Inhibition of the Nuclear Export Receptor XPO1 as a Therapeutic Target for Platinum-Resistant Ovarian Cancer

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Abstract

Purpose: The high fatality-to-case ratio of ovarian cancer is directly related to platinum resistance. Exportin-1 (XPO1) is a nuclear exporter that mediates nuclear export of multiple tumor suppressors. We investigated possible clinicopathologic correlations of XPO1 expression levels and evaluated the efficacy of XPO1 inhibition as a therapeutic strategy in platinum-sensitive and -resistant ovarian cancer.

Experimental Design: XPO1 expression levels were analyzed to define clinicopathologic correlates using both TCGA/GEO datasets and tissue microarrays (TMA). The effect of XPO1 inhibition, using the small-molecule inhibitors KPT-185 and KPT-330 (selinexor) alone or in combination with a platinum agent on cell viability, apoptosis, and the transcriptome was tested in immortalized and patient-derived ovarian cancer cell lines (PDCL) and platinum-resistant mice (PDX). Seven patients with late-stage, recurrent, and heavily pretreated ovarian cancer were treated with an oral XPO1 inhibitor.

Results: XPO1 RNA overexpression and protein nuclear localization were correlated with decreased survival and platinum resistance in ovarian cancer. Targeted XPO1 inhibition decreased cell viability and synergistically restored platinum sensitivity in both immortalized ovarian cancer cells and PDCL. The XPO1 inhibitor–mediated apoptosis occurred through both p53-dependent and p53-independent signaling pathways. Selinexor treatment, alone and in combination with platinum, markedly decreased tumor growth and prolonged survival in platinum-resistant PDX and mice. In selinexor-treated patients, tumor growth was halted in 3 of 5 patients, including one with a partial response, and was safely tolerated by all.

Conclusions: Taken together, these results provide evidence that XPO1 inhibition represents a new therapeutic strategy for overcoming platinum resistance in women with ovarian cancer.

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Introduction

Ovarian cancer is the most lethal reproductive tract malignancy in the United States, with over 22,000 cases and approximately 16,000 deaths annually (1). The high fatality-to-case ratio of ovarian cancer is directly related to the fact that most patients will develop resistance to platinum chemotherapy and eventually will die from their disease (2). New therapeutic targets and/or treatments restoring platinum sensitivity are urgently needed.

Nuclear–cytoplasmic transport plays a crucial role in maintaining normal cellular function (3), and defects in this process have been increasingly identified in solid and hematologic cancers (4, 5). One of the key nuclear transport proteins is exportin 1 (ref. 6; XPO1; also known as chromosomal regional maintenance 1, CRM1; Supplementary Fig. S1). XPO1 is one of eight known nuclear exporters, regulates the nuclear–cytoplasmic partitioning of a number of nuclear export sequence (NES) containing tumor suppressors, cell-cycle inhibitors, and oncogenes (7–9). XPO1 overexpression has been suggested to be a general feature of...
Ovarian cancer is the most lethal female reproductive tract malignancy worldwide. Although most patients appear to respond to first-line platinum-based chemotherapy, in reality, most of these women will have a recurrence of chemoresistant disease, with a 5-year survival of only approximately 30%. Here we show that overexpression of the nuclear export protein XPO1 correlates with worse survival and platinum resistance. Targeted inhibition of XPO1, using the small-molecule XPO1 inhibitors KPT-185 and KPT-330, results in apoptosis and synergistic cell death when used in combination with platinum, even in platinum-resistant ovarian cancer cell lines and PDX mice. Patients with late-stage, recurrent, and heavily pretreated ovarian cancer were treated with single-agent KPT-330. Tumor growth was halted in 3 of 5 patients, including 1 with a partial response. Together, these results provide evidence for XPO1 inhibition as a novel paradigm in overcoming platinum resistance in ovarian cancer.

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Patients, specimen collection, and patient-derived cell lines
Ovarian cancer tumor samples were collected, with patient consent, at the time of surgery at a single institution under an IRB-approved protocol. Following examination in pathology for confirmation and staging, tumor tissue sections were generated and used for establishing patient-derived cell lines and generating xenograft mouse models. Patient-derived cell lines were established based upon a previously published protocol (29).

