Specificity, biodistribution, tumor targeting, and pharmacokinetics of a novel humanized anti-Globo H antibody, OBI-888, for cancer immunotherapy

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INTRODUCTION

OBI-888 is a humanized monoclonal IgG1 antibody that binds to Globo H (GH), a tumor-associated carbohydrate antigen. It is being developed as a therapy to treat GH-positive cancers.[1] In the present study, we examined the binding specificity, binding epitope, antigen targeting ability, and pharmacokinetics of OBI-888.

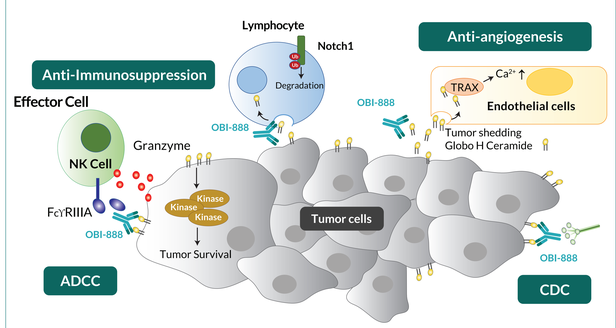
BACKGROUND

Table 1. Prevalence of Globo H in different cancer types

Indication	Total subjects	H-score ≥1	H-score ≥1 Prevalence	H-score ≥15	H-score ≥15 Prevalence
Pancreatic	72	55	76.4%	48	66.7%
Esophageal	64	42	65.6%	33	51.6%
Gastric	73	43	58.9%	30	41.1%
Breast	131	77	58.8%	49	37.4%
Lung	77	45	58.4%	28	36.4%
Colon	75	38	50.7%	24	32.0%
Liver	70	12	17.1%	5	7.1%

A total of 562 specimens were analyzed: 81 breast cancer, 24 pancreatic cancer, 29 lung cancer, 25 gastric cancer, 29 colon cancer, 23 liver cancer, and 24 esophageal cancer. Along with tissue specimens, tissue microarray (TMA) sections with 40–50 cores for each of the 7 cancer types mentioned above were also analyzed. Anti-Globo H monoclonal antibody (VK9) was used for immunohistochemistry staining. Globo H expression level was assessed by certified pathologists and results are quantified using an H-score system with results ranging from 0 to 300. H-score was used to present the expression level of Globo H on tumor tissue. H score is calculated by the following equation. H-score = percentage of weak intensity x 1 + percentage of moderate intensity x 2 + percentage of strong intensity x 3.

Potential Therapeutic Mechanisms of Action of the Globo H Monoclonal Antibody OBI-888



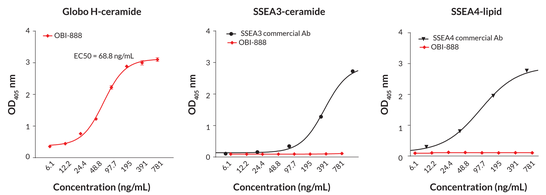
ADCC, antibody-dependent cell-mediated cytotoxicity; Ca^{2+}, calcium ions; CDC, complement-dependent cytotoxicity; NK, natural killer.

Chuang, et al. *PNAS* 2019;116(9):3518-3523.
Cheng, et al. *Cancer Res.* 2014;74(23):6856-6866.
Tsai, et al. *J Cancer Sci Ther.* 2013;5(7):264-270.
OBI Data on file.

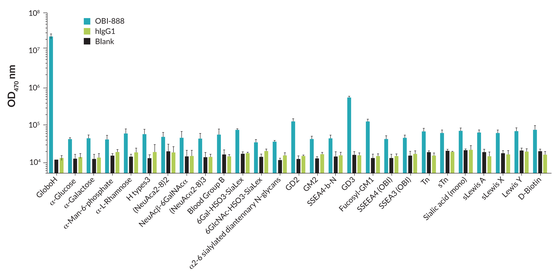
Specificity of OBI-888 *in vitro*

Figure 1. Binding specificity of OBI-888 *in vitro* by ELISA.

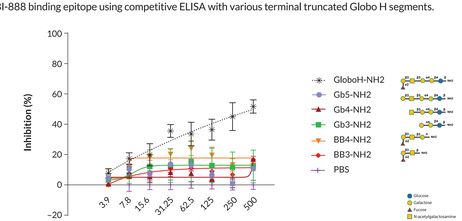
A. OBI-888 binds to Globo H-ceramide but not SSEA3-ceramide or SSEA4-lipid.



B. The specificity of OBI-888 against Globo H was demonstrated using a glycan array of 26 glycans.



C. Determination of OBI-888 binding epitope using competitive ELISA with various terminal truncated Globo H segments.



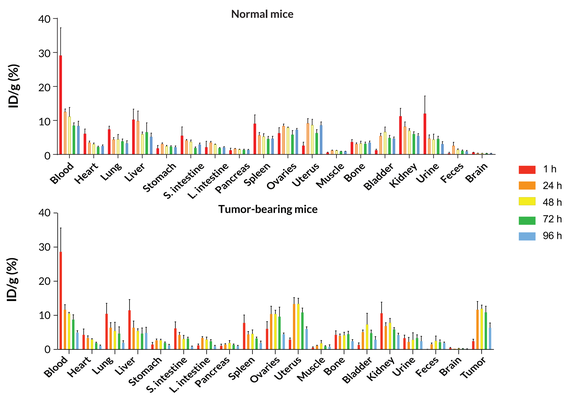
Ab, antibody; EC50, half maximal effective concentration; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline.

