# Globo H-Targeted CAR T Cell Cancer Immunotherapy

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AACR 2023
4084

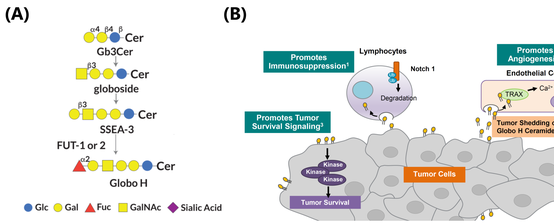
## BACKGROUND

- Globo H (GH), a Globo-series glycosphingolipid (GSL), is highly expressed in epithelial tumors, such as colon, endometrial, gastric, pancreatic, lung, prostate, and breast cancers. Aberrant expression of GH has been reported to be associated with the metastatic potential and poor prognosis of these cancers.
- In normal tissues, GH expression is limited to the secretory borders of apical epithelial cells, making it difficult to access by the immune system. GH is therefore a promising target for anticancer therapeutics.
- Clinical studies with the Globo H vaccines (OBI-822 and OBI-833), the humanized anti-Globo H antibody (OBI-888), and its antibody-drug conjugate (OBI-999) demonstrating an excellent safety profile.
- Predominant expression of GH in cancers and the clinical safety results suggest GH may provide a novel and unique cancer target for the development of Chimeric Antigen Receptor (CAR) T cell therapy.

## OBJECTIVE

The aim of this study is to develop efficacious CAR T targeting Globo H (obi-R007) to offer a safe and persistent anticancer cell therapeutic agent.

## 1. Introduction




(C)

| Products | Target | Indication | Discovery | Pre-Clinical | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|---|---|---|---|
| Adaglioxad Simolenin (Vaccine) | Globo H | Breast (TNBC) | GLORIA Global Phase 3 TNBC Study | | | | |
| OBI-999 (ADC) | Globo H | Multiple Cancers | | | | | |
| OBI-833 (Vaccine) | Globo H | Multiple Cancers | | | | | |

**Figure 1. Introduction of Globo H targeting for anticancer therapeutics.**
**(A)** Biosynthesis of Globo-series Glycosphingolipids (GSL) (Cer, Ceramide; GalNAc, N-Acetylgalactosamine) (Ref. 4).
**(B)** MOA of Globo H as promising target for anticancer therapeutics (Refs 1-4).
**(C)** First-in-class clinical pipeline for Globo H targeting therapeutics at OBI (Ref. 5-7).

## REFERENCES

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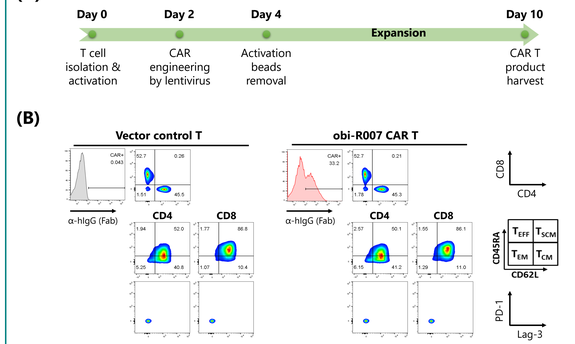
## RESULTS

