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Anti-tumor efficacy and potential mechanism of action of a novel therapeutic humanized anti-Globo H antibody, OBI-888

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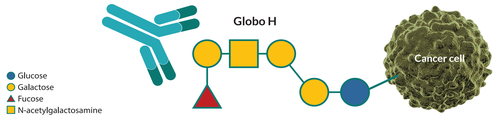
INTRODUCTION

OBI-888 is a therapeutic humanized monoclonal IgG1 antibody targeting Globo H, a cancer-specific hexasaccharide antigen overexpressed on a variety of cancer cells of epithelial origin such as colon, ovarian, gastric, pancreatic, endometrial, lung, prostate, and breast cancers.[1, 2, 3] In some normal tissues, Globo H is negligibly expressed on the apical epithelial cells at lumen borders, where accessibility to the immune system is restricted.[3] This study evaluated mechanisms of action and in vivo efficacy of OBI-888.

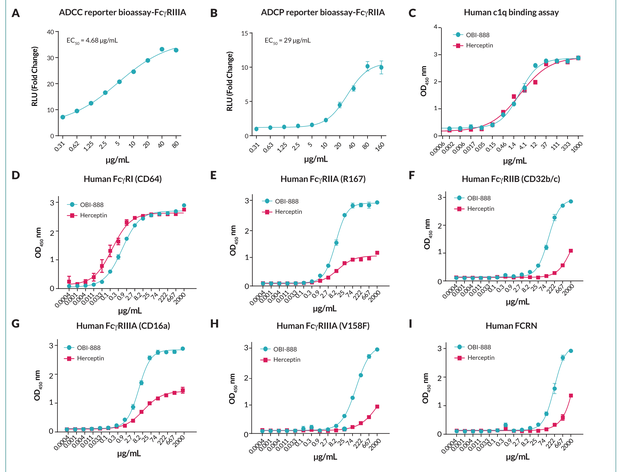
METHODS

We evaluated the potential therapeutic activity of the Globo H monoclonal antibody (OBI-888) *in vitro* to determine complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and antibody-dependent phagocytosis (ADCP) and T-cell suppression. We also evaluated it in xenograft models of various tumors to determine the effects on tumor growth and M2 macrophage reduction.



RESULTS

Figure 1. OBI-888-induced ADCC, ADCP, and CDC.

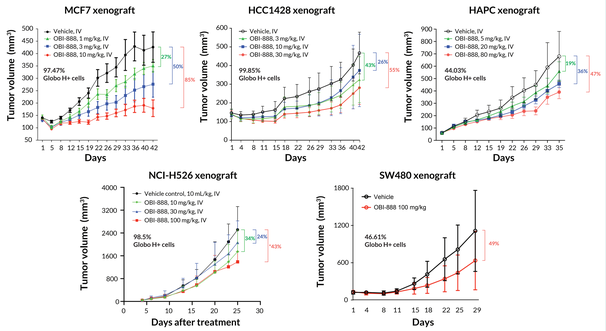


ADCC, antibody-dependent cell-mediated cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CDC, complement-dependent cytotoxicity; EC_{50}, half maximal effective concentration.

(**A**) ADCC reporter bioassay responses were collected from 9 serial dilutions from 80-0.31 μg/mL of OBI-888. (**B**) ADCP reporter bioassay responses were collected from 10 serial dilutions from 160-0.31 μg/mL of OBI-888. (**C**) Binding curve of OBI-888 to purified human complement c1q. Data were collected from 14 serial dilutions from 1,000-0.0006 μg/mL of OBI-888. (**D-I**) Binding curve of OBI-888 to purified human FcγRI, FcγRIIA, FcγRIIB, FcγRIIIA, FcγRIIIA (V158F) and FCRN. Analysis of FcγR assays was performed over various concentrations of OBI-888 ranging from 2,000-0.0004 μg/mL. Four-parameter logistic regression curve fit analysis was used. The OD values are shown as mean ± standard deviation. .

OBI-888 inhibited tumor growth in the xenograft models of various cancer types.

Figure 2. *In vivo* efficacy of OBI-888.

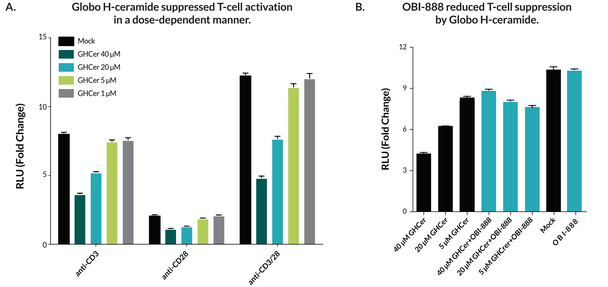


IV, intravenous.

Groups of Nude female mice were subcutaneously implanted with viable MCF7 (human breast adenocarcinoma), HCC1428 (human breast adenocarcinoma), HPAC (human pancreatic adenocarcinoma), NCI-H526 (human small cell lung cancer), or SW480 (human colorectal adenocarcinoma) cells. Tumor-implanted mice were then divided into treatment groups with 8 animals in each group. Intravenous administration of OBI-888 was initiated one day after tumor cell implantation. Tumor volumes are shown as mean ± standard deviation. **Tumor growth inhibition was calculated as (1 –[(Tn –T1)/ (Cn –C1)]) × 100%, where Tn represents tumor weight measured on Day n in the treatment group and Cn represents tumor weight measured on Day n in the control group.**

OBI-888 reduced Globo H-ceramide-induced T-cell inactivation.