Tissue microarray and immunofluorescence
Tissue microarrays (TMA) were constructed from 56 pathologist-selected, 1.0-mm tumor cores of formalin-fixed, paraffin-embedded (FFPE) ovarian cancer specimens (Beecher Instruments) in triplicate. All were patient samples of the Mount Sinai Hospital. Eighty-three percent of the samples were serous ovarian cancer, and all of these were defined as high grade (grades 2 and 3). In brief, TMA sections were immunostained with the XPO1 antibody SC-5595 (Santa Cruz Biotechnology) using the Benchmark XT (Ventana/Roche) with the UltraVIEW Universal DAB Detection Kit (Ventana/Roche). Isotype rabbit IgG1 was used as a negative control. The intensity of the XPO1 immunostaining in tumor cells was evaluated independently and then overseen by a senior pathologist (M. Donovan). All readers were blinded to patient outcomes. Intensity, percent positivity, and cellular localization (i.e., nuclear and cytoplasm) were analyzed for each core, with a calculated H-score [% positivity (0–100) multiplied by intensity (1–3+), maximum 300] determined for each core. Cut-off points were derived on the basis of maximizing sensitivity and specificity for predicting the outcomes. FFPE OVCAR3 cells were used as a positive control. Normal ovary, fallopian tube, and endometrial tissues, incorporated into the arrays, were evaluated as negative tissue controls along with whole sections of normal colon tissue, normal testis, and normal lung during XPO1 development assays.

For immunofluorescence studies, cells were first plated on coverslips. At designated time points, cells were fixed with 4% paraformaldehyde and permeabilized on ice with 0.5% Triton X-100 (Sigma) or 90% methanol for 10 minutes. After incubation with primary and secondary antibodies, cells were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted with Vectashield (Vector Laboratories). Images were acquired and analyzed using the Axion plan software on a Zeiss Axio microscope (Zeiss).

Antibodies
Anti-XPO1 (H-300), anti-p53 (DO-1), anti-lamin A/C (N-18), anti-GAPDH, and anti-NF-kB p65 were obtained from Santa Cruz Biotechnology. Anti-cleaved caspasases 8 (Asp391), -9 (Asp330), and -3 (Asp175), anti-cleaved-PARP (Asp214), anti-phospho-p53 (Ser15), anti-Erk1/2, anti-phospho-p44/42 Erk1/2 (Thr202/Tyr204), anti-Bcl-xl, anti-ix8, anti-phospho-p65 (Ser536), and anti-cIAP1/2 were obtained from Cell Signaling Technology.

Chen et al.

Translational Relevance
Ovarian cancer is the most lethal female reproductive tract malignancy worldwide. Although most patients appear to respond to first-line platinum-based chemotherapy, in reality, most of these women will have a recurrence of chemoresistant disease, with a 5-year survival of only approximately 30%. Here we show that overexpression of the nuclear export protein XPO1 correlates with worse survival and platinum resistance. Targeted inhibition of XPO1, using the small-molecule XPO1 inhibitors KPT-185 and KPT-330, results in apoptosis and synergistic cell death when used in combination with platinum, even in platinum-resistant ovarian cancer cell lines and PDX mice. Patients with late-stage, recurrent, and heavily pretreated ovarian cancer were treated with single-agent KPT-330. Tumor growth was halted in 3 of 5 patients, including 1 with a partial response. Together, these results provide evidence for XPO1 inhibition as a novel paradigm in overcoming platinum resistance in ovarian cancer.
Cell culture and reagents
The ovarian cancer cell lines A2780, CP70, OVCAR3, and SKOV3 were purchased from ATCC and cultured in DMEM supplemented with 10% FBS. Immortalized human normal fallopian tube epithelial cell line FT33-shp53-R24C was purchased from Applied Biological Materials Inc (ABM) and cultured in Prigrow IV Medium with 10% FBS in PriCoat T25 Flasks (ABM). Human fibroblast cell lines IMR-90 and WI-38 were purchased from ATCC and cultured in minimum essential medium (MEM) with 10% FBS. Human fibroblast cell lines AG060062 and GM17071A were maintained in our laboratory (30). KPT-185 and KPT-330 were provided by Karyopharm Therapeutics. DMSO and KPT-185 were delivered three times per week via intraperitoneal injection. For these models, 6-week-old female Rag1 knockout mice (C.129S7(B6)-Rag1tm1Mom/J) and Balb/c nude mice were purchased from Jackson Laboratories. Once tumors developed in mice, a multicenter phase I clinical trial was performed to assess the safety, tolerability, and efficacy of selinexor (KPT-330) in late-stage, platinum-resistant ovarian cancer patients. Approval was obtained from the respective Institutional Review Boards at participating sites. Patients were eligible for study participation if they met the following criteria: 18 years of age or older, had ovarian cancer resistant or refractory to standard therapy, and Eastern Cooperative Oncology Group performance status (ECOG PS) ≤2, and measurable disease by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). In addition, adequate renal, hepatic, and hematologic functions were required. Patients received single-agent treatment with 30–35 mg/m² oral KPT-330 2–3 times weekly (8–10 doses/4-week cycle). Response was evaluated every 2 cycles (RECIST 1.1). Dose-limiting toxicity was defined as events occurring in the first 28 days at the target dose. These events included: severe nausea/vomiting/diarrhea and fatigue lasting >5 days, hematologic toxicities, and liver/renal function abnormalities. Treatment could be discontinued in the event of dose-limiting toxicities, and dose modifications secondary to toxicities were not allowed. Information regarding the statistical design of the overall phase I study is available (33).

