OBI-992, a Novel TROP2-Targeting Antibody–Drug Conjugate, Displayed Excellent Antitumor Efficacy in Various Animal Models

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BACKGROUND

- Antibody–drug conjugates (ADCs) consist of a tumor-specific monoclonal antibody connected via a chemical linker to a cytotoxic payload, allowing for the targeted delivery of potent chemotherapy agents.[1]
- One target for anticancer drug delivery is trophoblast cell surface antigen 2 (TROP2), a transmembrane glycoprotein that is overexpressed in various tumor types relative to normal tissue.[2]
- The ADC pairing of a TROP2-directed antibody and topoisomerase 1 (TOP1) inhibitor payload has demonstrated clinical success; however, the TROP2-TOP1 ADCs currently approved or under clinical investigation (sacituzumab govitecan [SG] and datopotamab deruxtecan [Dato-DXd], respectively) are limited by linker stability and/or toxicity profiles.[3,4]
- Thus, a TROP2-targeting therapy with better stability and less toxicity would be an important addition to existing ADC therapeutic options.
- OBI-992 is a new ADC derived from a novel TROP2 antibody linked with the TOP1 inhibitor exatecan through conjugation with a hydrophilic enzyme-cleavable linker; ex vivo serum stability and in vitro cytotoxicity studies found that OBI-992 had a more stable linker and lower off-target toxicity than Dato-DXd (see AACR 2024 posters 7179 and 3130), warranting further investigation of OBI-992 antitumor activity in vivo.

OBJECTIVE

- To evaluate the antitumor activity of OBI-992 in vitro and in vivo, including cell-based cytotoxicity, as well as cell line–derived xenograft (CDX) and patient-derived xenograft (PDX) models of various cancer types.

METHODS

In vitro cytotoxicity of OBI-992

- Several cancer cell lines were treated with serially diluted OBI-992 for 6 days, and cell proliferation was analyzed by CellTiter-Glo® (Promega Corporation); IC_{50} was calculated with GraphPad Prism v. 6.0 (GraphPad Software).
- TROP2 RNA levels in cancer cell lines were derived from the Human Protein Atlas,[5] and TROP2 protein levels were determined by quantitative flow cytometry.

In vivo antitumor activity of OBI-992 (CDX, PDX, multidrug resistance, and synergy with PARP inhibitors)

- For CDX models, cancer cells were subcutaneously injected into BALB/c nude mice.
- For PDX models, tumor fragments harvested from host mice were subcutaneously implanted into BALB/c nude or NOD.CB17-Prkdc/J (NOD/SCID) mice.
- Tumor growth was monitored twice weekly. Tumor-bearing mice were divided into treatment groups and treated with OBI-992, SG, Dato-DXd, olaparib, talazoparib, or combinations when average tumor volume reached 150-200 mm^3 for standard models and when tumor volume reached 500-550 mm^3 for large-tumor models.
- Antitumor activity was evaluated by tumor growth inhibition (TGI), calculated according to the following formula: TGI (%) = [1 – (Ti – T1)/(Ci – C1)] × 100%. Ti and Ci indicate the mean tumor volume in the treatment group and vehicle control at the end of the study, respectively. T1 and C1 indicate the mean tumor volume in the treatment group and vehicle control at the beginning of treatment administration, respectively.

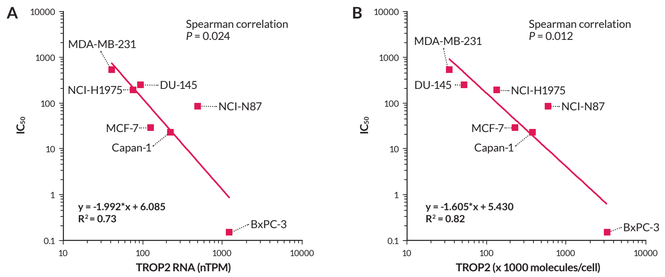
Bystander killing

- ES-2/GFP cells (TROP2-negative) were cultured alone or co-cultured with BxPC-3 cells (TROP2-positive) at different ratios and then treated with serially diluted OBI-992 or control IgG1 ADC.
- Viability of ES-2/GFP cells in vitro and ES-2/GFP-specific tumor growth in vivo (alone or mixed with BxPC-3 cells at a specific ratio) were measured by immunofluorescent signals.