## 2. CAR T Production and Characterization




| Healthy Donor | Expansion Fold | CAR⁺ % | CD4/CD8 Ratio | CD4 | | CD8 | |
|---|---|---|---|---|---|---|---|
| | | | | Tscm | Tcm | Tscm | Tcm |
| PBMC01 | 56 | 25.1 | 1.6 | 15.6 | 79.6 | 63.2 | 28.5 |
| PBMC02 | 164 | 27.4 | 1.2 | 20.6 | 68.0 | 57.0 | 31.6 |
| PBMC03 | 50 | 31.1 | 0.6 | 16.2 | 74.4 | 55.9 | 38.2 |
| PBMC04 | 52 | 42.5 | 1.6 | 27.7 | 62.3 | 59.8 | 22.5 |
| PBMC05 | 64 | 48.1 | 0.6 | 15.4 | 74.4 | 52.4 | 42.8 |
| PBMC06 | 25 | 49.0 | 1.5 | 9.6 | 74.4 | 27.3 | 49.8 |
| PBMC07 | 183 | 14.5 | 0.7 | 37.4 | 48.5 | 79.6 | 7.7 |
| PBMC08 | 245 | 17.0 | 0.9 | 62.6 | 35.2 | 88.8 | 10.9 |
| PBMC09 | 168 | 30.0 | 1.3 | 18.4 | 60.8 | 42.7 | 36.0 |
| **Average** | **112** | **31.6** | **1.1** | **24.8** | **64.2** | **58.5** | **29.8** |

**Figure 2. Production and characterization of GH CAR T (obi-R007)**
**(A)** Process flow of obi-R007.
**(B)** Immunophenotypes of obi-R007 ($T_{SCM}$+$T_{CM}$ > 80%, without exhaustion markers).
**(C)** Characterization of obi-R007 (summary table from 9 healthy donors).

## 3. *In Vitro* Potency

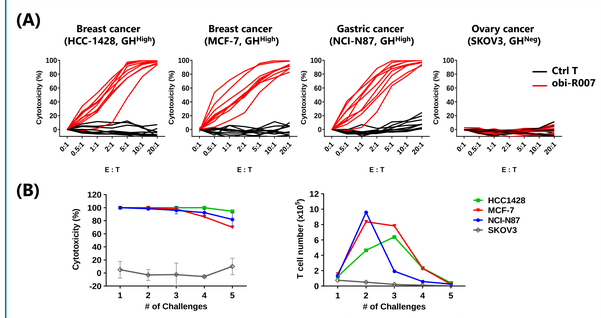



**Figure 3. *In vitro* cytotoxicity and repeated killing potency of obi-R007.**
**(A)** Cytotoxicity of obi-R007 against bioluminescent-tagged tumor cell lines for 24 hours ($EC_{50}$ range: E/T ratio= 0.5:1 ~ 5:1).
**(B)** Cytotoxicity and CAR T cell expansion by repeated tumor cell challenges for 5 rounds (3 days interval; E/T ratio= 1:1).

## 4. *In Vitro* Activity

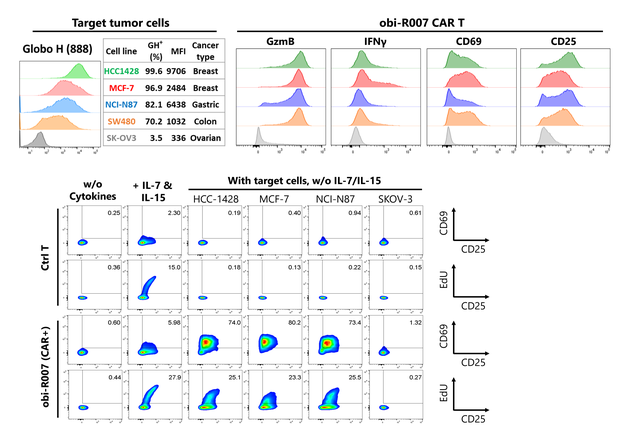



**Figure 4. *In vitro* effector function of GH CAR T (obi-R007)**
**(A)** Expression of effector molecules after 24 hours co-culture at E/T ratio= 4:1.
**(B)** Target cell/cytokines-specific activation ($CD69^+CD25^+$) and proliferation ($EdU^+$) of obi-R007.