Statistical analysis
Significant differences between groups were determined using the Student t test. Survival data analysis was performed using the Kaplan–Meier and log-rank tests (GraphPad Prism software v5.0).

Results
XPO1 overexpression and nuclear localization are correlated with decreased survival and platinum resistance
In seeking to gain an understanding of the potential value of XPO1 as a therapeutic target in ovarian cancer, we first analyzed in silico the correlation between XPO1 RNA expression levels and progression-free survival (PFS) and overall survival (OS) in ovarian cancer using the largest independent, multicenter high-grade ovarian cancer sample dataset available. Using the cBio Cancer Genomics Portal (34), we interrogated all 489 cases of platinum-resistant or refractory to standard therapy, an Eastern Cooperative Oncology Group performance status ≤2, and measurable disease by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). Dose-limiting toxicity was defined as events occurring in the first 28 days at the target dose. These events included: severe nausea/vomiting/diarrhea and fatigue lasting >5 days, hematologic toxicities, and liver/renal function abnormalities. Treatment could be discontinued in the event of dose-limiting toxicities, and dose modifications secondary to toxicities were not allowed. Information regarding the statistical design of the overall phase I study is available (33).

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We next used Kaplan–Meier plotter (35) to further explore the association between XPO1 overexpression and survival using Gene Expression Omnibus (GEO) and TCGA datasets. Again we focused exclusively on high-grade serous ovarian cancer and included only those individuals treated with a platinum agent as platinum agents are the gold-standard treatment for primary disease. Because surgical debulking status remains one of the most
significance contributors to overall survival (36), we first analyzed all patients together regardless of debulking status and then independently examined those with optimal and suboptimal debulking. XPO1 expression levels ranged from low (30%, 1/t to 1/0) to high (90%, 3+ nuclear and cytoplasmic staining). The representative fields shown were viewed at 200× magnification.

Finally, we quantitated and localized XPO1 protein levels in situ across a wide range of patient-derived ovarian cancer tissues using a tissue microarray (TMA) representing patient samples from individuals treated at our institution. We analyzed samples from 56 patients with high-grade serous ovarian cancer representing 143 evaluable cores (Supplementary Table S2). Nuclear and cytoplasmic staining were analyzed independently. XPO1 expression levels ranged from low (30%, 1+ cytoplasmic staining; left) to high (90%, 3+ nuclear and cytoplasmic; right) across the sample set (Fig. 1C, left and right). Clinically relevant clinicopathologic associations were identified (37) based on XPO1 expression levels and nuclear versus cytoplasmic distribution. Increased nuclear XPO1 expression correlated with shorter 5-year PFS (concordance index of 0.40; HR = 2.0, cut-off point 90, P < 0.003; Fig. 1D) and with platinum resistance (score = 0.227; scale of -1 to +1). A correlation with overall survival was also present but was weaker (concordance index of 0.44; not shown). Conversely, XPO1 cytoplasmic staining was positively correlated (score 0.262; scale of -1 to +1) with those patients who were survivors including those who were with no evidence of disease (NED) and alive-with-disease (AWD). There was a statistically
significant difference in cytoplasmic expression in cancer survivors versus those who had died of their disease ($P = 0.0042$).

