Targeting BMI1 mitigates chemoresistance in ovarian cancer

Resistance to chemotherapy is a prominent clinical problem in high grade serous ovarian cancer (HGSOC). An inadequate understanding of adaptive signaling coupled with limited treatment options for a chemoresistant tumor are likely causes for poor outcomes. We previously reported that BMI1, a stem-cell factor is instrumental in regulating chemoresistance. However, to advance anti-BMI1 therapy from the bench to the bedside, efficacy needs to be tested in patient-derived chemoresistant HGSOC models, which is lacking. Here, we report generation and characterization of a chemoresistant, patient-derived xenograft (PDX) model of HGSOC that recapitulates carboplatin and paclitaxel resistance observed in the patient. We demonstrate that the combination of standard therapy (carboplatin and paclitaxel) along with PTC596 also known as Unesbulin, a clinical inhibitor of BMI1 mitigates chemoresistance and significantly decreases the tumor growth compared to either therapy alone. Mechanistically, PTC596 treatment decreases expression of cancer stem-like markers in tumor tissues, along with a decrease in proliferative and an increase in apoptotic markers, thereby overcoming chemoresistance. Thus, patients with chemoresistant cancer that have limited treatment options may benefit from combination of anti-BMI1 strategies with standard chemotherapy.

We screened four PDX models for BMI1 expression that were largely generated internally. The OV119 and OV63 models were developed from relapsed peritoneal metastatic tissues of serous ovarian cancer patients that had previously received carboplatin and paclitaxel treatment. PDX-0113 was developed from a patient whose serous ovarian tumor was resistant to carboplatin and paclitaxel with disease recurrence within 3 months and PDX-0081 was responsive to treatment with disease recurrence by 14 months of therapy. Resected tumor tissue was processed and either implanted in mice, “fresh” or cryopreserved as viable fragments.

Compared to the non-malignant human ovarian surface epithelial (OSE) cells, the expression of BMI1 was higher in the cisplatin-resistant CP20 and in the OV90 ovarian cancer cell lines as well as in all the PDX tumors tested (Fig. 1A), which is consistent with our previous findings that BMI1 is often overexpressed in HGSOC patient tissues. For immediate studies, we chose PDX-0113 and PDX-0081 because they demonstrated higher expression of BMI1 and based on the respective patient tumor responses, allowing us to test if drug sensitivity can be recapitulated. To determine if the histo-morphologic features of the original tumor were preserved in the PDX tissues, immunohistochemistry (IHC) was performed. Haematoxylin and eosin (H&E) staining demonstrated preservation of morphologic features (Fig. 1B, C). IHC for relevant serous epithelial markers, including pan-cytokeratin (CK), paired-box 8 (PAX8) and Wilms’ tumor 1 (WT1) showed similar staining, supporting histologic fidelity upon passaging (Fig. 1B, C).

Both the PDXs were expanded into a larger cohort for drug treatment experiments. The animals were monitored regularly and tumors were typically measured twice per week. For advanced ovarian cancer, carboplatin and paclitaxel comprise standard first-line chemotherapy. When the tumors reached ~200 mm³, carboplatin 50 mg/kg by intra-peritoneal (IP) and nano-albumin bound (nab)-paclitaxel 10 mg/kg by intra-venous (IV) routes were administered once weekly for two weeks and tumor growth monitored up to ~13 weeks (Fig. 1D). The effective doses of carboplatin and paclitaxel in mice were selected based on prior reports. Recapitulating the patient response, PDX-0113 continued to grow despite treatment while PDX-0081 showed regression and did not grow beyond ~200 mm³ (Fig. 1D). Based on these results further experiments were performed in the chemoresistant, PDX-0113 model.

