OBI-999, an anti-Globo H antibody drug conjugate, exhibits synergistic anti-tumor effect in combination with pembrolizumab

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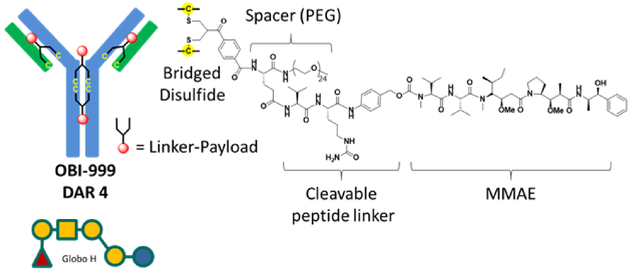
Background

Globo H, a glycosphingolipid, is highly expressed in a variety of epithelial tumors with a limited expression in normal tissues. OBI-999 is an anti-Globo H antibody drug conjugate, which consists of a Globo H-specific monoclonal antibody conjugated with monomethyl auristatin E (MMAE) through a cleavable linker. MMAE is known to induce Immunogenic cell death (ICD). ICD involves the activation of cytotoxic T lymphocyte-driven adaptive immunity with long-term immunological memory. Given the capability of inducing ICD and creating more accessible tumor microenvironment, this study aims to investigate whether OBI-999 in combination with pembrolizumab can have synergistic antitumor effect in animal models. Results showed that OBI-999 and pembrolizumab had a significant synergistic efficacy in various animal models. OBI-999 is currently in Phase 1/2 clinical trial for advanced solid tumors (NCT04084366).

Methods

The ICD effects of OBI-999 were examined *in vitro* by incubation of the Globo H expression cells with OBI-999 followed by the detection of damaging-associated molecular patterns (DAMPs) such as calreticulin (CRT), high mobility group box 1 (HMGB1), and ATP. The ICD-related immunity induced by OBI-999 was assessed *in vivo* using advanced severe immunodeficient mice that were reconstituted with human peripheral blood mononuclear cells (PBMCs). Antitumor effect of OBI-999 in combination with pembrolizumab was evaluated in several cancer types of xenograft tumor models using PBMC-humanized mice.

Structure of OBI-999



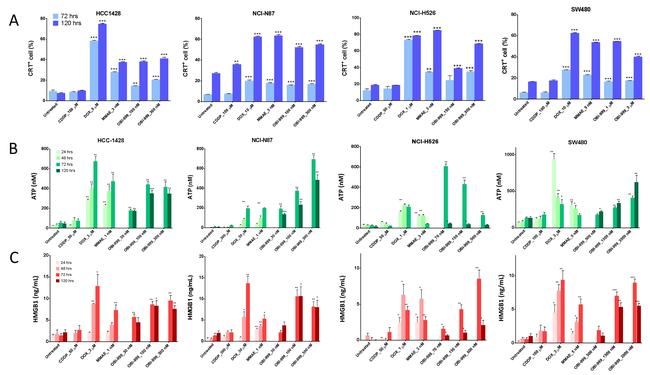
OBI-999 consists of a Globo H targeting antibody plus a novel linker ThioBridge® and a tubulin inhibitor payload MMAE. The ThioBridge® is designed to form site-specific disulfide bonds through cross-linking to the reduced cysteines in the Fab and hinge regions of the antibody rendering a homogeneous drug-antibody ratio (DAR).

