vaccination; expansion of germinal centers of B cells to Globo H series tumor antigens; preferential expansion of germinal centers containing high affinity anti-Globo H antibodies; induction of low frequency B cells normally not present in sufficient numbers to produce meaningful responses with single (or low repetition of exposure to antigen) (e.g., they may bind to epitopes to which few antibodies bind); expansion of antibody secreting plasma cells ("antibody secreting plasma cells" are what B cells differentiate into) and memory B cells which may be important for long term maintenance of the anti-tumor response; increase kinetics of Ab class switching [need good T cell helper function or B cells will not switch to IgG]; increase in kinetics of antibody response (e.g., successive vaccination may lead to more rapid expansion of antibody than obtained without either conjugation to KLH, without co-administration of cyclophosphamide, and/or without repeated vaccinations; reduction of Treg activity that interferes with the development and maintenance of the above anti-tumor response; induction of Antibody-Dependent Cellular Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity (CDC) for tumor killing; induction of anti-Globo series antigens IgM/IgG immune response to elicit CDC and ADCC mediated tumor cell killing; induction of anti-Globo series antigens antibodies trap Globo series antigens-ceramide shedding from tumor cells to block translin-associated factor X (TRAX)-dependent angiogenesis; induction of anti-Globo series antigens antibodies to block Globo series antigens-ceramide induced Notch 1-dependent immunosuppression and thereby enhancing T cell proliferation and cytokine production; induction of anti-Globo series antigens antibodies to lead to apoptosis; Inhibition of Globo series antigens induced angiogenesis; OBI-822 vaccination induces apoptosis; Induction of CTLs (cytotoxic lymphocyte).

BRIEF DESCRIPTION OF THE DRAWINGS

[10] Figure 1. OBI-822: Active cancer immunotherapy induces ADCC and CDC for tumor killing. Globo H-ceramide depletion by OBI-822 effectively blocks TRAX-dependent angiogenesis and achieves tumor regression. Tumor-secreted soluble factors bind to their respective receptors on endothelial cells, trigger phospholipase C (PLC) activation and intracellular calcium release, and promote proliferation, migration, and tube formation of endothelial cells. PLC serves as an "early generator" of second messengers, driving early stages of angiogenesis. PLC activity is regulated by several binding partners, including translin-associated factor X (TRAX), which blocks PLCβ1 activity. The molecular mechanism of this event involves the binding of Globo-H ceramide to TRAX with consequent release and activation of PLCβ1.

[11] Figure 2. OBI-822 depletes Globo H-ceramide, which in turns increases Notch 1 degradation, blocking the tumor immunosuppression effect, leading to tumor regression. The Notch signaling pathway is an evolutionarily conserved cell signaling system in most organisms, and it can regulate cell proliferation, differentiation, apoptosis, and survival. The regulation of Notch 1 is controlled by the E-protein transcription factor E2A and its natural inhibitor ID3. It is degraded by ubiquitination though E3 ubiquitin ligase, ITCH. Addition of Globo-H ceramide to immune cells could inhibit their proliferation and cytokine or immunoglobulin secretion. Globo-H ceramide could induce immunosuppression which involved inhibition of Notch 1 signaling through the induction of ID3 and EGR2/3 accompanied ITCH expression.

[12] Figure 3. OBI-822 leads to apoptosis. Apoptosis (programmed cell death) contributes to the normal development and tissue remodeling of multicellular organisms. Focal adhesion kinase (FAK) has been implicated in the integration of signals from integrins, oncogenes, and neuropeptides. Proteolytic cleavage of FAK by caspase-3 has been reported during growth factor deprivation-induced apoptosis in human umbilical vein endothelial cells, which implies an association between FAK and apoptosis.

[13] Figure 4 illustrated the Progression-Free Survival (mITT) after received 9 Injections of Study Drugs (Stratified Log-rank Test, p=0.0542).

[14] Figure 5 illustrated the Progression-Free Survival (mITT) after received 9 Injections of Study Drugs and with Disease at Baseline (Stratified Log-rank Test, P=0.0362).