(**A**) In titration ELISA, serial dilutions of OBI-888, rat anti-SSEA3-IgM antibody (BioLegend, Cat# 330302), and mouse anti-SSEA4-IgG antibody (BioLegend, Cat# 330402) were added to Globo H, SSEA3-ceramide, or SSEA4-lipid pre-coated plates (0.2 ug/well, OBI). Dose-response binding curves were shown.(**B**) Biotinylated sugars at 1 ng/mL were coated onto the plate and incubated with 781 ng/mL of OBI-888. The amount of OBI-888 bound was examined by chemiluminescent sandwich ELISA analysis. (**C**) In competitive ELISA, serial dilutions of each Globo-series glycan and truncated glycan terminal antigens were mixed with 0.195 μg/mL OBI-888 and added to GH-ceramide coated plates. % Inhibition = 100 – (antigen with OBI-888/PBS with OBI-888)*100% .

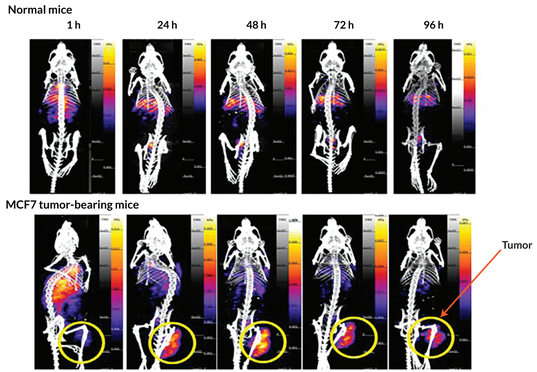
Biodistribution of In^{111}-DTPA-OBI-888 in normal and tumor-bearing mice

Figure 2. Biodistribution and microSPECT images of In^{111}-DTPA-OBI-888

A. Biodistribution of In^{111}-DTPA-OBI-888 in different organs



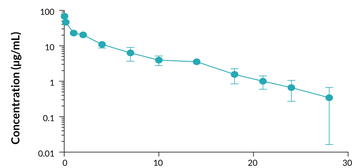
B. MicroSPECT images of In^{111}-DTPA-OBI-888 biodistribution in normal and tumor-bearing mice



(**A**) OBI-888 was conjugated with p-SCN-Bn-DTPA and labeled with Indium-111. Normal mice and MCF7 tumor-bearing mice received a single intravenous injection of In^{111}-DTPA-OBI-888 and tomographic single-photon emission computed tomography (SPECT)/computed tomography (CT) images were recorded at 1, 24, 48, 72, and 96 hours post injection. The intensity of radioactivity was measured for each organ. (**B**) Tomographic SPECT/CT imaging of MCF7 tumor-bearing and normal mice at 1, 24, 48, 72, and 96 hours post injection of Indium-111-labeled OBI-888.

Pharmacokinetics of OBI-888 in mice after IV administration

Figure 3. Mean concentration-time profile of OBI-888 in mice.



Groups of female nude (nu/nu) mice (n = 5/time point) were intravenously injected with a single dose of OBI-888 at 5 mg/kg. Serum samples were collected at the 1st and 4th hour on Day 1 and on Day 2, 4, 7, 10, 14, 18, 21, 24, and 28 post injection for ELISA assay.

Table 2. Pharmacokinetic parameters of OBI-888 from the non-compartmental analysis

	T_{max} (day)	Half-lives (day)	C_{max} (μg/mL)	AUC_{0-t} (day*μg/mL)	AUC_{INF} (day*μg/mL)	CL (mL/kg/day)	V_d (mL/kg)	V_{ss} (mL/kg)	MRT_{0-t} (day)
Mean	0.04	5.1	68.7	167.1	172.2	29.1	213.9	179.3	5.3
SD	0.00	0.6	8.0	8.4	7.7	1.3	30.6	19.4	0.5

AUC_{0-t}, area under the concentration-time curve from time 0 to last measurable concentration; AUC_{INF}, area under the concentration-time curve to infinity; CL, clearance; C_{max}, maximum concentration; MRT_{0-t}, mean residence time from time 0 to infinity; SD, standard deviation; T_{max}, time of maximum concentration; V_d, volume of distribution; V_{ss}, volume of distribution at steady state.

CONCLUSIONS

Specificity of OBI-888

- Titration ELISA showed that OBI-888 specifically binds to Globo H-ceramide, but not other Globo series antigens such as SSEA3-ceramide and SSEA4-lipid.
- Cross-reactivity testing showed that OBI-888 is highly specific to Globo H, but not to other tumor-associated carbohydrates.
- Competitive ELISA showed that OBI-888 does not bind to truncated structures of Globo H, and instead, requires a full hexasaccharide structure of Globo H.

Biodistribution of OBI-888

- In^{111}-OBI-888 was preferentially localized to the tumor site. The tumor/muscle ratio peaked at 11.26 ± 1.90 % ID/g at 72 hours post injection.
- The distribution of OBI-888 in other organs was comparable to that of non-tumor-bearing normal mice, except in the urine. Conceivably, the radioactive Indium could be sequestered intra-tumorally after OBI-888 was internalized by tumor cells.

Pharmacokinetic profile of OBI-888 in mice

- After single-dose IV administration of 5 mg/kg the elimination half-life of OBI-888 was 5.1 days. The estimated clearance (CL) and steady state volume of distribution (V_{ss}) were 29.1 mL/kg/day and 179.3 mL/kg, respectively.

Clinical trial

- A first-in-human clinical trial of OBI-888 (NCT03573544) has been initiated.

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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