RESULTS

- The cytotoxicity of OBI-992 increased with increasing TROP2 RNA or protein expression levels in different cancer cell lines (**Figure 1**).

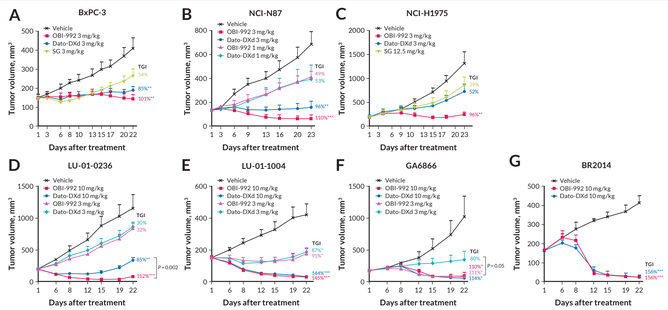
Figure 1. OBI-992 Cytotoxicity Significantly Correlated With TROP2 Expression



The IC_{50} of OBI-992 treatment versus TROP2 (A) RNA and (B) protein expression levels in various cancer cell lines. Statistical significance of the association was determined by Spearman's correlation.
IC_{50}, half-maximal inhibitory concentration; nTPM, transcripts per million; TROP2, trophoblast cell surface antigen 2.

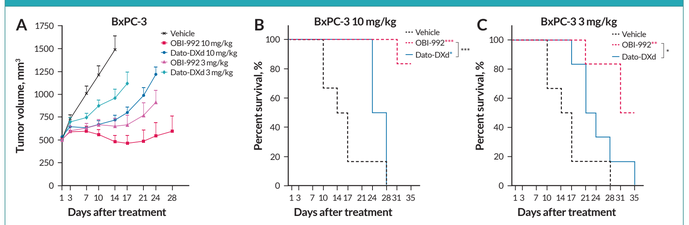
- In various CDX and PDX models, a single dose of OBI-992 at 3 or 10 mg/kg exhibited significant antitumor activity relative to the vehicle control (**Figure 2**).
- Notably, the level of tumor growth inhibition (TGI) of OBI-992 surpassed that of SG and Dato-DXd across different CDX and PDX models, including a large-tumor model (**Figure 2; Figure 3**).

Figure 2. OBI-992 Exhibited Better Antitumor Activity Than SG and Dato-DXd in Multiple CDX and PDX Models



Tumor volume versus number of days post-treatment for (A) pancreatic cancer BxPC-3, (B) gastric cancer NCI-N87, and (C) lung cancer NCI-H1975 CDX mice and (D-E) lung cancer LU-01-0236 and LU-01-1004, (F) gastric cancer GA6866, and (G) breast cancer BR2014 PDX mice treated with the indicated doses of vehicle, OBI-992, SG, or Dato-DXd. TGI was calculated for each drug as a measure of antitumor activity. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ relative to vehicle control treatment.
CDX, cell line–derived xenografts; Dato-DXd, datopotamab deruxtecan; PDX, patient-derived xenografts; SG, sacituzumab govitecan; TGI, tumor growth inhibition.

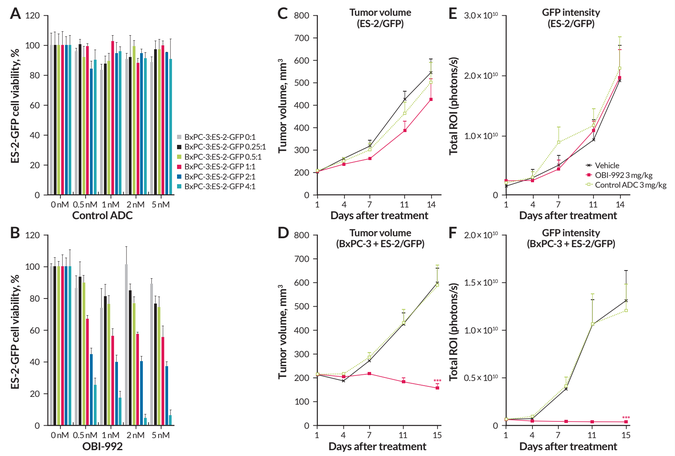
Figure 3. OBI-992 Exhibited Better Antitumor Activity Than Dato-DXd in a Large-Tumor Model



