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Novel Globo H-targeting antibody-drug conjugate with binding specificity and anti-tumor efficacy in multiple cancer types

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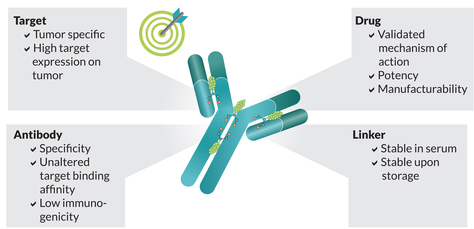
INTRODUCTION

The globo series is a unique class of glycosphingolipids involved in tumor development and survival. Globo series antigens SSEA3 (Gb5), SSEA4 (sialyl-Gb5), and Globo H (fucosyl-Gb5) are expressed on multiple epithelial cancer cells. Globo H, a cancer-specific hexasaccharide antigen, plays an essential role in tumor survival by promoting immunosuppression, tumor survival signaling, and angiogenesis. Globo H has been reported to be highly expressed in multiple cancer types but is expressed to a lesser extent in normal tissues.[1] Therefore, Globo H may be a potential target for cancer immunotherapy. OBI-999 is an antibody-drug conjugate (ADC), which consists of a Globo H-specific monoclonal antibody, OBI-888; and Thiobridge™ conjugated with monomethyl auristatin E (MMAE), a synthetic antineoplastic agent.

OBI-999: MMAE conjugated Globo H targeting-monoclonal antibody.



OBJECTIVES

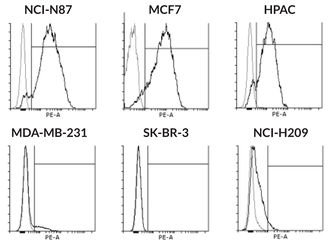
- The present study investigates antigen specificity, drug internalization, anti-tumor efficacy, and pharmacokinetic profiles of OBI-999.

METHODS

- The binding specificity and internalization of OBI-999 were determined by flow cytometry and confocal microscopy, respectively. In vivo anti-tumor efficacy was studied in conventional xenograft and patient-derived xenograft models. Pharmacokinetic parameters were evaluated in normal and tumor-bearing xenograft mice.

RESULTS

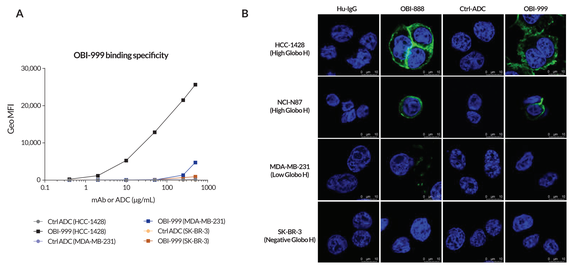
Figure 1. Globo H expression on multiple human cancer cell lines.



Cell line	Tumor type	Globo H expression
MCF7	Breast	93.5% (+++)
HCC-1428	Breast	98.8% (+++)
MDA-MB-231	Breast	10.6% (+)
SK-BR-3	Breast	0.1% (-)
NCI-N87	Gastric	94% (+++)
NCI-H526	Lung	68.9% (++)
NCI-H209	Lung	3.8% (+)
HPAC	Pancreatic	88.4% (+++)

Globo H expressions were determined for several human cancer cell lines, including gastric, breast, lung and pancreatic cancer, using flow cytometry staining. Cell surface flow cytometry staining is conducted using anti-Globo H antibody VK9 (ThermoFisher) incubated for 60 minutes on ice. The positive percentage (black) is indicated compared with isotype control antibody mouse IgG staining (gray).

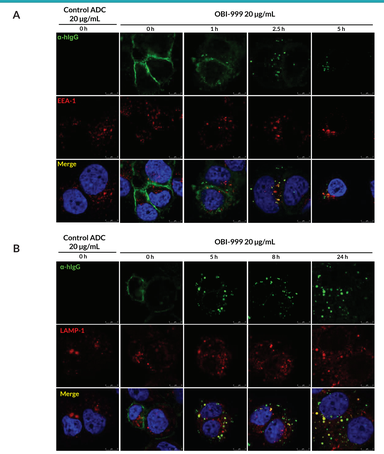
Figure 2. Binding specificity of OBI-999 to Globo H-expressing tumor cells.



ADC, antibody-drug conjugate; DAPI, 4′,6-diamidino-2-phenylindole; mAB, monoclonal antibody; MFI, mean fluorescence intensity.

