receptor; Nuclear Hormone Receptor; Chemotherapy Resistance

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Conflict of Interest

JTV is currently an employee of GlaxoKlineSmith. GFF reports honorarium for talk from Curio Science, advisory board for GSK, uncompensated advisory board for TTC, and PI of industry-sponsored study for Corcept, Abbvie, Genentech/Roche, Tesaro/GSK, Syndax, 47 inc, Iovance, Syros, Astex, Merck, Sanofi, Sermonix, Compugen, INcyte, Hoffman LaRoche, Eisai. The authors acknowledge supply of Relacorilant from Corcept Therapeutics. SDC and The University of Chicago have been issued patents on methods of measuring GR expression in triple-negative breast cancer (TNBC) and using GR antagonists in TNBC and prostate cancer.

Synopsis

This study found that a selective glucocorticoid receptor (GR) modulator (SGRM) added to carboplatin increased high-grade serous ovarian cancer response and lengthened time to primary tumor regrowth in a subset of GR-expressing patient-derived xenografts. GR expression alone did not predict SGRM benefit and thus, additional biomarkers are needed.

INTRODUCTION

Glucocorticoid receptor (GR) is a nuclear hormone receptor and transcription factor activated by endogenous cortisol and pharmacologic glucocorticoid treatment. A recent in-depth immunohistochemical analysis of epithelial ovarian cancer (OvCa) GR expression demonstrated that 133 of 349 patients (39%) had high tumor GR expression defined as >1% of tumor cells with 2+ or 3+ intensity staining [1]. This study also found that high tumor GR protein expression is associated with a worse prognosis in epithelial OvCa patients. High GR expression in tumor cells has also been shown to correlate with decreased duration of patient survival in estrogen receptor (ER)–negative breast cancer [2] and endometrial cancer [3,4], suggesting that GR expression in tumor cells may have prognostic value in a variety of cancer types.

Glucocorticoids reduce the efficacy of chemotherapy-induced cytotoxicity by activating anti-apoptotic mechanisms in GR-positive (GR+) cell lines in vitro and in cell line xenograft models in vivo [5-11]. Dexamethasone and subsequent GR transcriptional activation has been found to induce expression of anti-apoptotic genes including *SGK1*, *DUSP1*, *cIAP2*, and *MCL1* in OvCa cell lines [7,10]. In addition, in a randomized pilot study, upregulation of *SGK1* and *DUSP1* mRNA was observed in resected ovarian tumor tissues from patients undergoing surgery for suspected OvCa who received intra-operative dexamethasone vs. normal saline [12]. Glucocorticoid treatment or endogenous cortisol and subsequent GR activation may also modulate GR+ tumor cell adhesion and proliferation to promote tumor cell invasion and metastasis [13-15].

These findings have led to translational interest in exploring cortisol competitive ligands or "selective GR modulators" (SGRMs) as adjuncts to anti-cancer therapy to improve tumor sensitivity when co-administered with chemo- and/or immunotherapy. Relacorilant (CORT125134, Corcept Therapeutics) is a potent SGRM with high affinity for GR and no significant binding to progesterone receptor, ER, or androgen receptor [16]. In vitro studies have shown that relacorilant competitively inhibits the effect of glucocorticoid on the expression of several pro-tumorigenic GR target genes [7,16,17]. Relacorilant was found to be safe and well-tolerated in a first-in-human clinical study in healthy volunteers [18]. In a phase I/II study of 72 patients with advanced solid tumors treated with relacorilant the day before, day of, and the day after nab-paclitaxel infusion (NCT02762981), a recommended phase II dose of relacorilant in combination with nab-paclitaxel was established [19]. Thirty-one percent (31%) of the 13 patients with OvCa receiving this combination had disease control lasting greater than 24 weeks [19]. Subsequently, a randomized phase II study of nab-paclitaxel with or without relacorilant in platinum-resistant OvCa patients (NCT03776812) showed a significant improvement in progression-free survival (PFS) in patients receiving 2 pre- and one post-chemotherapy relacorilant doses compared to chemotherapy alone [20]. Interestingly, this disease-specific difference in PFS was observed in the absence of a response rate difference [20].

Author Contributions

Conceptualization: V.J.T., W.S.J., F.G.F., C.S.D.; Data curation: T.M., H.X., K.N., L.M.C., M.M.J., H.E.P., O.A.L., C.S.D.; Formal analysis: T.M., H.X., V.J.T., L.M.C., M.M.J., H.E.P., C.H., L.R., O.A.L., F.G.F., C.S.D.; Funding acquisition: V.J.T., W.S.J., F.G.F., C.S.D.; Investigation: T.M., V.J.T., W.S.J., F.G.F., C.S.D.; Methodology: T.M., H.X., V.J.T., O.A.L., W.S.J., F.G.F., C.S.D.; Project administration: F.G.F., C.S.D.; Resources: W.S.J., C.S.D.; Supervision: W.S.J., F.G.F., C.S.D.; Validation: W.S.J., F.G.F., C.S.D.; Writing - original draft: T.M., V.J.T., W.S.J., F.G.F., C.S.D.; Writing - review & editing: T.M., H.X., K.N., L.M.C., O.A.L., W.S.J., F.G.F., C.S.D.