(A) Tumor volume versus number of days post-treatment for pancreatic cancer BxPC-3 CDX mice with large tumors (tumor volume before treatment initiation was ~2.5x greater than in the standard CDX models) treated with the indicated doses of vehicle, OBI-992, or Dato-DXd. Percent survival of the large-tumor CDX mice versus number of days post-treatment with (B) 10 mg/kg and (C) 3 mg/kg doses of vehicle, OBI-992, or Dato-DXd. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ relative to vehicle control treatment.
CDX, cell line–derived xenografts; Dato-DXd, datopotamab deruxtecan.

- OBI-992 demonstrated a bystander killing effect, as OBI-992 treatment killed cell cultures and xenograft tumors that were heterogenous for TROP2 expression (**Figure 4**).

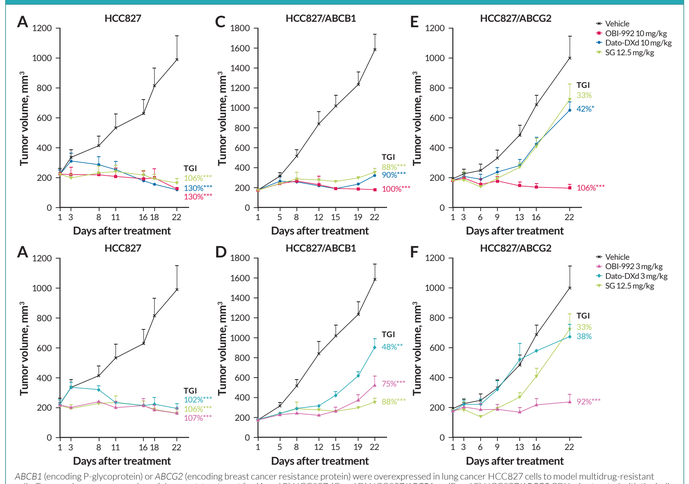
Figure 4. OBI-992 Demonstrated Strong Bystander Killing In Vitro and In Vivo



(A and B) Increasing ratios of TROP2-positive BxPC3 cells were co-cultured with TROP2-negative ES-2/GFP cells. ES-2/GFP cell viability versus treatment concentration of (A) control IgG1 ADC and (B) OBI-992 is plotted. (C and D) Tumor volume and (E and F) GFP intensity versus number of days post-treatment of (C and E) ES-2/GFP CDX mice and (D and F) BxPC-3 + ES-2/GFP CDX mice (to model a tumor heterogeneous for TROP2 expression) treated with indicated doses of vehicle, OBI-992, or control IgG1 ADC. $^{***}p < 0.001$ relative to vehicle control treatment.
ADC, antibody–drug conjugate; CDX, cell line–derived xenografts; GFP, green fluorescent protein; ROI, region of interest; TROP2, trophoblast cell surface antigen 2.

- OBI-992 treatment significantly suppressed tumor growth in CDX models of *ABCB1* (encoding P-glycoprotein [P-gp]) or *ABCG2* (encoding breast cancer resistance protein [BCRP]) overexpression (**Figure 5**).
  - In the HCC827/ABCB1 model, the TGI of OBI-992 treatment was similar to SG and better than Dato-DXd.
  - In the HCC827/ABCG2 model, OBI-992 had greater TGI than both SG and Dato-DXd.
- These findings suggest that OBI-992 was not affected by P-gp- and BCRP-mediated multidrug resistance.