The binding specificity of OBI-999 was tested on tumor cell lines with different Globo H expression levels. (**A**) Flow cytometry cell surface binding of multiple doses (0.4, 2, 10, 50, 250, 500 μg/mL) of OBI-999 or anti-CD30 control ADC (Ctrl ADC) to high, low, and negative Globo H-expressing breast cancer cell lines HCC-1428, MDA-MB-231, and SK-BR-3, respectively. (**B**) Confocal microscopy staining results of10 μg/mL OBI-888 or 20 μg/mL OBI-999 to high, low, and negative Globo H-expressing tumor cells. OBI-888, OBI-999, or Ctrl-ADC were incubated with cells for 1 hour at 4°C, followed by further detection with anti-human IgG-AF488 (green) antibody for 1 hour. Cell nuclei were stained with DAPI (blue). The images were acquired by Leica SP8, and processed with LAS X software.

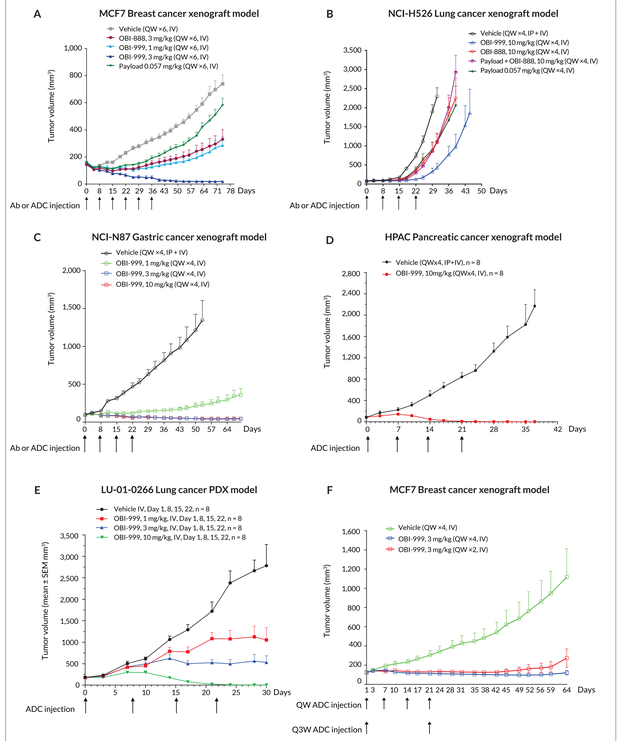
Figure 3. Endosome- and lysosome-dependent internalization of OBI-999.



ADC, antibody-drug conjugate; DAPI, 4′,6-diamidino-2-phenylindole; EEA-1, early endosome antigen 1; LAMP-1, lysosomal-associated membrane protein.

The internalization process of OBI-999 was demonstrated on the HCC-1428 breast cancer cell line and detected by confocal microscopy. OBI-999 or anti-CD30 ADC (control ADC) were incubated at 37°C for various time periods and stained for (**A**) endosome marker EEA-1 or (**B**) lysosome marker LAMP-1. OBI-999 or control ADC, EEA-1, and LAMP-1 were detected using anti-human IgG-AF488 (green), anti-EEA-1 IgG-AF568 (red), and anti-LAMP-1 IgG-AF568 (red) antibodies, respectively. Cell nuclei were stained with DAPI (blue). The images were acquired by Leica SP8, and processed with LAS X software.

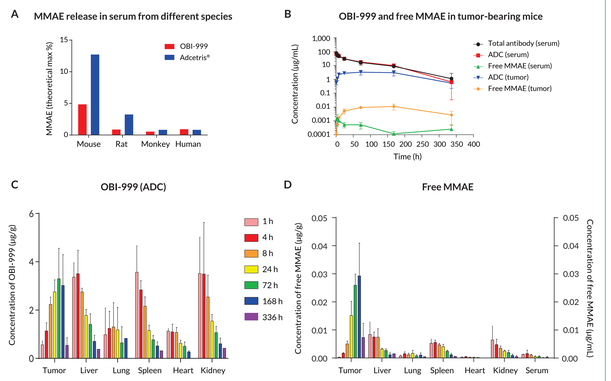
Figure 4. Anti-tumor efficacy of OBI-999 in multiple cancer types.



AAALAC, Assessment and Accreditation of Laboratory Animal Care; Ab, antibody; ADC, antibody-drug conjugate; IACUC, Institutional Animal Care and Use Committee; IP, intraperitoneal; IV, intravenous; MMAE, monomethyl auristatin E; QW, every week.