To further understand the potential benefit of adding a SGRM to chemotherapy in OvCa in vivo, we used a well-established OvCa patient-derived xenograft (PDX) model system, wherein tumors are implanted intraperitoneally into SCID-Bg mice, and tumor size following 4 weeks of treatment is measured longitudinally by peritoneal ultrasonography [21]. High-grade serous (HGS) OvCa PDXs maintain their original tumor heterogeneity and gene expression patterns in these model systems [21-23]. We hypothesized that pre-treatment with a SGRM would result in increased carboplatin-induced tumor shrinkage and/or increased time to tumor regrowth in GR-expressing HGS OvCa PDXs. In one of the 6 models, we found that tumor shrinkage following carboplatin (as measured by peritoneal transcutaneous ultrasonography) showed a trend towards improvement with the addition of the SGRM. Moreover, when tumors in this model were followed longitudinally for tumor regrowth after completion of chemotherapy +/− SGRM, addition of the SGRM was associated with a significantly improved progressive disease (PD, defined as the time for a regrowing tumor to reach 1.44 fold the post-treatment nadir as measured by the largest cross sectional diameter) and PFS (defined as either time from treatment initiation (day 0) to detection of PD, unexpected animal death or unplanned sacrifice) compared to animals receiving chemotherapy alone.

MATERIALS AND METHODS

1. PDX study

In accordance with the Mayo Clinic Institutional Review Board (IRB), fresh tissue from consenting patients with ovarian, primary peritoneal, or fallopian tube cancer was collected at the time of debulking surgery as previously described [21], and coded with a patient heterotransplant (PH) number in accordance with the Mayo Clinic IRB and the Health Insurance Portability and Accountability Act regulations. An IRB-approved written informed consent form was documented in the electronic medical record of every patient. All animal procedures were approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC), consistent with all policies of the American Veterinary Medical Association. Tumors were established by intraperitoneal injection into female SCID-Bg mice (C.B.-17/IcrHsd-Prkdcscid Lystbg; ENVIGO) as previously described [22]. Briefly, approximately 0.3 mL of minced patient tumor was mixed 1:1 with McCoy's 5a medium containing rituximab (10 mg/kg, Genentech, Inc., South San Francisco, CA, USA) to prevent lymphoma development [24]. Mice were monitored for engraftment, and PDX tumors were expanded into additional mice when the tumor dimension was assessed as greater than or equal to 10% of body weight or if humane endpoints were met (humane endpoints: weight loss greater than or equal to 20% of body weight, inability to ambulate, inability to reach for food and/or water, tumors that had ulcerated, or a body condition score of 5 or less using the IACUC approved scoring system). PDX tumors were formalin fixed and cryogenically preserved for future experiments [22]. The minimal information standard for OvCa PDX models is provided in **Table 1**.

For in vivo PDX drug studies, 6 selected GR+ HGS OvCa PDX tumors were minced and injected into the peritoneal space of SCID-Bg mice, as described above for initial tumor inoculations. When tumors reached a minimum threshold of 0.3–0.5 cm^2 cross-sectional area by ultrasound, animals were randomized into 4 groups of mice per model. For PDX model (PH423, PH454, PH462): 1) control vehicle injected, 2) relacorilant alone (15 mg/kg) 24 hours and 1 hour prior to carboplatin treatment, 3) carboplatin (51 mg/kg) weekly for 4 weeks; and 4) combination of carboplatin and relacorilant. This schedule was chosen based on preclinical data in xenograft models, with the intention of reducing anti-apoptotic GR

Table 1. Patient and ovarian tumor characteristics

PDX model	Patient age (yr)	Histology	Stage of tumor at resection for PDX	Neoadjuvant therapy prior to resection	Clinical platinum status*	*TP53* mutation	*RAD51C*, *RAD51D*, *BRCA1/2* mutation (HRR status)
PH423	55	HGS carcinoma	IIIC	No	Sensitive	Not detected	Not detected
PH454	44	HGS carcinoma	IIIC	No	Sensitive	Not detected	Not detected
PH462	48	HGS carcinoma	IV	Yes	Sensitive	*TP53*, c.323_329del7; p53, p.Val107fs	Not detected
PH160	65	HGS carcinoma	IIIC	No	Sensitive	*TP53*, c.277delC; p53, p.Leu93fs; *TP53*, c.733G>A; p53, p.G245S	RAD51C, c.956G>A *RAD51C*, R319Q *BRCA1/2*: not detected
PH756	65	HGS carcinoma	IVB	No	Sensitive	*TP53*, c.722C>A; p53, p.S241Y	Not detected
PH817	40	HGS carcinoma	IVB	No	Sensitive	Not detected	*RAD51D*, c.796C>T *RAD51D*, p.Arg266Cys *BRCA1/2*: not detected

Characteristics of patient and ovarian tumors from which PDX models were derived. Tumor histology, stage at time of resection for PDX generation, neoadjuvant therapy status, clinical platinum status (clinical platinum sensitivity defined as disease progression >6 months following upfront platinum-based chemotherapy), and *TP53, RAD51C, RAD51D, BRCA1/2* status are shown.
HGS, high-grade serous; HRR, homologous recombination repair; PDX, patient-derived xenograft; PH, patient heterotransplant.
*Clinical platinum-sensitive defined as disease progression >6 months following the completion of the primary platinum-based chemotherapy.

transcriptional activity with SGRM treatment prior to chemotherapy infusion [7]. PH160 and PH756 received the same 4 treatments, consisting of relacorilant and/or a reduced dose of 5 mg/kg of carboplatin. PDX model PH817 received the same 4 treatments, consisting of an increased dose of 20 mg/kg of relacorilant and/or 25 mg/kg carboplatin. For model PH817 we administered relacorilant 24 hours before, 1 hour before and 24 hours after carboplatin treatment. The additional dose after chemotherapy was added to mimic the treatment schedule used in the randomized phase II study of nab-paclitaxel with or without relacorilant in platinum-resistant OvCa [20].