ThioBridge® is the registered trademark of Abzena

Results

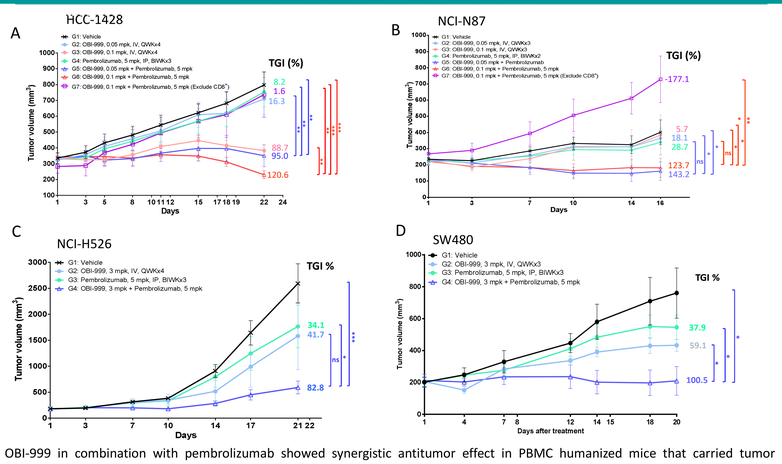
Incubation of OBI-999 with high Globo H expression cancer cell lines (HCC-1428, NCI-N87, and NCI-H526) and mid Globo H expression cancer cell line (SW480) induced the release of a panel of DAMPs including CRT, ATP, and HMGB1 in dose- and time-dependent manners. The detection of the hallmark DAMPs indicated that OBI-999 induced ICD *in vitro*. Furthermore, OBI-999 showed a synergistic antitumor effect in combination with pembrolizumab in several xenograft tumor models using PBMC-humanized mice. In high Globo H expression human breast cancer cell line HCC-1428 xenograft model, OBI-999 (0.05 mg/kg; once a week) plus pembrolizumab (5 mg/kg; twice a week) exhibited significantly stronger inhibition on tumor growth (TGI 95.0%) compared to the treatment with OBI-999 (TGI 16.3%) or pembrolizumab (TGI 8.2%) alone. Similar synergistic effects of the combination therapy were observed in other cancer types of xenograft models as well, including gastric cancer (NCI-N87), small cell lung cancer (NCI-H526) and colorectal cancer (SW480). Analysis of tumor-infiltrating lymphocytes (TILs) in HCC-1428 xenograft model showed that OBI-999 combined with pembrolizumab treatment induced the populations of cytotoxic CD8 T-cells and mature dendritic cells. In addition, pembrolizumab treatment decreased PD-1 expression on CD8 and CD4 cells, and OBI-999 treatment decreased PD-L1 expression on tumor cells, which reversed the exhausted status of immune cells and alleviate the immunosuppression microenvironment.

Figure 1. OBI-999 induced immunogenic cell death as shown by a panel of DAMPs



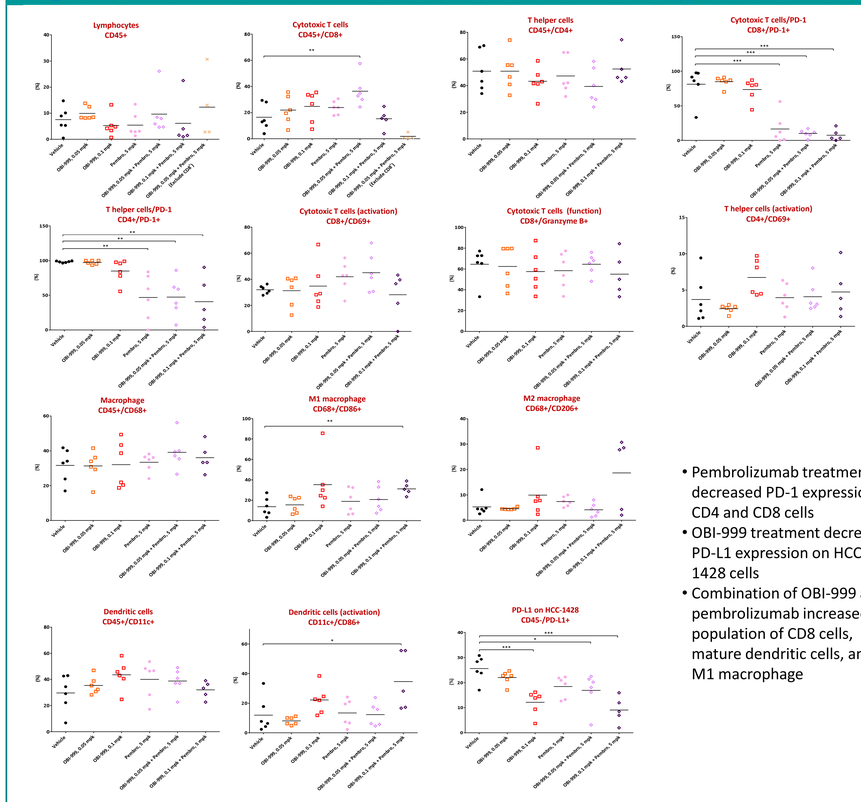
OBI-999 induced a panel of damage-associated molecule patterns (DAMP) *in vitro*. (A) Ecto-calreticulin (CRT) was detected by flow cytometry on HCC-1428, NCI-N87, NCI-H526, and SW480 cells after treatment with various concentrations of OBI-999 for 72 or 120 hours. (B) Extracellular ATP was measured in the culture supernatant after cells were treated with OBI-999 for 72 or 120 hours. (C) High mobility group box 1 (HMGBI) was detected by ELISA in all tested cell lines after OBI-999 treatment for 72 or 120 hours. Doxorubicin was used as a positive control and cisplatin as a negative control for ICD induction. Data are presented as mean ± S.D. Significant difference between treated and control was analyzed by Student's t-test. *p < 0.05, **p < 0.01, ***p < 0.001.