Tumor growth inhibition of OBI-999 was evaluated in MCF7, NCI-H526, NCI-N87, and HPAC xenograft models, as well as the lung cancer patient-derived xenograft (PDX) model. (**A**) MCF7 breast cancer tumor-bearing mice were intravenously treated with OBI-888 (3 mg/kg), OBI-999 (1, 3 mg/kg), or MMAE only (0.057 mg/kg) once a week for 6 weeks. (**B**) NCI-H526 lung cancer tumor-bearing mice were intravenously treated with OBI-888 (10 mg/kg), OBI-999 (10 mg/kg), or MMAE only (0.057 mg/kg) or MMAE+OBI-888 once a week for 4 weeks. (**C**) NCI-N87 gastric cancer tumor-bearing mice were intravenously treated with 1, 3, or 10 mg/kg of OBI-999 once a week for 4 weeks. (**D**) HPAC pancreatic cancer tumor-bearing mice were intravenously treated with 10 mg/kg OBI-999 once a week for 4 weeks. (**E**) PDX LU-01-0266 lung cancer bearing mice were intravenously treated with 1, 3, or 10 mg/kg OBI-999 weekly for 4 weeks. (**F**) MCF7 breast cancer tumor-bearing mice were intravenously treated with 3 mg/kg OBI-999 weekly for 4 weeks (QW) or once every 3 weeks for 2 doses (Q3W). The anti-tumor efficacy was evaluated via tumor size measurement. Above studies were conducted at an AAALAC-accredited laboratory animal facility; operating procedures were reviewed and approved by IACUC.

Figure 5. Serum stability, pharmacokinetics, and tissue distribution of OBI-999.



ADC, antibody-drug conjugate; ELISA, enzyme-linked immunosorbent assay; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MMAE, monomethyl auristatin E.

(**A**) The *in vitro* serum stability of OBI-999 was conducted at 37°C for 14 days. (**B**) Pharmacokinetic studies were conducted at 5 mg/kg in NCI-N87 tumor-bearing mice. (**C, D**) Organs and tumor tissues from 5 mg/kg OBI-999-injected tumor-bearing mice (n = 5) were harvested and homogenized to evaluate OBI-999 and free MMAE concentration at various time points from 0 to 336 hours post injection. The concentrations of OBI-999 total antibody and ADC were determined by ELISA, and MMAE concentration was evaluated by LC-MS/MS.

Table 1. Pharmacokinetic parameters of OBI-999 in tumor-bearing mice

Dosage	Total Ab[a]				Intact ADC[a]				Free MMAE[b]		
	$T_{1/2}$(d)	C_{max}	AUC	CL	$T_{1/2}$(d)	C_{max}	AUC	CL	$T_{1/2}$(d)	C_{max}	AUC
5 mg/kg	3.2	80.8	158.8	30.1	2.3	73.4	164.6	30.2	3.7	1.8	4.7

Ab, antibody; ADC, antibody-drug conjugate; AUC, area under the concentration-time curve; CL, clearance; C_{max}, maximum concentration; MMAE, monomethyl auristatin E; $T_{1/2}$, half-life.
[a]For total Ab and ADC, C_{max} = μg/mL, AUC = d^*μg/mL.
[b]For MMAE, C_{max} = ng/mL, AUC = d^*μg/mL, CL = mL/kg/day.

CONCLUSIONS

- OBI-999 binds specifically to Globo H-expressing tumor cells, is internalized and trafficked to the endosomes and lysosomes. The drug payload, MMAE, is cleaved in a lysosomal-dependent manner.
- OBI-999 shows excellent anti-tumor efficacy in multiple cancer types, including breast, pancreatic, lung, and gastric xenograft models that express high levels of Globo H.
- PK profile demonstrates good serum stability, i.e., very low level of free MMAE was detected in serum over time.
- There was a gradual and long retention of OBI-999 in the tumor region, reaching maximum concentration at 168 hours and rapidly decreasing at perfusion-rich organs after 72 hours.
- OBI-999 is entering Phase I clinical trials in 2019.

Reference

1. Zhang S, Cordon-Cardo C, Zhang HS, Reuter VE, Adluri S, Hamilton WB, et al. Selection of tumor antigens as targets for immune attack using immunohistochemistry: I. Focus on gangliosides. *Int J Cancer.* 1997;73:42–9.

Disclosures

All authors are employees of OBI Pharma Inc.

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