## 5. *In Vivo* Efficacy and Persistence

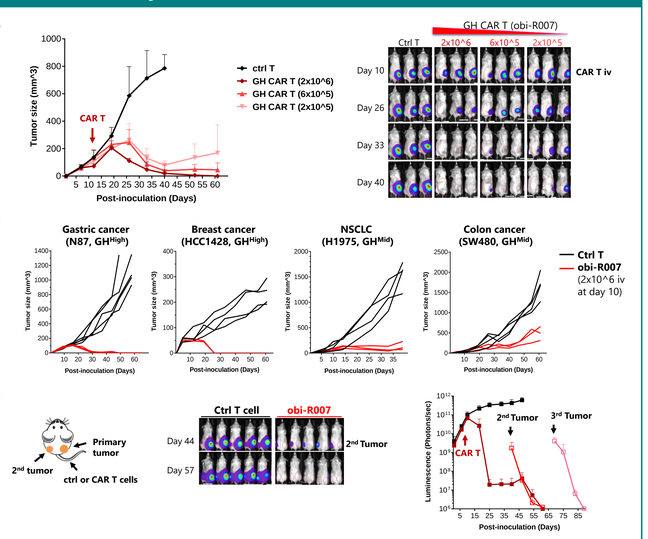



**Figure 5. *In vivo* efficacy and persistence of GH CAR T (obi-R007)**
**(A)** Dose-dependent efficacy of obi-R007 in N87 gastric xenograft model (ASID mice).
**(B)** *In vivo* efficacy of obi-R007 is demonstrated in multiple tumor xenograft models.
**(C)** Persistence of obi-R007 by repeated tumor challenges in N87 xenograft model.

## 6. Biodistribution and Acute Toxicity

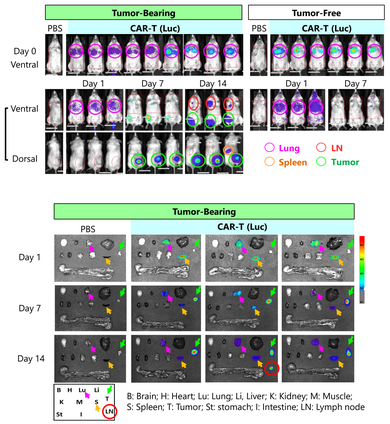



**Figure 6. *In vivo* and gross necropsy images of GH CAR T.**
**(A)** *In vivo* image of bioluminescent-tagged GH CAR T after intravenous injection in N87 xenograft model.
**(B)** Images of gross necropsy show predominate trafficking of GH CAR T in tumor and lymphoid organs.

## SUMMARY

1. *Ex vivo* expansion of obi-R007 from healthy donors ranged from 40- to 200-fold post-activation for 10 days.
2. There was less differentiation in the $CD62L^+$ population ($T_{SCM}$ and $T_{CM}$) in greater than 80% of obi-R007 cells, and no significant exhaustion markers (PD-1, LAG-3, etc.) were detected.
3. obi-R007 showed specific cytotoxicity against Globo H-positive tumor cells with an E/T ratio of 0.5:1~2:1 and exhibited continuously killing potency for 10 days *in vitro*.
4. obi-R007 exhibited Globo H-specific activation through the expression of CD69, CD25, and Granzyme B as well as the release of Interferon γ and IL-2.
5. The activation and proliferation of obi-R007 were dependent on the presence of target cells. All the *in vitro* results indicate the specific targeting of obi-R007 to Globo H resulting in cytotoxic T cell responses.
6. In the *in vivo* study, the efficacy of obi-R007 was demonstrated by adoptive cell transfer in several xenograft models and obi-R007 was shown to be persistent under multiple tumor challenges in the NCI-N87 gastric cancer model.
7. Luminance labelling of CAR T cells showed that the distribution of CAR T cells in mice was specific to the tumor site and lymphoid organs suggesting a homing activity of the CAR T cells. Furthermore, no obviously physiological toxicity was observed *in vivo*.

## CONSCLUSIONS

**In conclusion, our *in vitro* and *in vivo* pharmacology and preliminary toxicology studies support clinical development of Globo H CAR T immunotherapy for patients with various cancers.**