2. RNA sequencing

RNA was extracted from snap-frozen PDX tumor tissues using the standard protocol for Qiagen RNeasy mini kit (Cat #74106; Qiagen, Venlo, Netherlands) with on-column DNA digestion for measuring *NR3C1* mRNA expression. Purity of total RNA and concentration was determined on a Thermo Scientific NanoDrop 2000c UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). All samples met RNA integrity number and validated Agilent (Agilent Technologies, Santa Clara, CA, USA) criteria.

A total of 100–200 ng of RNA was used for library preparation. The Mayo Clinic sequencing core (Rochester, MN, USA) prepared the paired-end sequencing libraries with the TruSeq Stranded Total Sample Preparation kit (Illumina, San Diego, CA, USA) and performed quality control, cluster generation and sequencing on the Illumina HiSeq 2000 platform. The reads were de-multiplexed and converted to FASTQ format using CASAVA software from Illumina (by the Mayo Clinic core) and read alignment was performed with TopHat [25]. From the aligned BAM files, expression data were summarized at the gene level and converted to normalized RPKM values by an RNA-seq workflow named MAP-RSeq [26].

3. GR protein expression by western blot

Cryopreserved tissues were homogenized in RIPA lysis buffer with phosphatase and protease inhibitors (Roche Diagnostics). Samples were clarified at 15,000 rpm for 10 minutes, and resulting lysate was quantified using Pierce BCA Protein Assay (Thermo Scientific), per the manufacturer's instructions. Protein (30 μg per sample) was denatured with Laemmli buffer

and resolved with SDS-PAGE. Membranes were blocked with 5% BSA (Sigma-Aldrich, St. Louis, MO, USA) in TBST and immunoblotted with anti-GR antibody at 1:1,000 (XP D8H2, Cell Signaling), and anti-a-tubulin 1:2,000 (DM1A, Cell Signaling). They were then washed in TBST and probed with Alexafluor-conjugated anti-rabbit or anti-mouse secondary antibodies (Alexafluor 680 goat anti-rabbit IgG A21109 [Invitrogen, Waltham, MA, USA] and Alexafluor 800 anti-mouse [LI-COR]) and imaged on the Odyssey infrared imaging system (LI-COR).

4. Tumor harvesting, fixation, and GR immunohistochemistry (IHC)

Residual tumors were harvested from PDX mice immediately after the observation phase (i.e. beginning at day 28 post-treatment and ending with tumor progression or animal sacrifice/unexpected death). Tumors treated with carboplatin + relacorilant were harvested from all 6 models, while tumors treated with carboplatin alone could only be harvested from 5 of the 6 models (PH423, PH462, PH160, PH756, and PH817). Tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin. Immunohistochemical analysis was performed on 5 μm sections using a Dako Autostainer Link 48 system. Briefly, the slides were baked for 30 minutes at 60°C, then deparaffinized and hydrated before the antigen retrieval step. Heat-induced antigen retrieval was performed for 20 minutes in a Dako PT Link. The tissue was incubated with a peroxidase block followed by an antibody incubation (anti-GR antibody, Cell Signaling, clone D8H2, 1:500 dilution) for 20 minutes. The staining was visualized using the EnVision FLEX or EnVision+ Rabbit HRP visualization system. Tissues from PH454 PDX mice could not be collected due to carboplatin sensitivity which left an insignificant amount of tumor to collect.

Anti-GR IHC staining was scored by the collaborating pathologist (HC), to evaluate the percentage of tumor cells with positive nuclear staining and the average staining intensity, according to the following scale: 0 (no staining), 1+ (weak), 2+ (moderate), and 3+ (strong). An H-score was then calculated by multiplying the percent of tumor cells with nuclear staining by the average staining intensity in each case to obtain an H-score ranging from 0 to 300. H score = [1 × (percentage of cells with 1+ intensity) + 2 × (percentage of cells 2+) + 3 × (percentage of cells 3+)]. Graphs were plotted on GraphPad Prism software version 10. Statistical analysis was performed using an unpaired 2-tailed Student's t test considering $p \leq 0.05$ as the threshold for statistical significance.

5. Statistical methods

Repeated measures implemented via linear mixed effects models were used to compare tumor growth trajectories between arms on the natural log scale separately for each PDX as we previously described [27]. PD was defined as ultrasound measurement with a 1.44 times increase in tumor area relative to the tumor size at its nadir. This is akin to a 20% increase in greatest length dimension, as used clinically in the RECIST criteria for patient tumor assessment [28,29]. Since a tumor size less than 0.1 cm^2 in a mouse is difficult to reliably measure by ultrasound (limit of detection), PD was only called when the raw tumor value was at least twice the limit of detection. In contrast, PFS was defined as time from treatment initiation (day 0) to detection of PD, unexpected animal death or unplanned sacrifice. Overall survival (OS) was defined as time from treatment initiation (day 0) to unexpected animal death or unplanned sacrifice. Kaplan-Meier methods were used to generate plots and confidence intervals.

The treatment phase (weeks 0–4) and observation phase (week 4+) were modeled separately with day (centered), an indicator of treatment arm, and the 2-way interaction as fixed effects.

For the treatment phase, a quadratic centered day effect and interaction with treatment arm were added for PH756 and PH817. Due to initial growth followed by treatment response in PH423, squared (interaction with treatment arm) and cubic centered day fixed effects were added. For the observation phase, a quadratic centered day effect and interaction with treatment arm were added for PH423 and PH454. Spatial (power) correlation structure was used to account for the correlation between repeated observations. A single 3-degree of freedom (df) coincident curve test was used for PH423's treatment encompassing the mean, linear and quadratic slopes. A single 2-df hypothesis test of coincident curves (accounts for both mean and slope) was performed for all other cases. Mean values in figures are estimates from these models.