Figure 2. Combination of OBI-999 and pembrolizumab showed synergistic antitumor effect in various cell-derived xenograft models



OBI-999 in combination with pembrolizumab showed synergistic antitumor effect in PBMC humanized mice that carried tumor xenograft of (A) human breast cancer HCC-1428 (B) human gastric cancer NCI-N87 (C) human lung cancer NCI-H526 and (D) human colon cancer SW480. The antitumor effect was mediated through CD8+ cells (A and B). Human PBMCs (2 x 10^6) mixed with tumor cells were implanted subcutaneously in the left flank of advanced severe immunodeficient mice. CD8+ T cells were removed by magnetic beads before injected into mice in the CD8-exclusion groups. OBI-999 was administered intravenously once a week for 3 or 4 weeks as indicated. Pembrolizumab was given intraperitoneally twice a week for 2 or 3 weeks. Each group contained 6 mice. Data are presented as mean ± S.E. Significant difference between groups was analyzed by Student's t-test. *p < 0.05, **p < 0.01, ***p < 0.001.

Figure 3. Immune cells infiltration and activation in HCC-1428 tumors after OBI-999/pembrolizumab treatment



Tumor infiltrated lymphocytes (TILs) analysis was performed at the end of the study in HCC-1428 xenograft model. On Day 22, HCC-1428 tumors of all groups were collected and digested. The primary cells collected from the tumors were stained for corresponding immune cell markers and analyzed by flow cytometry. Each group contained 6 tumor-bearing mice that were reconstituted with human PBMCs. Each dot represents one individual mouse. Statistically significant differences between groups were analyzed using student's t-test. P*<0.05, P**<0.01, P***<0.001.

- Pembrolizumab treatment decreased PD-1 expression on CD4 and CD8 cells
- OBI-999 treatment decreased PD-L1 expression on HCC-1428 cells
- Combination of OBI-999 and pembrolizumab increased the population of CD8 cells, mature dendritic cells, and M1 macrophage

Conclusions

- OBI-999 exhibited synergistic antitumor effect with pembrolizumab in various xenograft models
- The synergistic effect may be attributed to the capability of OBI-999 to induce immunogenic cell death
- Tumor-infiltrating lymphocytes and activated dendritic cells suggested a tumor microenvironment that favors the function of immune checkpoint inhibitors like pembrolizumab
- A combination therapy of OBI-999 with anti-PD-1 in clinical study is warranted