Preclinical Characterization of a Novel SSEA-4-Targeting Antibody-Drug Conjugate, OBI-998

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OBI PHARMA

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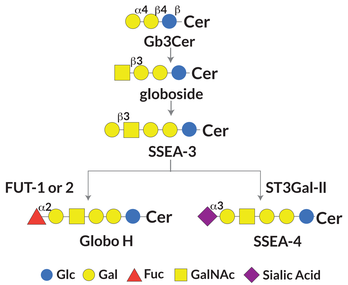
BACKGROUND

Stage-specific embryonic antigen-4 (SSEA-4):

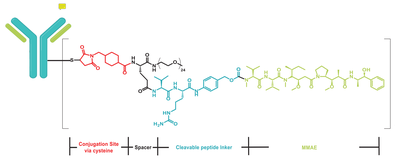
- A Globo-series hexasaccharide
- Overexpressed in multiple cancers, including teratocarcinoma, glioblastoma multiforme, renal cell carcinoma, basaloid lung cancer, epithelial ovarian carcinoma, breast cancer, and oral cancer[1-6]
- A potential cancer stem cell marker[7,8]

Biosynthesis of Globo-series Glycosphingolipids



OBI-998 is an antibody drug conjugate (ADC) comprising the humanized anti-SSEA-4 antibody (OBI-898) that is conjugated to the highly potent microtubule-disrupting agent monomethyl auristatin E (MMAE) through maleimide and PEGylated cleavable linkers

OBI-998 Structure



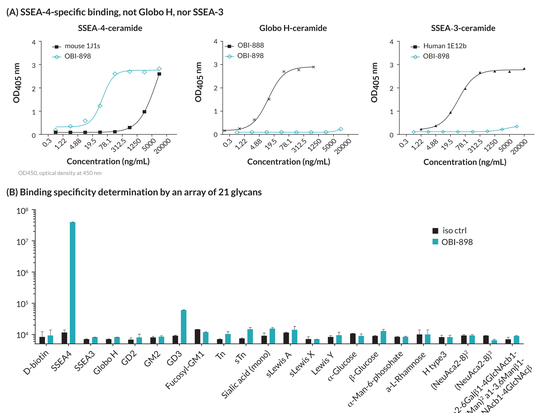
OBJECTIVE

We investigated a novel antibody-drug conjugate (ADC) targeting SSEA-4 to evaluate its potential as a therapeutic agent for cancer treatment.

RESULTS

Binding specificity

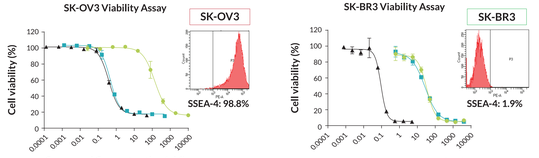
Figure 1. Binding specificity of antibody part of OBI-998.



(A) In titration enzyme-linked immunosorbent assay (ELISA), serial dilutions of anti-SSEA-4 immunoglobulin G (IgG) (OBI-898, the antibody part of OBI-998 ADC) were incubated with 20 μg SSEA-4-ceramide, Globo H-ceramide, or SSEA-3-ceramide. Mouse anti-SSEA-4 IgG (1J1s), human anti-Globo H antibody (OBI-888), and human anti-SSEA-3-IgG antibody (1E12b) were used as a positive control, respectively. Dose-response binding curves were shown. **(B)** Biotinylated sugars at 50 ng were coated onto the plate and incubated with 2.5 μg/mL of OBI-898 or isotype control (iso ctrl). The amount of OBI-898 bound was examined by chemiluminescent sandwich ELISA analysis.

Cytotoxicity

Figure 2. Cytotoxicity of OBI-998.