RESULTS

1. GR is variably expressed in HGS OvCas and corresponding PDXs

We identified OvCa PDX models in the Mayo Clinic cohort [21] by screening PDX RNA-seq data for high GR mRNA expression. We defined high GR mRNA as a read count above the median expression of GR (*NR3C1*) in 84 available Mayo OvCa models. Of these 42 high GR models, 6 HGS models without *BRCA* mutation and with carboplatin sensitivity were chosen. **Fig. 1A** shows the 6 PDX GR (*NR3C1)* mRNA steady-state expression levels, and **Fig. 1B** shows their relative GR protein levels by western blot analysis. As shown, we found a positive correlation between high GR mRNA and protein. Clinical and pathological characteristics of the patients who donated their tumor tissue are listed in **Table 1**. All 6 tumors had been diagnosed as HGS OvCa. Five of 6 of the models were from chemotherapy-naïve patients undergoing debulking

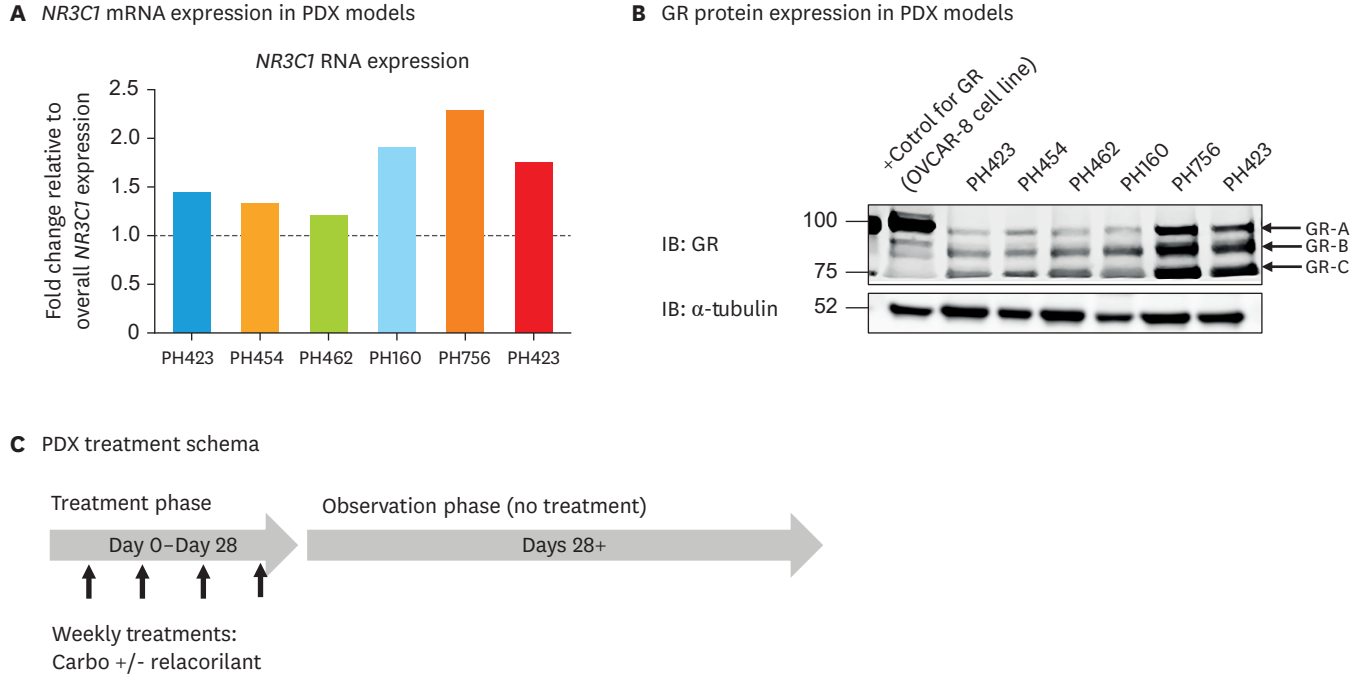

Fig. 1. PDX GR expression and treatment schema. (A) Levels of *NR3C1* gene expression in OvCa PDX tumors by RNA sequencing. (B) Immunoblot of OvCa PDX tumors depicting varying levels of GR isoforms. (C) PDXs randomized into treatment arms (day 0), were treated weekly for 4 cycles with carboplatin +/– relacorilant. Ultrasounds were performed weekly during both treatment and observation phases.
GR, glucocorticoid receptor; OvCa, ovarian cancer; PDX, patient-derived xenograft; PH, patient heterotransplant.

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surgery, and one patient (PH462) underwent surgical debulking following neoadjuvant therapy containing carboplatin. Three out of 6 tumors had *TP53* mutations detected, while the other 3 had no *TP53* mutation detected. Interestingly, PDX PH160 came from a germline *RAD51C*-mutated patient whose tumor also had both a p53 frameshift mutation (Leu93fs) and a p53 G245S DNA binding domain missense mutation. All 6 PDX tumors were considered "platinum-sensitive" because they were resected from patient's tumors that recurred more than 6 months after adjuvant (or neoadjuvant, PH462) chemotherapy. All 6 PDX tumors also showed carboplatin sensitivity in prior mouse studies [21].