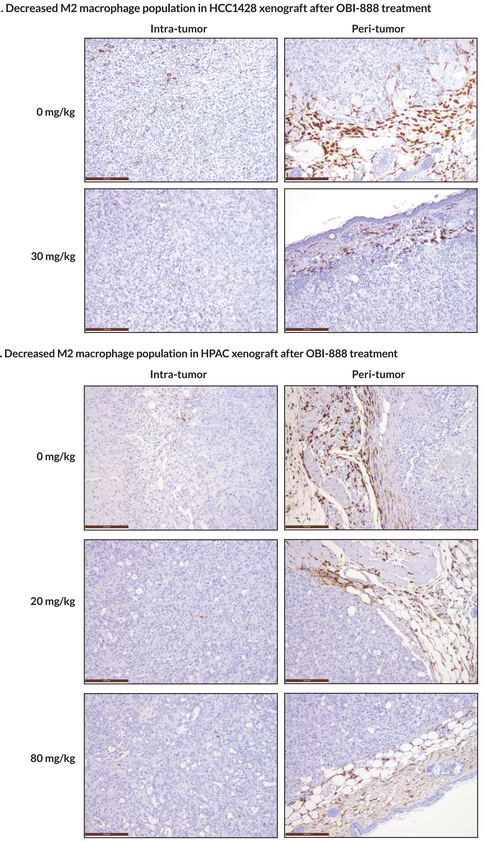
Figure 3. T-cell activation assay.



(**A**) Globo H ceramide (GHCer) inhibited T-cell activation in a dose-dependent manner. After pre-incubation with various concentrations of Globo H-ceramide, Jurkat/NFAT-Re Luc cells were washed once with medium, and added to anti-CD3, anti-CD28, or anti-CD3/CD28 pre-coated plate. Bio-Glo luciferase assay reagent was then added to the plate. Luminescence was measured at 470 nm. Fold of induction was calculated by $RLU_{activated}/RLU_{unstimulated}$.

(**B**) OBI-888 reduced Globo H-ceramide-induced T-cell inactivation. Different concentrations of Globo H-ceramide were incubated with 8.7 μM of OBI-888. After centrifuge, the supernatants were then added to Jurkat/NFAT-Re Luc cells. After incubation, the cells were washed once with medium and added to anti-CD3/CD28 pre-coated plate. Bio-Glo luciferase assay reagent was then added to the plate. Luminescence was measured at 470 nm. Fold of induction was calculated by $RLU_{activated}/RLU_{unstimulated}$.

Figure 4. Representative images of CD163+ M2 macrophages in peri-tumor and intra-tumor regions of HCC1428 (A) and HPAC (B) xenograft.



BALB/c Nude male mice subcutaneously implanted with HCC1428 or HPAC cells were intravenously administrated with OBI-888 at 0 and 30 mg/kg twice weekly for 6 weeks or at 0, 20, and 80 mg/kg twice weekly for 5 weeks, respectively. At day 42 for HCC1428 xenografts and day 35 for HPAC xenografts, the xenografts were fixed, sectioned, and stained with CD163 and hematoxylin.

Decreased M2 macrophage population after OBI-888 treatment

Table 1. Intra-tumoral and peri-tumoral infiltrations of CD163+ macrophages

		Intra-tumoral infiltrations	Peri-tumoral infiltrations
Xenograft model	OBI-888 dose (mg/kg)	M2 macrophage CD163+ cell count[1]	M2 macrophage CD163+ cell count[1]
MCF-7	0	4.60 ± 6.13	60.73 ± 26.5
	3	0.30 ± 0.34	68.03 ± 17.4
	10	1.17 ± 0.71	44.87 ± 24.32
HPAC	0	12.00 ± 4.81	105.6 ± 3.96
	20	6.80 ± 0.42	97.85 ± 32.46
	80	1.90 ± 0.57**	58.30 ± 4.24
HCC-1428	0	19.68 ± 4.77	103.6 ± 29.77
	30	6.30 ± 7.17*	62.70 ± 8.23*

[1]Average cell count of 10 fields under microscopy.
The results are presented as mean ± standard deviation of each treatment group.
* $P < 0.05$
** $P < 0.01$
Student's t-test

CONCLUSIONS

ADCC, ADCP, and CDC

- OBI-888 can trigger ADCC, ADCP, and CDC, mechanisms utilized by therapeutic antibodies to induce tumor lysis.
- The ability to trigger ADCC and ADCP was further supported by the binding affinity of OBI-888 to multiple FcγRs, including FcγRIIIA V176F, which can potentially benefit those patients with low-affinity genotype.

In vivo efficacy

- Treatment with OBI-888 induced tumor growth inhibition in various types of tumor models that express Globo H, suggesting that OBI-888 has substantial therapeutic potential.

Decreased M2 macrophage

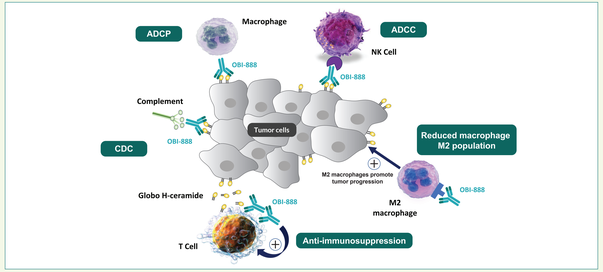
- *In vivo* tumor growth inhibition with decreased M2 population was observed in various Globo H-expressing xenograft models treated with OBI-888, suggesting the potential mechanism of action is through immune modulation.

Anti-immunosuppressive effect

- Globo H-ceramide displayed an immunosuppressive effect.
- OBI-888 reversed the immunosuppressive effect of Globo H-ceramide.

Clinical trial

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



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Disclosures

All authors are employees of OBI Pharma Inc.

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