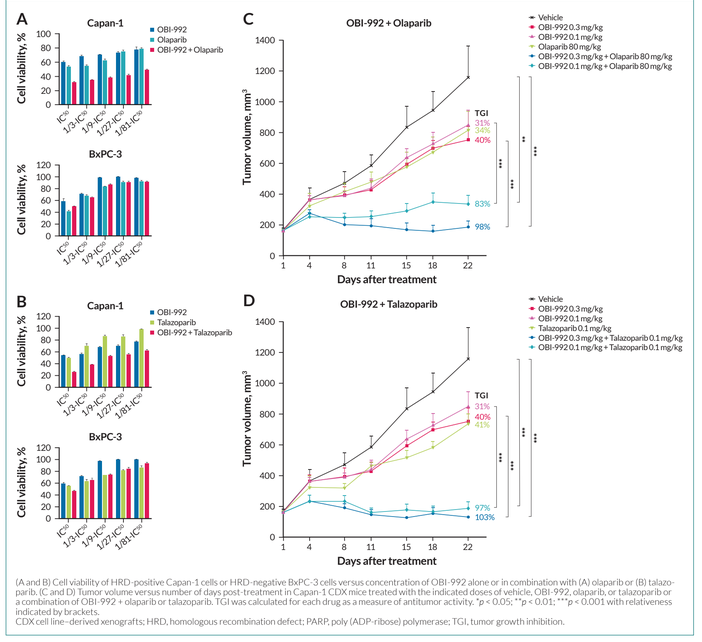
Figure 5. OBI-992 Maintained Antitumor Activity in CDX Models of Multidrug Resistance via *ABCB1* and *ABCG2* Overexpression



ABCB1 (encoding P-glycoprotein) or *ABCG2* (encoding breast cancer resistance protein) were overexpressed in lung cancer HCC827 cells to model multidrug-resistant cells. Tumor volume versus number of days post-treatment for (A and B) HCC827, (C and D) HCC827/*ABCB1*, or (E and F) HCC827/*ABCG2* CDX mice treated with the indicated doses of vehicle, OBI-992, SG, or Dato-DXd. TGI was calculated for each drug as a measure of antitumor activity. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ relative to vehicle control treatment.
CDX, cell line–derived xenografts; Dato-DXd, datopotamab deruxtecan; SG, sacituzumab govitecan; TGI, tumor growth inhibition.

- In cell lines with homologous recombination deficiency (HRD), combined OBI-992 plus poly (ADP-ribose) polymerase (PARP) inhibitor treatment (olaparib or talazoparib) demonstrated a synergistic reduction in cell viability (**Figure 6**).
- In the HRD CDX model, suboptimal doses of OBI-992 combined with olaparib or talazoparib synergistically suppressed tumor growth (**Figure 6**).

Figure 6. Synergistic Effects of OBI-992 in Combination With PARP Inhibitors



(A and B) Cell viability of HRD-positive Capan-1 cells or HRD-negative BxPC-3 cells versus concentration of OBI-992 alone or in combination with (A) olaparib or (B) talazoparib. (C and D) Tumor volume versus number of days post-treatment in Capan-1 CDX mice treated with the indicated doses of vehicle, OBI-992, olaparib, or talazoparib or a combination of OBI-992 + olaparib or talazoparib. TGI was calculated for each drug as a measure of antitumor activity. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ with relativeness indicated by brackets.
CDX cell line–derived xenografts; HRD, homologous recombination defect; PARP, poly (ADP-ribose) polymerase; TGI, tumor growth inhibition.

CONCLUSIONS

- OBI-992 exhibited strong antitumor activity and outperformed the SG and Dato-DXd benchmarks in CDX and PDX models of various cancer types.
- A strong bystander killing effect suggests that OBI-992 will be effective for tumors with heterogenous expression of TROP2.
- OBI-992 retained its antitumor effect in models of P-gp and BCRP overexpression, suggesting that OBI-992 may overcome these mechanisms of multidrug resistance.
- A synergistic effect was observed with the combination of OBI-992 and PARP inhibitors; combined OBI-992 and PARP inhibitor therapy may benefit patients with HRD-positive cancers.
- These compelling in vivo results warrant further development and investigation of OBI-992 in the clinical setting.

References

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Disclosure

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