2. Platinum-sensitive GR+ HGS OvCa PDX models show variable treatment response

Having confirmed that the selected GR+ PDXs remained platinum-sensitive during preliminary experiments at the Mayo core, we initiated treatment with either carboplatin, relacorilant + carboplatin, relacorilant alone, or control (vehicle). **Fig. 1C** shows the treatment schema. We started treatment with 51 mg/kg/week of carboplatin, given intraperitoneally (based on the previously determined maximum tolerated dose for SCID-Bg mice) [30]. Using this dose, we measured tumor response in the first 3 models. All 3 PDXs showed tumor shrinkage close to 100% after 4 weekly carboplatin or relacorilant + carboplatin treatments. This prevented our ability to detect an increase in tumor shrinkage with the addition of relacorilant (PH454, PH462, PH423, **Fig. 2A-C**). Therefore, we reduced our carboplatin dose by 90% (5.1 mg/kg per week) for the remaining 3 GR+ models (PH160, PH756, PH817, **Fig. 2D-F**). PDX PH160 did not show a significant shrinkage with carboplatin alone compared to control treatment (carbo 10% vs. control, p=0.431, **Table 2**). However, PDX PH160 did shrink significantly when relacorilant was added to carboplatin (relacorilant + carbo 10% vs. control, p=0.044, **Table 2**). Taken together, these results indicate that the majority of the high GR-expressing PDXs we selected were exquisitely sensitive to carboplatin.

3. Measurement of PFS, PD, and OS in GR-expressing PDX models following carboplatin +/– relacorilant treatment

PDX models in immunocompromised mice are usually used to measure tumor shrinkage while the mice are on therapy. However, women with OvCa often relapse despite a "complete response," both radiographically and by a greatly reduced tumor marker, after surgery and adjuvant chemotherapy [31]. The time period to tumor "regrowth" or PD is an important aspect of PFS and quality of life for patients. We therefore decided to monitor for PDX regrowth by ultrasonography, morbidity necessitating sacrifice, or unexpected death as a measure of PFS as well as PD, and OS. Tumor regrowth curves during the observation phase are shown in **Fig. 2G-L**. We observed a significantly longer PFS with the addition of

Table 2. The p-value comparison of treatment groups (days 0–28)

PDX model	Carbo vs. control	Relacorilant + carbo vs. control	Relacorilant + carbo vs. carbo
PH423*	**0.000**	**0.000**	0.481
PH454*	**0.015**	**0.002**	0.706
PH462*	**0.000**	**0.000**	0.998
PH160†	0.431	**0.044**	0.154
PH756†	**0.000**	**0.000**	0.801
PH817†	**0.000**	**0.000**	0.652

Endpoint for treatment phase was tumor size at day 28. Comparisons assess whether tumor shrinkage was significantly improved by treatment with carboplatin compared to control, relacorilant + carboplatin compared to control, and relacorilant + carboplatin compared to carboplatin alone. Statistically significant values are bolded. Statistical criteria for obtaining p-values are described in the methods section.
PDX, patient-derived xenograft; PH, patient heterotransplant.
*Model treated with full dose carboplatin (51 mg/kg); †Model treated with carboplatin 10% (5 mg/kg).

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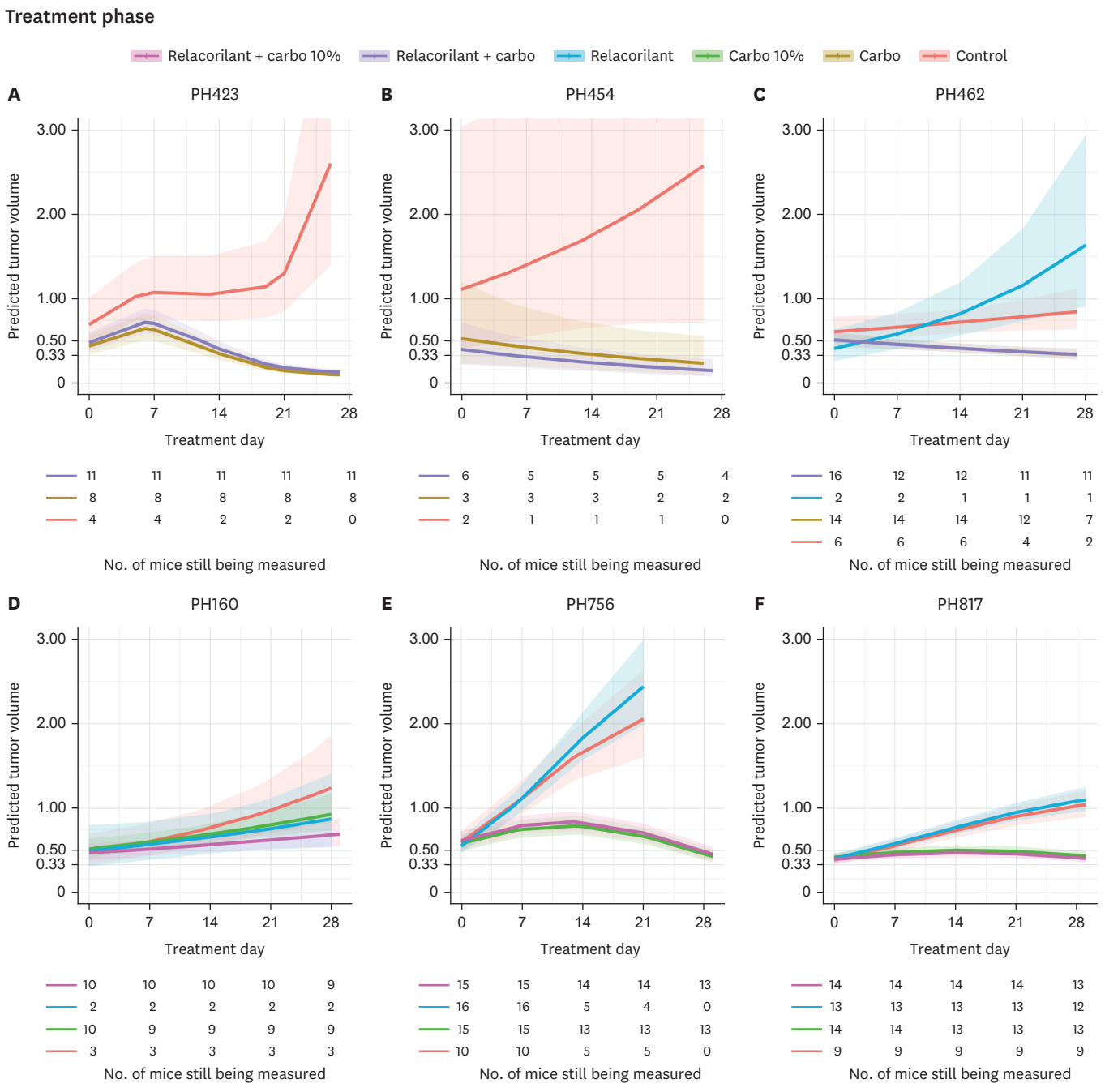