KPT-185 selectively inhibits proliferation and induces apoptosis even in platinum-resistant ovarian cancer cells

Having established these associations, we next investigated the anticancer efficacy of XPO1 inhibition, particularly in the context of platinum resistance. We first examined the effects of the XPO1 inhibitor KPT-185 on cisplatin-sensitive A2780 cells, A2780-derived cisplatin-resistant CP70 cells (38), and cisplatin-resistant OVCAR3 (39) and SKOV3 (40) cells. Submicromolar KPT-185 concentrations significantly inhibited growth of all cell lines (Fig. 2A). The IC$_{50}$ of these cell lines ranged from 46.53 nmol/L to 328.7 nmol/L. Selinexor, with a similar molecular weight as KPT-185, achieves serum levels $>1.5$ mmol/L at doses below the MTD in patients with cancer, indicating that these concentrations are clinically relevant. In marked contrast, and highlighting a specificity for the cancer-derived cell lines, a KPT-185 concentration of 4,000 nmol/L failed to kill noncancerous IOSE527 cells and concentrations greater than 10,000 nmol/L did not have a demonstrable toxic effect on the other noncancerous cell lines tested (Fig. 2A). We further corroborated this finding of an increased cancer cell sensitivity by testing the effect of KPT-185 on patient-derived primary tumor cells versus patient-derived benign cells. Forty-four patient-derived high-grade serous ovarian cancer (HGSOC) cell lines and 6 patient-derived benign ovarian cell lines were tested. While the HGSOC-derived cell lines had an average IC$_{50}$ of 3.2 mmol/L, the average IC$_{50}$ for the benign cell lines (19.1 mmol/L) was nearly six times greater ($P < 0.001$; Supplementary Fig. S2). Taken together, these findings in both the immortalized and
patient-derived cell lines are in accord with previous findings that 40 μmol/L of KPT-185 or related SINEs are not toxic to normal lymphocytes, bone marrow cells, primary hepatocytes, and other normal cells (25, 26). Finally, and given that many of the commonly used ovarian cancer cell lines do not share the same genetic profiles of native tumors, most notably that unlike tumors, the cell lines are TP53 wild-type, we also investigated the efficacy of KPT-185 in patient-derived primary ovarian cancer cell lines. In addition to testing the efficacy of KPT-185 in the above forty-four patient-derived cells, primary tumor cell lines derived from 17 different ovarian cancer patients with either primary or recurrent disease and platinum-sensitive and -resistant tumors (Supplementary Table S3) were treated with KPT-185. Regardless of platinum or other chemotherapy resistance status, all patient-derived ovarian cancer cell lines were significantly growth inhibited by KPT-185 (Fig. 2F, red line).

To determine the mechanism of KPT-185–mediated decrease in cell viability, we first determined the degree of cell-cycle arrest in each cell line. While KPT-185 treatment resulted in G1 phase arrest in A2780 cells (P = 0.006) no significant degree of arrest was noted for CP70, OVCAR3, or SKOV3 cells (Supplementary Fig. S3). In these latter three cell lines, we next quantitated the degree of apoptosis induction. All three cell lines initiated apoptosis following KPT-185 treatment and apoptosis could be almost completely blocked by the use of the antiapoptotic agent Z-VAD-FMK (Fig. 2B). KPT-185 treatment resulted in marked increases in caspases 3, -8, and -9, as well as PARP cleavage (Fig. 2C), suggesting activation of the apoptotic cascade through both the intrinsic and extrinsic pathways.

KPT-185 acts synergistically with platinum to increase chemosensitivity independent of cellular p53 status

Because platinum resistance is the foremost obstacle to long-term successful ovarian cancer treatment, we explored the effect of combining SINEs and cisplatin. While the well-studied isogenic ovarian cancer lines A2780 and CP70 were originally derived to study cisplatin resistance differences, they are both p53 WT. In contrast, nearly 100% of late-stage serous ovarian cancer tumors are deficient for p53 or possess a mutated p53 (41). Thus, for clinical relevance, SINE compounds must be effective even in p53-mutated/deficient tumor cells. We determined the effect of increasing concentrations of cisplatin alone or in combination with KPT-185 on cell viability in the four immortalized ovarian cancer cell lines and also the set of patient-derived ovarian cancer cell lines, which were established from both platinum-sensitive and -resistant tumors, as described above.