We performed a pilot study and determined that the 12.5 mg/kg dose of PTC596 could be favorably combined with carboplatin and paclitaxel (Fig. S1A). In a cohort of 80 NSG female mice, PDX-0113 was expanded subcutaneously. When the tumors reached ~200 mm³, the animals were randomized into 4 groups of 20 mice each. The groups...
Anti-BMI1 strategies have the potential to alleviate chemoresistance when applied in combination with conventional chemotherapy in chemoresistant PDX model of HGSOC. (A) Expression of BMI1 in normal immortalized ovarian surface epithelium (OSE), different PDXs and in malignant ovarian cells. (B) PDX-0113 and PDX-0081 (C) demonstrate similar staining pattern of hematoxylin and eosin (H&E) and also IHC for relevant serous epithelial markers, including pan-cytokeratin (CK), paired-box 8 (PAX8) and Wilms’ tumor 1 (WT1), supporting histologic fidelity upon passaging with the original HGSOC patient. (D) Both PDX-0113 and PDX-0081 were expanded sub-cutaneously to 10 female NSG mice for each PDX. Carboplatin 50 mg/kg body weight, via intra-peritoneal (IP) and NAB-Paclitaxel 10 mg/kg body weight, via intra-venous (IV) routes were administered once weekly for two weeks when the tumors reached ~200 mm³ and tumor growth monitored up to ~13 weeks. (E) PDX-0113 was expanded sub-cutaneously to 80 NSG mice and randomized into 4 different groups (20 mice in each group) when tumors reach ~200 mm³. The groups were treated with: (a) Vehicle treated Control; (b) Carboplatin + NAB-Paclitaxel [Carbo + Pax]; (c) PTC596; (d) PTC596 [Carbo + Pax]. Mice were monitored for tumor volume, tumor regression until around ~11 weeks. After completion of 2 cycles of each treatment, 10 mice from each group were taken out for further molecular characterization. Data represent mean ± SEM. *, P < 0.05. (F–I) Molecular characterization of the therapy response in the chemoresistant tumor. PDX-0113 tumor tissues after completion of 2 cycles of each treatment (as described in Fig. S2), 10 mice from each group were taken out for IHC staining of tumor xenografts for (F) BMI-1, (G) Ki67, (H) TUNEL positivity and (I) CD31. Scale bar represents 100 μm. After quantification, fold changes with respect to the control are shown graphically on the right. Con is vehicle treated control; C + P is Carboplatin + NAB-Paclitaxel; PTC is PTC596 and Com is combination of PTC596 along with Carboplatin + NAB-Paclitaxel. *, P < 0.05 compared with the control group by one-way ANOVA. (J, K) Determining the mechanism of chemosensitization. As shown in Fig. S2, after completing two weeks of treatment, 10 mice from each group were euthanized and respective tumors collected for (J) mRNA expression of various stem cell markers by quantitative polymerase chain reaction analysis. 18S ribosomal RNA (rRNA) was used as an internal control. Control mRNA levels were set to 1 and data represent mean ± SD of three independent experiments performed from 4 different animals per group. *P < 0.05 when P < 0.05 compared with the control group by one-way ANOVA. (K) Protein level expression of the oncogenic stem-marker cMYC and efflux transporter MDR1 were determined by immunoblotting from 4 different animals per group. HSP70 was used as a loading control. Con is vehicle treated control; C + P is Carboplatin + NAB-Paclitaxel; PTC is PTC596 and Com is combination of PTC596 along with Carboplatin + NAB-Paclitaxel. (L) Schema of anti-BMI1 strategies to alleviate chemoresistance when applied in combination with conventional chemotherapy in chemoresistant HGSOC. Carboplatin and paclitaxel therapy failed to restrain tumor growth with prominent expression or even upregulation of certain stem-markers but combining PTC596 with standard carboplatin and paclitaxel therapy sensitizes the chemoresistant HGSOC-PDX tumor to the therapy. Cytotoxic therapy eliminated some of the more differentiated cancer epithelial cells while a smaller fraction of chemoresistant cancer stem cells (CSC) were not targeted and continued to expand the tumor. Another possibility is that cytotoxic therapy eliminated some cancer epithelial cells while others were reprogrammed to express stem-like factors manifesting resistance. PTC-596 mediated anti-BMI1 therapy sensitizes HGSOC to chemotherapeutics to abrogate chemoresistance and prevent recurrent tumor growth.
received (a) vehicle; (b) carboplatin + nab-paclitaxel (Carbo + Pax); (c) PTC596; (d) PTC596 + (Carbo + Pax) (Fig. 52). After completion of 2 cycles of treatment, 10 mice from each group were removed for molecular characterization. Around ~11 weeks, the vehicle treated mice were euthanized because their tumor burden reached humane endpoint per IACUC guidelines. Concurrently, animals from all the other treatment groups were euthanized. Compared to the control, Carbo + Pax treatment showed a ~35% reduction, PTC596 alone showed a ~25% reduction and the combination of PTC596 with Carbo + Pax showed a ~92% reduction in tumor growth (Fig. 1E). Importantly, the combination treatment did not exhibit any sign of tumor recurrence (Fig. 1E). No obvious toxicity was noted in the animals as assessed by mean body weight (Fig. S1B, S3). These results indicate that PTC596 can be combined with and sensitizes the tumor to Carbo + Pax therapy.