	IC_{50} (nM)
Ctrl ADC	140.3
OBI-998	0.4684
MMAE	0.3871

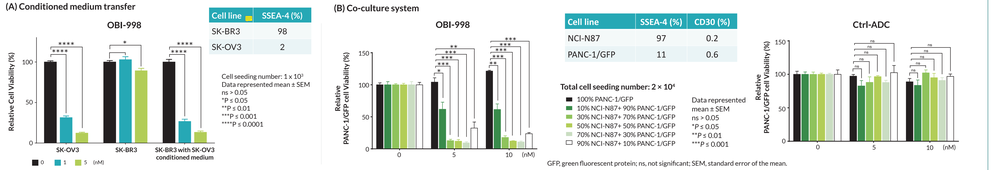
	IC_{50} (nM)
Ctrl ADC	33.4
OBI-998	27.29
MMAE	0.09322

OBI-998 showed potent cytotoxic activity on SSEA-4 high expression cancer cell line, SK-OV3, but not on low expression cell, SK-BR3.

SSEA-4 expression level of SK-OV3 and SK-BR3 cell lines are determined using flow cytometry (FACS) surface staining. The SK-OV3 and SK-BR3 cells were treated with OBI-998, anti-CD30 ADC (Ctrl ADC), or MMAE at respective concentrations for 4 days. The cell viability was determined by CellTiter-Glo® viability assay kit.

In vitro bystander effect

Figure 3. *In vitro* bystander effect of OBI-998 in conditioned medium transfer (A) and co-culture system (B).



Cell line	SSEA-4 (%)
SK-BR3	98
SK-OV3	2

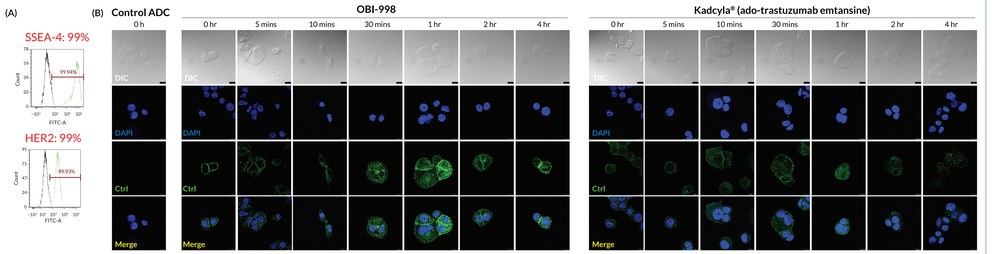
Cell line	SSEA-4 (%)	CD30 (%)
NCI-N87	97	0.2
PANC-1/GFP	11	0.6

(A) SSEA-4 expression levels of SK-OV3 and SK-BR3 cell lines are determined using FACS surface staining. SK-OV3 cells were treated with 0, 1, and 5 nM of OBI-998 for 5 days; then the supernatant was transferred to treat SK-BR3 cells for another 5 days (**SK-BR3 with SK-OV3 conditioned medium**). The SK-OV3 and SK-BR3 cells were treated with OBI-998 at respective concentrations for 5 days as positive (**SK-OV3**) and negative (**SK-BR3**) controls, respectively. The cell viability of treated cells relative to corresponding untreated cells (0 nM) was determined by CellTiter-Glo® viability assay kit. Statistically significant differences were indicated ($P < 0.05$).

(B) SSEA-4 and CD30 expression levels in NCI-N87 and PANC-1 transfected with green fluorescent protein (GFP) (PANC-1/GFP) were determined using FACS surface staining. PANC-1/GFP cells alone or co-cultured with NCI-N87 cells in different ratios were treated with either OBI-998 or Ctrl-ADC (anti-CD30-MMAE) at various concentrations (0, 5, 10 nM) for 8 days. The cell viability of PANC-1/GFP cells relative to corresponding untreated cells (0 nM) was calculated by GFP fluorescence intensity. Statistically significant differences were indicated ($P < 0.05$).

Internalization

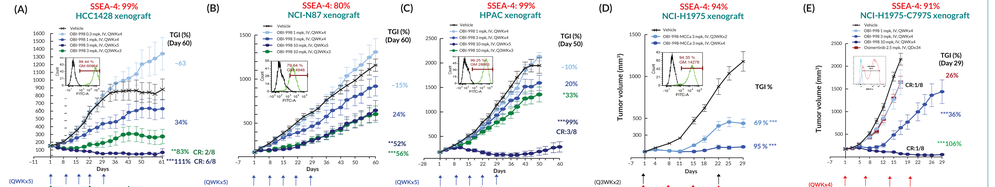
Figure 4. Internalization kinetics of OBI-998 in HCC1428 cells.