Regardless of p53 status, KPT-185 enhanced the efficacy of cisplatin in all cell lines (Fig. 2D–F). In cisplatin-resistant CP70 cells, combinatorial treatment resulted in an IC50 similar to that of the isogenic cisplatin-sensitive parent line (A2780), indicating an increase in platinum sensitivity (Fig. 2D and E). The combination index (CI) was calculated to determine the degree of synergy. KPT-185 and cisplatin were shown to be synergistic as demonstrated by their low CI values at all concentrations explored (Supplementary Fig. S4). Isobologram analysis revealed that the combination of drugs was consistent with a synergistic, not simply additive effect. The CI in the immortalized cell lines ranged from 0.51 to 0.69.

Synergy between KPT-185 and cisplatin was also demonstrated in all the patient-derived cell lines that were tested (Fig. 2F). Even doses as low as 500 nmol/L of KPT-185, an average IC45 dose, effectively lowered cisplatin IC50 levels, on average, >4-fold (13.53 μmol/L vs. 3.15 μmol/L; Fig. 2F). The combined CI value was 0.55, indicating synergy between these two drugs.

SINE compounds induce apoptosis through p53-dependent and independent pathways

As described above, KPT-185 effectively induced apoptosis in all the ovarian cancer cell lines tested regardless of their p53 status. This together with the role of p53 in apoptosis and as a recognized XPO1 cargo protein (7) led us to explore whether the mechanisms of SINE-induced apoptosis could be mediated through both p53-dependent and independent pathways.

We first examined the effect of KPT-185 treatment on the p53 wild-type (WT) CP70 cell line. p53 nuclear accumulation and upregulated expression were readily apparent following KPT-185 treatment (Fig. 3A, left and 3B). Increased phospho-p53 (Ser15) expression was confirmed via Western blot analysis (Fig. 3C). Previous studies have demonstrated that the Ras– Raf– ERK signaling cascade functionally interacts with p53 activation in apoptosis (42); therefore, we also investigated the degree, if any, of ERK1/2 activation. After KPT-185 treatment, phosphorylated ERK1/2 levels increased in the nucleus (Fig. 3C). The use of the highly selective p53 inhibitor Pifithrin-alpha (PFT-aα; ref. 27) suppressed phosphorylated-ERK1/2 expression (Fig. 3C), consistently increased Bcl-XL expression (Fig. 3C, bottom), and decreased apoptosis (Fig. 3D). We also examined the effect of targeted p53 knockdown using siRNA. CP70 cells were transfected with either p53 siRNA or control scrambled siRNA (scRNA) and cells treated with KPT-185 (Supplementary Fig. S5). The effect of p53 loss on apoptosis was then measured by FACS analysis. While treatment with KPT-185 in a p53 WT background resulted in >25% apoptosis, silencing p53 markedly blocked this response such that cell death was essentially at the background levels observed in the control cells. Together, these findings provide evidence for p53-dependent SINE-mediated ERK1/2 activation, suggesting that in CP70 cells, ERK1/2 is a downstream regulator of p53 during KPT-185–mediated apoptosis.

As an independent method to confirm the role of p53 in the response of CP70 cells to KPT-185, and do so in an agnostic manner, we performed a whole-genome RNA analysis. CP70 cells treated with KPT-185 were compared with sham-treated cells and total RNA isolated and interrogated by microarray (Illumina, HumanHT12, v4 Expression BeadChip). The Upstream Regulator Analysis (URA) of Ingenuity Pathway Analysis (IPA) was used to identify the predicted state (activation/inhibition) and cascade of upstream transcriptional regulators that could explain observed gene expression changes following KPT-185 treatment. In total, more than 20 activated pathways were identified. The most activated pathway in this analysis was p53. The activation Z-score was 3.5, and P value, 1.67E–05. The p53 network (i.e., p53 downstream targets) and its direction of activation are shown in Supplementary Fig. S6.