Fig. 2. Tumor growth during treatment phase and observation phase. Statistical models with estimated average tumor size during treatment phase (A-F) and observation phase (G-L) as a function of time are shown for each PDX model, with shading indicating 95% confidence bands. Color indicates treatment arm as in the legend. Number remaining for each arm at each time point is depicted under the graph.
PDX, patient-derived xenograft; PH, patient heterotransplant.

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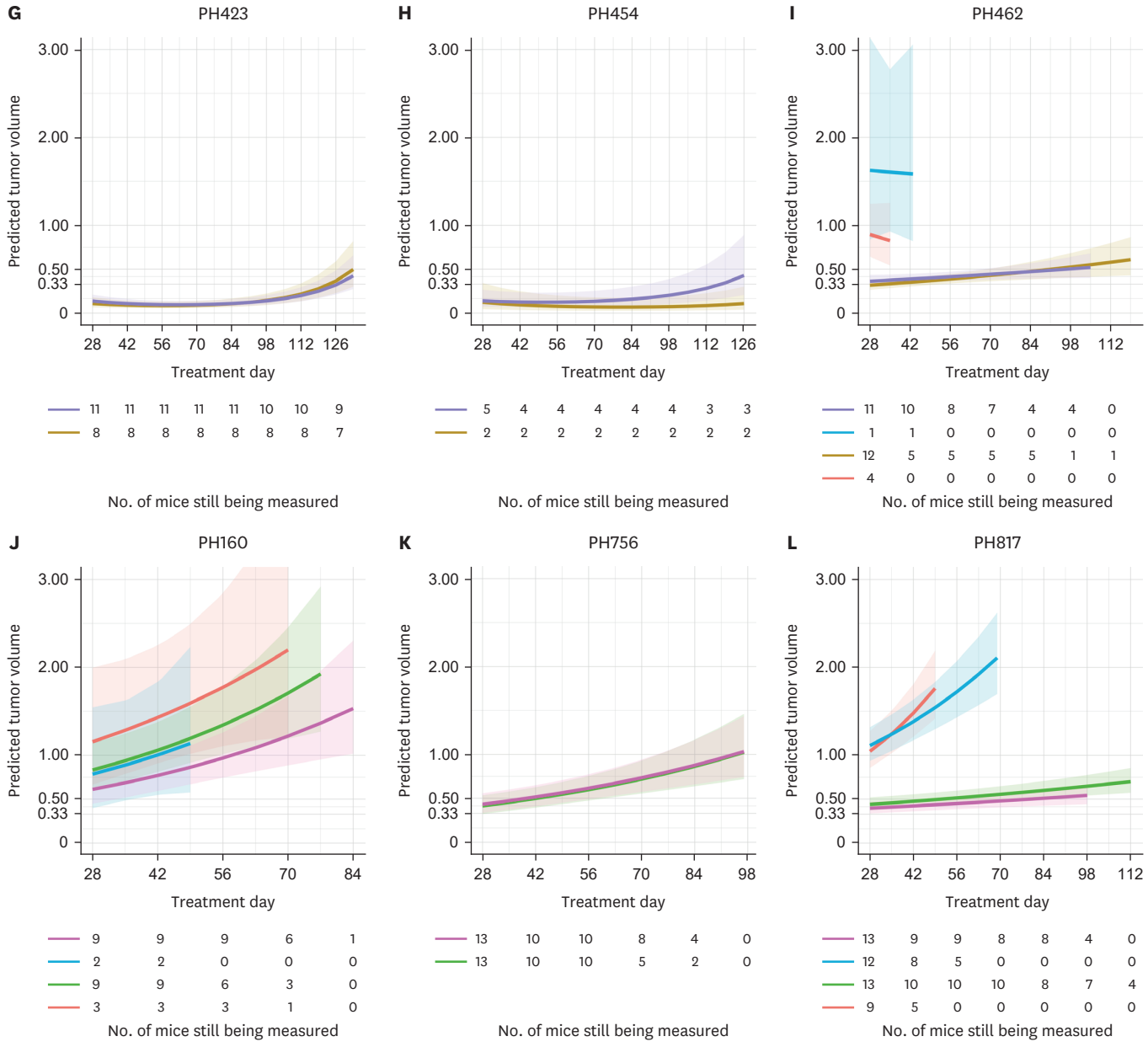