To characterize the molecular mechanism of response and sensitization by PTC596, we evaluated the following markers. As determined by IHC, compared to the control, the distinct nuclear expression of BMI1 was reduced 4-fold in the PTC596 treated group and by ~10-fold in the combination with Carbo + Pax group (Fig. 1F). The nuclear tumor proliferation marker Ki67, decreased 2.5-fold and 5-fold respectively in the PTC596 alone and the combination group (Fig. 1G). A significant increase in the TUNEL positive nuclei in the PTC596 combination treated group, compared to either therapy alone or with the vehicle control indicated enhanced apoptosis (Fig. 1H). Additionally, there was a significant ~2-fold decrease in the CD31 positive microvessel density (Fig. 1I) in the combination group which may be a reflection of the smaller tumor volume. These results indicate that PTC596 treatment decreases the expression of BMI1 in vivo and sensitizes the chemoresistant tumor to standard therapy, causing apoptotic death. Interestingly, determination of stem cell markers revealed that Carbo + Pax treatment significantly upregulated SOX 2 (SRY-related HMG-box family of transcription factor 2) by ~1.7-fold, OCT4 (Octamer-binding transcription factor 4) by ~1.5-fold and KLF4 (Kruppel like factor 4) by ~2.6-fold while ALDH1A (aldehyde dehydrogenase 1 family member A1) remained unchanged, compared to the control (Fig. 1J). In the PTC596 only treated group, all of these markers were significantly downregulated compared to the control (Fig. 1J), suggesting inhibition of a stem-like phenotype, despite significant tumor burden (Fig. 1E). Strikingly, compared to the Carbo + Pax treatment only, combining with PTC596 demonstrated a robust reduction of SOX 2 by ~110-fold, OCT4 by ~5-fold, KLF4 by ~17-fold and ALDH1A by ~5-fold respectively that was accompanied with a reduced tumor burden and increased apoptosis. Furthermore, protein levels of the oncogenic stem-marker cMYC (MYC proto- oncogene) and efflux transporter MDR1 were prominently decreased in the PTC596 alone or in the combination treatment group (Fig. 1K). An interpretation of our main findings are represented in the schema, Figure 1L.

Recently, PTC596 has entered Phase1 clinical trials including advanced HGSOC (NCT03206645) and upon completion the maximum tolerated dose and recommended Phase2 dose will be determined. In this context, our preclinical study has revealed that targeting the tumor by PTC596 not only decreases BMI1 levels but also reduces expression of a number of stem-cell factors including MDR1 which we have demonstrated is a direct target. Importantly, downregulation of these stem-cell factors sensitizes the chemoresistant tumor towards standard therapy and may prove to be useful therapy response markers in the ongoing clinical trial.

Conflict of interests

The corresponding author has no financial interest to declare, however, PTC596 is being developed and was provided by PTC Therapeutics. NJ. M.W. and J.D.B. are employees of PTC Therapeutics.

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Appendix A. Supplementary data

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