(A) SSEA-4 and human epidermal growth factor receptor-2 (HER2) expression levels of HCC1428 cell line are determined using FACS surface staining. **(B)** HCC1428 cells were incubated with OBI-998 or Kadcyla® (ado-trastuzumab emtansine; 1 μg/mL) for 1 hr at 4°C and then fixed (0 hr) or transferred to prewarmed media and maintained at 37°C for 5 mins, 10 mins, 30 mins, 1 hr, 2 hr, and 4 hr before fixation. They were then fixed and incubated with Alexa Fluor® 488-conjugated anti-human immunoglobulin G (IgG) for detection of SSEA-4 or HER2. Cells were incubated with Control ADC, anti-CD30-MMAE (1 μg/mL) as a negative target-binding control (ctrl) and only observed at 0 hr. Cell nuclei were stained by DAPI.

Anti-tumor efficacy

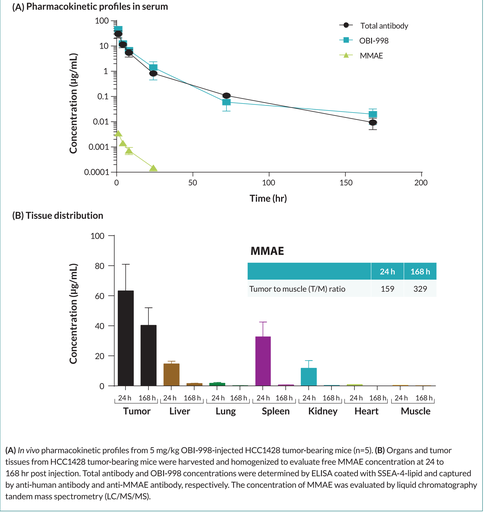
Figure 5. Anti-tumor efficacy of OBI-998 in multiple cancer types.



SSEA-4 expression levels in HCC1428 breast cancer **(A)**, NCI-N87 gastric cancer **(B)**, HPAC pancreatic cancer **(C)**, NCI-H1975 lung cancer cell line (EGFR L858R/T790M mutation and is sensitive to osimertinib) **(D)**, and NCI-H1975-C797S lung cancer (EGFR exon 19 deletion/T790M/C797S mutation and is resistant to osimertinib) **(E)** cell lines are determined by FACS surface staining. 6- to 8-week-old BALB/c nude female mice were subcutaneously implanted with viable respective cancer cells. Tumor implanted mice were divided into treatment groups, each group containing 8 animals. Dose administrations were initiated at 11 days **(A)** or 7 days **(B-D)** post tumor cell implantation (denoted as day 1). Vehicle and OBI-998 at indicated doses were administered intravenously once weekly or once every 3 weeks. Tumor size was monitored and recorded twice weekly for 61 days **(A)**, 60 days **(B,C)**, or 29 days **(D,E)**. Tumor growth inhibition (TGI) rate was calculated at the end of study. Data are represented as mean ± SEM. CR: complete regression.

Pharmacokinetics and tissue distribution

Figure 6. Pharmacokinetic profile of OBI-998.



(A) *In vivo* pharmacokinetic profiles from 5 mg/kg OBI-998-injected HCC1428 tumor-bearing mice (n=5). **(B)** Organs and tumor tissues from HCC1428 tumor-bearing mice were harvested and homogenized to evaluate free MMAE concentration at 24 to 168 hr post injection. Total antibody and OBI-998 concentrations were determined by ELISA coated with SSEA-4-lipid and captured by anti-human antibody and anti-MMAE antibody, respectively. The concentration of MMAE was evaluated by liquid chromatography tandem mass spectrometry (LC/MS/MS).

CONCLUSIONS

- OBI-998 is a novel ADC targeting SSEA-4 that possesses desired properties such as high target specificity, rapid internalization, potent cytotoxicity, and significant bystander effects
- OBI-998 showed a high level of deposition and a persistent presence of MMAE in tumors and significant anti-tumor efficacy in a variety of animal models
- The results support the further development of OBI-998 as a therapeutic agent for SSEA-4-targeting cancer therapy

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