In contrast, we hypothesized that during p53-independent apoptosis, in ovarian cancer cells with inactive or mutated p53, the proapoptotic effect of KPT-185 could be mediated by an alteration of the NF-kB prosurvival pathway. This was based on the complementary findings that: (i) NF-kB suppresses programmed cell death and promotes tumor growth (43), (ii) NF-kB activity can be downregulated by XPO1 inhibition in mantle cell lymphoma (23), and (iii) NF-kB has been linked to ovarian cancer (44). In untreated SKOV3 and OVCAR3 cells, NF-kB p65 and IκBα were abundantly expressed in the cytoplasm. KPT-185...
treatment altered this subcellular localization such that nuclear accumulation of IkBα and p65 was readily apparent (Fig. 4A and B) with concomitant slight decreases of their cytoplasmic expression. As an indication of suppressed NF-κB activity, KPT-185 treatment reduced phosphorylated p65 levels with decreased expression of p65 target genes, cIAP1 and cIAP2 (Fig. 4C).

As KPT-185 induced nuclear translocation of both p65 and IkBα and this correlated with decreased NF-κB activity, we asked whether this inhibition was the result of IkBα directly binding p65 in the nucleus. In support of this translocation-based mechanism, IkBα bound NFκB only in immunoprecipitates from the nuclear fractions of KPT-185 treated cells (Fig. 4D). Loss of IkBα, through siRNA-mediated silencing, reversed the suppression of p65 activity (Fig. 4E), increased cIAP1 and cIAP2 expression (Fig. 4E), and decreased apoptosis (Fig. 4F). Thus, these results indicated that this p53-independent apoptosis induced by KPT-185 is mediated by increased nuclear levels of IkBα.

XPO1 inhibition increases platinum sensitivity and survival in ovarian cancer–bearing mice

We assessed the in vivo antitumor efficacy of SINE compound KPT-330 using two complementary ovarian cancer mouse models. First, we tested an intraperitoneal model of ovarian cancer growth and dissemination using cisplatin-resistant CP70 cells.
Seventy tumor-bearing mice were divided into four groups: vehicle control (n = 16), cisplatin treated (n = 17), KPT-330 treated (n = 16), and combination cisplatin/KPT-330 treated (n = 21). We confirmed the highly chemoresistant nature of the CP70 cell line by demonstrating a slight (4-day) increase in median survival among cisplatin-treated mice compared with control mice (Fig. 5A, P < 0.0005). However, oral (gavage) selinexor treatment significantly increased the median survival compared with control (32 vs. 24 days, P < 0.0001) and cisplatin-treated (32 vs. 28 days, P < 0.005; Fig. 5A) mice. The greatest survival benefit was achieved with the selinexor–cisplatin combination, which had a median survival of 37 days—an approximately 30% increase in survival compared with cisplatin alone (P < 0.0001) and an approximately 15% increase in survival compared with selinexor alone (P = 0.01). More than 60% (13/21) and approximately 30% of cotreated mice survived beyond the maximum survival date of the cisplatin-only (31 days) and selinexor-only treated (43 days) mice, respectively. Immunohistochemical staining of ex vivo tumor samples following treatment is shown (Fig. 5B).

We next assessed the antitumor efficacy of oral selinexor on ovarian cancer xenograft (PDX) mice. The PDX ovarian cancer model has recently been reported to maintain histologic, genomic, and, importantly, platinum-treatment response profiles similar to those of the donor ovarian cancer tumor (45). Our PDX mice were derived from 7 independent patients with high-grade serous ovarian cancer. Each patient had received a minimum of six rounds of chemotherapy, and at least platinum, taxol, and bevacizumab. Five patients had recurrent disease and failed cisplatin treatment, and one each had cisplatin-sensitive or -resistant primary tumors (Supplementary Fig. S7). Our PDX models maintained the histologic features of the original donor tumor (top), and, as demonstrated by directed DNA sequencing of the human/murine tumor pairs and the patient’s germline DNA, also maintained their p53 and BRCA mutation profiles (bottom).