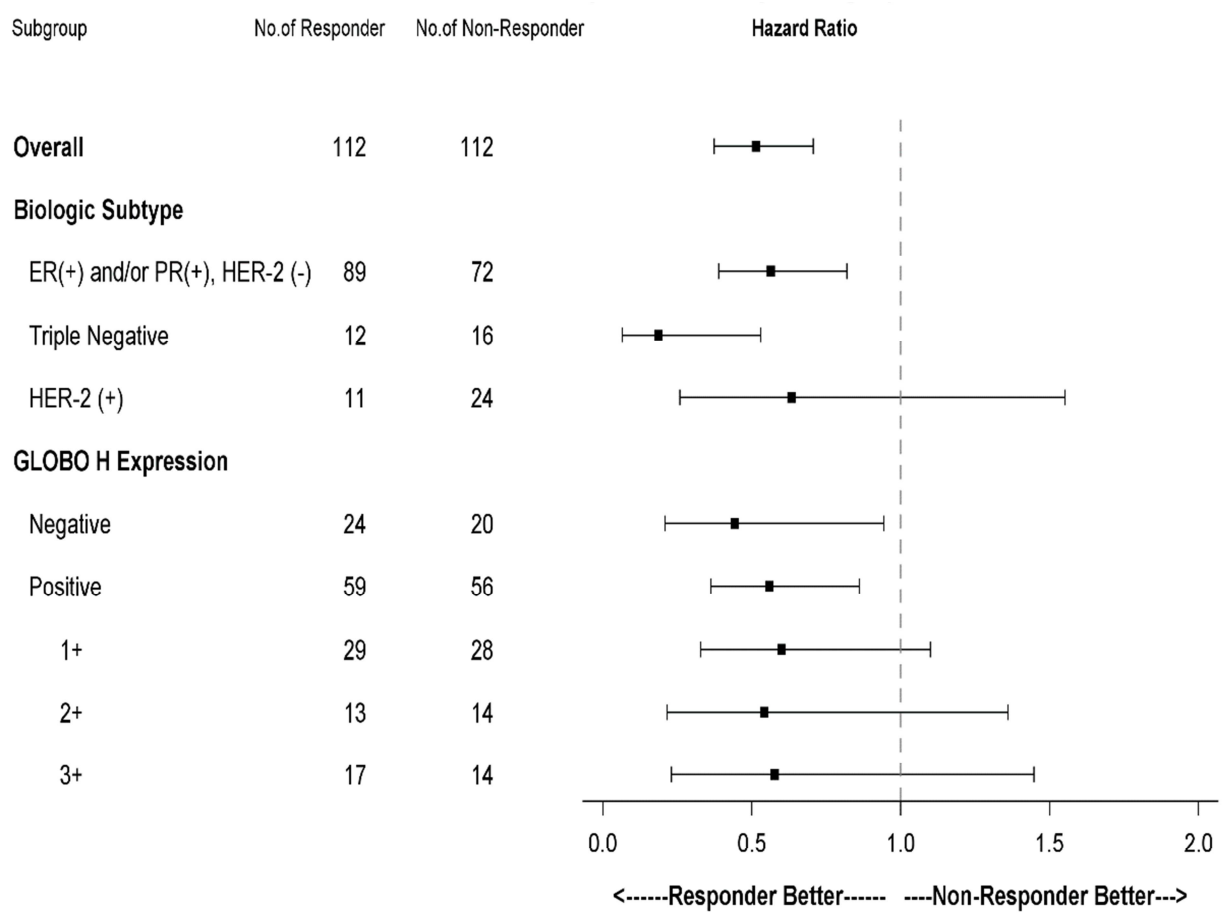
[15] Figure 6 illustrated the Progression-Free Survival (mITT) of Study Drugs Treatment with/without IgG immune response (IgG Criteria 1:640) vs placebo.

[16] Figure 7 illustrated the Overall Survival (mITT) of Study Drugs Treatment with/without IgG immune response (IgG Criteria 1:640) vs placebo.

[17] Figure 8 illustrated the Progression-Free Survival (mITT) after received one injection of Study Drug on Week 2 with/without early IgM immune response (IgM Criteria) vs placebo.

[18] Figure 9 showed there was a high anti-KLH IgG immune response after patients received the injections of OBI-822 vaccine.

[149] Table 2B. PFS OBI-822/821 Immune Responder vs OBI-822/821 Non-Responder



[150] Figures 4 and 5 showed the Progression Free Survival (PFS) after received 9 injections of Study Drugs. It indicated that OBI-822 vaccine could extend the survival rate of breast cancer patients with a longer time.

[151] Figures 6 and 7 showed the Progression Free Survival (PFS)/Overall Survival (OS) after received of Study Drugs with/without IgG immune response (IgG Criteria 1:640). It indicated that OBI-822 vaccine could induce IgG immune response and extend the survival rate of breast cancer patients with a longer time.

[152] Clinical trial data of human Anti-Globo H IgM titer determination by Glycan Array

Background

μg/mL of human IgM. The linear regression of the internal curve of each chip was used to calculate the slope and the intercepts. In certain examples, the Ab level (μg/mL) = [(raw data-intercept)/slope]×0.1.

[157] Figure 8 showed the Progression-Free Survival (PFS) after received one injection on Week 2 of Study Drug with/without early IgM immune response. It indicated that OBI-822 vaccine could induce early IgM immune response and extend the survival rate of breast cancer patients with a longer time.

[158] Demonstration of Efficacy using Clinical trial data Based on Human Anti-Keyhole Limpet Hemocyanin (KLH) IgG Titer Assay

[159] Human Anti-Keyhole limpet hemocyanin (KLH) IgG titer assay was used to show that the immunological-reactions generated by the administration of Globo series antigens vaccine could be induced by the Globo series antigens vaccine, the Globo series antigen and/or the carrier protein.

Reagent/Buffer Preparation

[160] KLH (Sigma-Aldrich, Cat #H1158, ocean harvest, stock concentration 5 mg/mL); Coating buffer: carbonate-bicarbonate buffer pH 9.2 (Sigma-Aldrich, Cat #C3041-50CAP); Secondary antibody: Goat anti-human IgG-HRP (KPL, Cat #474-1002); 10X PBS, pH 7.4 (Gibco, Cat #70011-044); Tween-20 (Sigma-Aldrich, Cat #P2287); TMB Substrate Solution (Clinical, Cat #01016-1-500); Blocking buffer (Sigma-Aldrich, Cat #B6429); PBST: 0.05% tween-20 in PBS; Stop solution: 1N H_2SO_4..

Assay Procedure

[161] KLH was diluted to 4 μg/mL with coating buffer. Fifty microliter of diluted KLH solution was added into each well of a standard well. The plate was incubated at 4 °C overnight. The plates was decanted and washed with PBST at 200 μL/per well for three times. 100 μL blocking buffer was added to each well and incubate at room temperature for 30 minutes. The plate was decanted and washed with PBST 200 μL/per well for three times.

[162] Serum sample dilution: samples were two-fold serial diluted with blocking buffer ranging from about 1000, 2000, 4000, 8000, 16000, 32000, 64000 to 128000 folds (1 μL of serum sample was added to 999 μL blocking buffer to make 1000 fold dilution). 50 μL of samples were added into each well in coated and un-coated plates, then incubated at room temperature for 1.5 hours. The plates were decanted and washed with PBST 200 μL/well for