Fig. 2. (Continued) Tumor growth during treatment phase and observation phase. Statistical models with estimated average tumor size during treatment phase (A-F) and observation phase (G-L) as a function of time are shown for each PDX model, with shading indicating 95% confidence bands. Color indicates treatment arm as in the legend. Number remaining for each arm at each time point is depicted under the graph.
PDX, patient-derived xenograft; PH, patient heterotransplant.

relacorilant (vs. carboplatin alone) in the PH160 model (p=0.008, hazard ratio=0.16, **Table 3**). Additionally, we examined time to PD in the models, and only PH160 showed a significantly favorable time to PD in the relacorilant + carboplatin treatment group compared to carboplatin alone (p=0.012, **Table S1**). We did not see a significant improvement in OS in any of these aggressive HGS OvCa models with the addition of relacorilant to carboplatin (**Table S2**). Taken together, these results suggest that the least carboplatin-sensitive tumor (PH160) benefitted the most from the addition of relacorilant to carboplatin treatment.

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Table 3. The p-value comparison of PFS

PDX model	Relacorilant + carbo vs. carbo	HR	HR lower confidence level	HR upper confidence interval
PH423	0.482	1.45	0.51	4.10
PH454	0.228	3.88	0.43	35.24
PH462	0.871	0.94	0.42	2.08
PH160	**0.008**	0.16	0.04	0.62
PH756	0.696	1.17	0.53	2.58
PH817	0.587	1.27	0.54	3.00

PFS was an endpoint for the post-treatment observation phase. Comparisons assess whether PFS was significantly improved with the addition of relacorilant to carboplatin compared to carboplatin alone. HRs are shown. Statistically significant values are bolded. Statistical criteria for obtaining p-values are described in the methods section.
HR, hazard ratio; PDX, patient-derived xenograft; PFS, progression-free survival; PH, patient heterotransplant.

4. GR immunohistochemical expression in carboplatin vs. relacorilant + carboplatin-treated PDX tumors at progression

Nuclear localization of GR is associated with its transcriptional activity. GR expression might be expected to be higher after chemotherapy compared to pre-treatment due to the postulated relative chemo-resistance of highly active GR. With the addition of relacorilant, nuclear GR might be expected to be relatively less abundant compared to chemotherapy alone because relacorilant could preferentially improve cytotoxicity of GR+ tumor cells. We therefore measured nuclear (active) GR by IHC H-score in the 5 available models with tumor at progression (**Fig. 3**). Interestingly, there was no significant difference in the very high nuclear GR expression in PDX PH160, the only PDX that showed an improvement in PFS and PD with the addition of relacorilant (PH160, **Fig. 3**). In other models that showed no improvement in PFS and PD, there were variable nuclear GR expression changes with the addition of relacorilant (PH423, PH756, PH462, PH817, **Fig. 3**). Taken together, these data suggest that GR expression alone is unlikely to be a useful determinant of relacorilant sensitivity in GR+ HGS OvCa.

DISCUSSION

Understanding mechanisms of OvCa relapse following chemotherapy is critically important to improving survival in this disease. Our initial hypothesis was that all GR-expressing HGS OvCa PDXs would demonstrate increased carboplatin sensitivity shown by greater tumor shrinkage and/or a significantly prolonged duration of remission before regrowth (PD or PFS) when treated with a SGRM in addition to chemotherapy. The 6 models selected were from patients with clinically platinum-sensitive tumors because the majority of patients with *de novo* and initially recurrent OvCa have tumors with some platinum-sensitivity. However, the one model (PH160) that did not show a significant difference in tumor shrinkage with chemotherapy compared to control (vehicle) treatment, did show a significant difference in tumor shrinkage with the SGRM added to chemotherapy compared to chemotherapy alone. This finding suggests that the SGRM improved PH160 sensitivity to carboplatin.

In addition to initial tumor shrinkage with chemotherapy, tumor regrowth following chemotherapy is clinically relevant to the natural history of OvCa [32,33]. However, many laboratory OvCa models assessing novel therapies do not measure OvCa time to regrowth beyond the time of active chemotherapy treatment. Because tumor regrowth after initial debulking surgery and adjuvant chemotherapy is such an important aspect of patient survival statistics and quality of life [34], we examined tumor regrowth rates (PD and PFS) following the completion of chemotherapy in our carboplatin-sensitive PDX studies. While these