Within 1 to 5 months after tumor tissue implantation, tumors developed in each of the mice. Selinexor-treated mice experienced significant and prolonged tumor regression, which was in marked contrast to controls (P < 0.0001, Fig. 5C, top). Dramatic tumor shrinkage (average volume reduction ≥ 99%) was observed in nearly 90% (19/22) of selinexor-treated mice. Their tumors were undetectable or only barely evident, even with open exploration of the inoculation site (Fig. 5D). For the 3 mice without tumor shrinkage, tumor growth was nonetheless markedly delayed. As expected, tumor volume decreases were accompanied by survival...
KPT-330 displays active antitumor activity in mouse models of platinum-resistant ovarian cancer. A, survival curves of CP70 tumor mice treated with different regimens. *, KPT versus CDDP, P < 0.001; **, combination of KPT and CDDP versus KPT, P = 0.01. B, IHC staining of p53, Ki-67, and TUNEL from tumor sections treated as in A. C, Top, tumor growth of PDX mice following KPT-330 treatment. Bottom, survival curve of PDX mice following KPT treatment. D, representative picture demonstrating ex vivo tumor sizes. Arrows indicate the tumors. E, tumor growth curve in mice treated with different regimens. Inset highlights the growth curves of KPT-330-treated mice. At the conclusion of the experiment, tumor tissues were obtained from all mice for ex vivo analysis. Tumor histology was consistent with high-grade morphology (Supplementary Fig. S4), and immunohistochemical staining revealed that increased p53 nuclear expression, Ki-67 downregulation, and increased apoptosis were observed only in mice treated with KPT-330 (alone or with cisplatin; Fig. 5B).

Selinexor is safely tolerated by ovarian cancer patients and can decrease tumor volume

Selinexor is safely tolerated by ovarian cancer patients and can decrease tumor volume in patients with heavily pretreated, relapsed ovarian cancer refractory to platinum (Fig. 6A). Patients received 30–35 mg/m² of oral selinexor (the MTD; ref. 33). The most common adverse events were fatigue, nausea, anorexia, diarrhea, and vomiting, which were managed with supportive care (Fig. 6B). No adverse events required discontinuation of treatment. There were no major organ toxicities and no life threatening or grade 4 adverse events were reported. Pharmacokinetics achieved in patients was comparable with those observed in mice. Selinexor resulted in an up to 10-fold induction of XPO1 mRNA measured by qRT-PCR in patient leukocytes. In selected solid tumors, p53 and Ikβ nuclear accumulation and...
induction of apoptosis were confirmed in repeated tumor biopsies after 3–4 weeks of selinexor treatment.

Two patients withdrew for non-drug–related issues. Of the 5 evaluable patients, selinexor inhibited tumor growth in 3 (Fig. 6C and D). One patient (patient #043-815; days on study = 156) experienced a partial response, whereas the disease stabilized in two patients (patient #043-044, days on study = 30; patient #043-046, days on study = 30) with maximal CA-125 reduction 39%; patient #043-047, days on study = 40) with maximal CA-125 reduction 75%). The CA-125 levels over time are presented in Fig. 6E. The patient with partial response, a >30% decrease in tumor volume (Fig. 6C), was diagnosed in February 2000 and had already undergone eight chemotherapy regimens (Fig. 6D). The patient received treatment for a total of 156 days prior to probable disease progression with small-bowel obstruction. There was no radiographic evidence of tumor progression.

**Discussion**

The nucleus is a defining feature of eukaryotes, separating the cell into nuclear and cytoplasmic components. This physical division provides a unique degree of spatial regulation to protein function. XPO1 is one of the eight known nuclear export proteins that are required for nuclear:cytoplasmic transport of macromolecules through the nuclear pore complex. Notably, XPO1 is the sole nuclear exporter of a number of key cancer-associated proteins, including p53 and IkBα (46). While other cancer types are increasingly treated successfully, ovarian cancer mortality rates have remained relatively high over the past 50 years, with a 5-year survival rate at approximately 30% for patients diagnosed with stage III/IV diseases. Given that chemoresistance represents the major cause for ovarian cancer treatment failure (2), our studies were specifically focused on exploring the efficacy and clinical relevance of XPO1 inhibition in treating platinum-resistant ovarian cancer. We believe our findings support a novel role for the use of XPO1 inhibition in platinum-resistant ovarian cancer.

Our results demonstrate that inhibition of XPO1 by two SINE compounds (KPT-185 and selinexor/KPT-330) was significantly associated with increased tumor killing, regardless of tumor platinum sensitivity or p53 status. Notably, we demonstrated a synergistic effect between KPT-185 and cisplatin in all immortalized and primary patient-derived cell lines tested. Moreover, selinexor and cisplatin cotreatment yielded the greatest overall survival and reductions in tumor size in two ovarian cancer mouse models. Most dramatically, the oral XPO1 inhibitor selinexor was safely tolerated by 5 ovarian cancer patients and that for the majority (3/5) of these patients, even in the setting of late-stage, heavily pretreated, and chemoresistant disease, XPO1 inhibition...
stabilized the disease and/or shrank the tumor, with the longest treatment lasting over 1 year. Based upon our findings of drug synergy, combined usage of SINE and platinum agents in future clinical studies may achieve an even greater antitumor effect.

As nearly 100% of late-stage serous ovarian tumors have defects in p53 (41), it was necessary to define the mechanism through which XPO1 inhibition resulted in cell death in p53-mutated cells. In both SKOV3 and OVCAR3 cell lines, in which p53 is either null or mutated, respectively, XPO1 inhibition resulted in increased nuclear levels of IκBα with consequent physical interaction with p65 and inhibition of NF-κB activity. This is consistent with a number of previous findings that have demonstrated that inhibition of NF-κB activity can induce cell death (43). In general, NF-κB activity can be regulated through the shutting of key interacting proteins, most notably IκBα, between cytoplasm and nucleus (47). NF-κB signaling is known to be constitutively active (localized to the nucleus) in multiple cancers, including ovarian cancer, and this activation is not necessarily dependent upon intrinsic mutations in NF-κB (44). NF-κB’s regulator IκBα is sequestered in the cytoplasm and/or degraded by the proteasome. Forced retention of IκBα in the nucleus by SINEs leads to binding of IκBα to NF-κB and results in the termination of NF-κB binding to DNA, neutralizing its transcriptional activity. Our findings may be especially relevant in serous ovarian cancer as NF-κB has been shown to be not only continuously active in ovarian cancer, playing a role in the development and maintenance of ovarian cancer and chemotherapy resistance (48) but NF-κB activity may be further increased in p53-mutant cells (49). In contrast to this finding, in the two cell lines with wild-type p53, A2780, and CP70, XPO1 inhibition resulted in a marked nuclear shift of p53 and activation of its downstream target, ERK1/2. Activation of ERK1/2 generally promotes cell survival but can, under certain conditions (42), and as we have shown in our model system, have proapoptotic functions. Thus, SINE can drive both p53-dependent and -independent apoptosis pathways allowing for broad antitumor activity.

In addition to XPO1 a number of nuclear transport proteins are also under study as potential therapeutic targets in cancer including KPNA1-6, KPNB1, TNPO, and IPO (50). Driven in part by the current understanding that nuclear–cytoplasmic export is primarily driven by XPO1, a number of potential therapeutic agents targeting XPO1 have been developed and tested, and the small-molecule SINE has shown the most promising clinical efficacy and safety (3–6, 51). The XPO1 inhibitor KPT-330 (selinexor) is the only compound currently being evaluated in clinical trials in solid tumors and hematologic malignancies. Phase I results in 189 patients with advanced solid tumors was recently reported by members of our group (33). The ovarian cancer patients in that trial are reported herein. These current studies provide an opportunity to examine in depth the mechanisms underlying cell death in a specific cancer type. In this regard, it is important to note that in addition to our current study in ovarian cancer, a recent report also examined the effect of KPT-185 in ovarian cancer models (52). Intriguingly, and adding to the concept that SINE may achieve their effectiveness through a number of different pathways, these authors identified elf3A and IGF2BP1 as playing an important role in KPT-mediated cell death in vitro. Our studies did not highlight these two targets despite the fact that both used the A2780 cell line. The reason for these differences is currently unknown but will be important to pursue to better understand mechanisms of effect and resistance.

The full roster of cancer-relevant cargo transported by XPO1 and the resultant pathway dysregulation(s) caused by inappropriate nuclear–cytoplasmic partitioning resulting from XPO1 overexpression remains unknown. Our studies not only provide the rationale for XPO1 as a therapeutic target in platinum-resistant ovarian cancer, but also demonstrate that XPO1 acts synergistically with cisplatin and highlights that cell death is achieved through both p53-dependent and independent pathways. Thus, future clinical trials targeting platinum-resistant ovarian cancers, which are almost always p53 mutated, could potentially overcome this resistance through combination treatment using both a platinum agent and SINE.

Disclosure of Potential Conflicts of Interest

A.R.A. Razak reports receiving commercial research grants from Karyopharm. J.-N. Billaud is an employee of Qiagen. M. Kauffman is an employee of and has ownership interests (including patents) at Karyopharm Therapeutics Inc. No potential conflicts of interest were disclosed by the other authors.

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