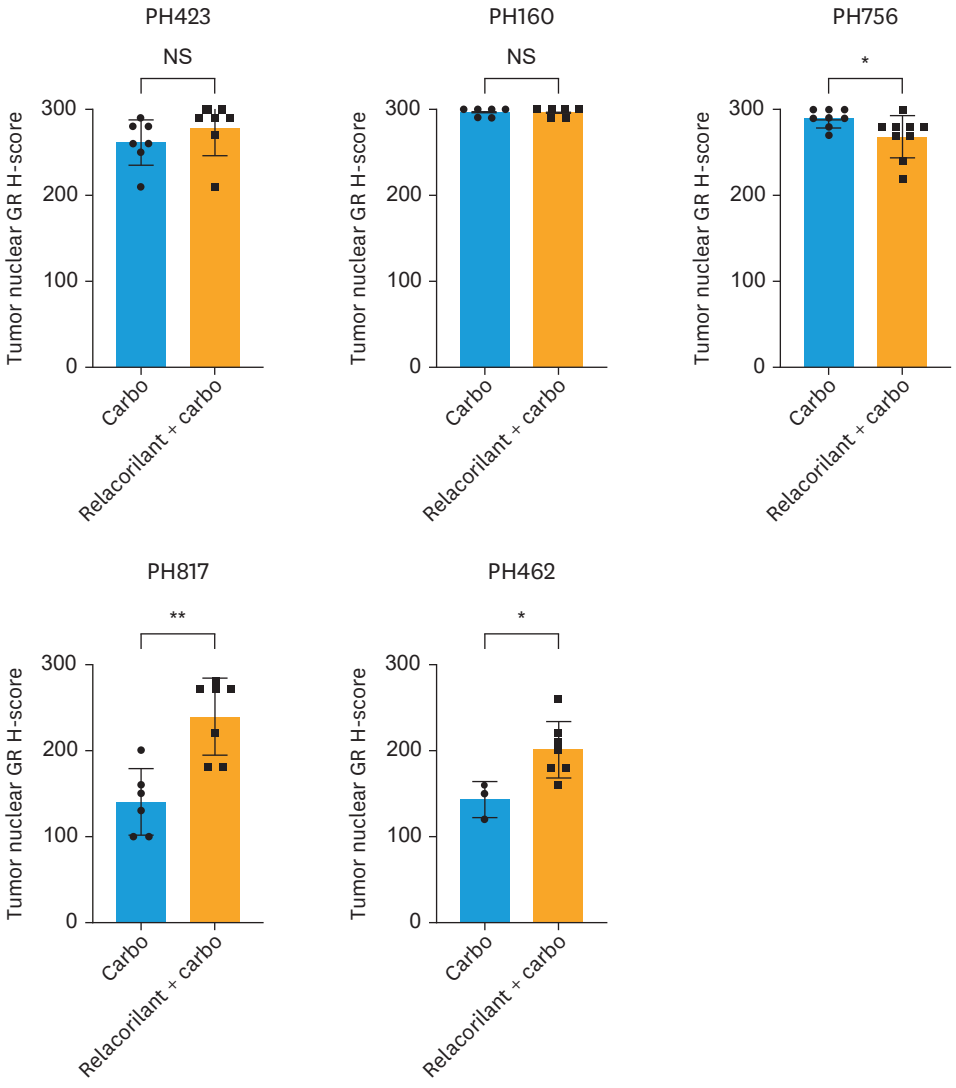

Fig. 3. Nuclear GR expression by immunohistochemistry in ovarian cancer PDXs. GR expression of residual PDX tumors from mice treated with relacorilant + carboplatin vs. carboplatin alone is shown. H-scores were calculated as described in the methods section.
GR, glucocorticoid receptor; NS, not significant; PDX, patient-derived xenograft; PH, patient heterotransplant. *p≤0.05, **p≤0.001.

laboratory studies were ongoing, the phase II trial of platinum-resistant/refractory OvCa treated with nab-paclitaxel +/- relacorilant revealed that tumor shrinkage was not statistically different between the arms, but PFS was significantly improved in patients treated with the addition of intermittent relacorilant to nab-paclitaxel [20]. Interestingly, one of the PDX models (PH160) in this study showed similar results with a significantly improved PFS following treatment with relacorilant and carboplatin compared to carboplatin alone. This model was also the least carboplatin-sensitive perhaps due to any of the various molecular mechanisms associated with chemotherapy-resistance (including having both a missense and a truncating *TP53* mutation) [35]. It is possible that PH160's 2 *TP53* mutations (whether co-occurring in single cells or individually in heterogenous tumor cells) led to relatively increased tumor GR transcriptional activity because p53 loss of wild type function has been previously associated with increased GR activity [36]. This hypothesis could be explored in

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the ongoing phase III clinical trial (NCT05257408) by examining the association of distinctive mutant *TP53* allele frequencies with improved PFS. Clinically, these data also suggest that OvCa tumors with reduced platinum-sensitivity are the subset that will likely demonstrate a measurable benefit from the addition of SGRMs. Finally, our study highlights the potential value of measuring PDX response during treatment as well as continuing an observation period following treatment to measure PD and PFS as co-primary endpoints in pre-clinical OvCa PDX studies assessing novel agents.

What remains unclear is whether pre-treatment tumor GR transcriptional activity could predict SGRM effectiveness [37]. Here we found that residual tumors can have high nuclear GR expression following treatment with relacorilant + carboplatin or carboplatin treatment alone irrespective of the benefit of the SGRM. Work is ongoing to identify an HGS OvCa GR-mediated transcriptional network or gene signature (analogous to the gene signatures derived to predict chemotherapy benefit in ER-positive early-stage breast cancer). A GR gene signature score may be superior to measuring nuclear GR protein expression to select high-grade serous ovarian cancers most likely to benefit from addition of a SGRM to chemotherapy. The significant improvement in PFS shown in the PH160 model after treatment with relacorilant and carboplatin underscores the need to identify subgroups (for example, specific *TP53* mutation +/− GR transcriptional activity) that are most likely to benefit from GR modulation.

The limitations of this study include the small sample number of PDX tumors and the use of platinum-sensitive models. A strength of this study is the inclusion of clinical endpoints beyond tumor shrinkage (including PD, PFS, and OS) in OvCa PDX models. These compelling methods may be of relevance in OvCa therapeutics due to the increasing use of maintenance therapies and the clinical use of PFS to assess for treatment response in patients without measurable disease at baseline.

SUPPLEMENTARY MATERIALS

Table S1

The p-value comparison of PD

Table S2

The p-value comparison of OS

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