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PHASE I TRIAL OF A BIVALENT GANGLIOSIDES VACCINE IN COMBINATION WITH β -GLUCAN:

FOR HIGH-RISK NEUROBLASTOMA IN SECOND OR LATER REMISSION

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Abstract

Background—To report on a phase I trial designed to find the maximally tolerated dose in children of the immunological adjuvant OPT-821 in a vaccine containing neuroblastoma-associated antigens (GD2 and GD3) (Clinicaltrials.gov NCT00911560). Secondary objectives were to obtain preliminary data on immune response and activity against minimal residual disease (MRD). Treatment also included the immunostimulant β -glucan.

Methods—Neuroblastoma patients in 2nd complete/very good partial remission received vaccine subcutaneously (weeks 1-2-3-8-20-32-52). Vaccine contained 30 μ g each of GD2 and GD3 stabilized as lactones and conjugated to the immunological carrier protein keyhole limpet hemocyanin; and OPT-821 which was dose-escalated as 50, 75, 100 and 150 μ g/m² per injection. Oral β -glucan (40 mg/kg/day, 14 days on/14 days off) started week 6.

Results—The study was completed with 15 patients because there was no dose-limiting toxicity at 150 μ g/m² of OPT-821 (the dosing used in adults). 13/15 patients received the entire protocol treatment including 12 who remain relapse-free at 24+ to 39+ (median 32+) months and one who relapsed (single node) at 21 months. Relapse-free survival was 80 $\pm$ 10% at 24 months. Vaccine and

β -glucan were well tolerated. 12/15 patients had antibody responses against GD2 and/or GD3. Disappearance of MRD was documented in 6/10 patients assessable for response.

Conclusions—This immunotherapy program lacks major toxicity and is transportable to any outpatient clinic. Patient outcome is encouraging but the efficacy is uncertain because of the complexity and heterogeneity of prior therapies. A larger phase II trial is underway.

Keywords

immunotherapy; gangliosides; vaccine; β -glucan; relapsed neuroblastoma

INTRODUCTION

Relapse of high-risk neuroblastoma (HR-NB) has long been viewed as tantamount to eventual death from progressive disease (PD) or toxicity.¹ We hypothesize that active humoral immunity could maintain second or subsequent remission even among these ultra-high-risk patients. This curative approach starts from diagnosis and has several components. First, patients in initial remission undergo close monitoring, with extent-of-disease evaluations at least every 3 months through 3 years from diagnosis; the aim is to detect focal relapse early, which may be more amenable to successful salvage than a widespread relapse with a large tumor burden.² Second, relapse is treated with a multimodality program that comprises surgery and/or radiotherapy for local control, plus salvage chemotherapy combining agents with well-established anti-NB activity and tailored to the patient's particular clinical context or past treatment history.^{3–8} Third, after achieving a maximal response with the standard modalities (chemotherapy, radiotherapy, surgery), remission is consolidated via immunotherapy using monoclonal antibody (MoAb) or vaccine, the latter being the subject of this report.

The vaccine is designed to induce antibodies against gangliosides GD2 and GD3 which are highly expressed on NB. Indeed, the intensity of ganglioside expression on NB is unique. Whereas melanoma (considered a ganglioside-rich tumor) yields a median of 43 mcg of GD3 per gram of tissue biopsied and 3.1 mcg of GD2, NB yields a similar level of GD3 but 10 $\times$ higher levels of GD2 (44 mcg and 31 mcg, respectively).^{9,10} GD2 and GD3 are also expressed on peripheral nerves and brain, and treatment with anti-GD2/GD3 MoAbs causes pain,^{11–13} but spares the central nervous system, presumably due to the blood-brain barrier.

Preclinical studies and clinical trials of vaccines in adults showed immunogenicity significantly augmented by 1) stabilizing GD2 and GD3 through lactone formation; 2) conjugating the resulting GD2L (L for lactone) and GD3L structures to the immunological carrier protein keyhole limpet hemocyanin (KLH); and 3) combining GD2L-KLH and GD3L-KLH with the immunological saponin adjuvant QS-21.^{14–19} For both GD2L and GD3L, the lowest optimal dose was 30 mcg per vaccine.^{18,19} For QS-21, doses of 50–200 mcg were tested and found safe, with side effects of grade 3 erythema and flu-like symptoms lasting 2–4 days.²⁰ When QS-21 became unavailable, we turned to OPT-821 which is a QS-21 equivalent based on ¹H and ¹³C nuclear magnetic resonance, mass spectrometry, high-performance liquid chromatography studies, and overlapping immunogenicity and toxicity profiles in mice.

We now report a phase I trial involving children with HR-NB in second or later remission, treated with a GD2/GD3 vaccine containing OPT-821. Patients also received β -glucan which has a broad range of immunostimulant effects^{21–23} and synergizes with anti-GD2/GD3 MoAbs in preclinical studies.^{24,25} β -glucans bind to CR3 (Mac-1), a complement receptor for iC3b, widely expressed on leukocytes.²¹ This receptor mediates the diapedesis of leukocytes through the endothelium and stimulates phagocytosis, degranulation, and tumor cytotoxicity. Many bacteria and fungi carry activating ligands for CR3, which renders them vulnerable to phagocytosis by neutrophils. Human tumors lack such molecules and CR3-mediated tumor lysis is suboptimal until it is activated by GM-CSF²⁶ or by ligands such as β -glucan.²⁷

METHODS

The primary objective of the Memorial Sloan-Kettering Cancer Center (MSKCC) protocol 05–075 phase I trial ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00911560) NCT00911560) was to find the maximally tolerated dose (MTD) in children of the immunological adjuvant OPT-821 in a vaccine containing two NB-associated antigens (GD2 and GD3). Secondary objectives were to obtain preliminary data on anti-GD2 and anti-GD3 immune response and anti-NB activity against minimal residual disease (MRD).

Patient selection

This study enrolled patients ≥ 21 years old with HR-NB who had previously relapsed but had achieved a $\geq 2^{\text{nd}}$ complete/very good partial remission (CR/VGPR). Disease status was confirmed ≥ 3 weeks after other therapy by computed tomography, magnetic resonance imaging (MRI), ^{123}I -metaiodobenzylguanidine (MIBG) scan, bone marrow (BM) histology (aspirates and biopsies from bilateral posterior and anterior iliac crests), and urine catecholamines. Using international criteria²⁸ expanded to include ^{123}I -MIBG findings, CR was defined as absence of NB by all studies, and VGPR was defined as the primary mass reduced $\geq 90\%$, no evidence of active distant disease, and urine catecholamines normal. There were no eligibility criteria/limits regarding prior therapy. Major organ toxicity was required to be $\leq$ grade 3, except neurologic status had to be $\leq$ grade 1, by the Common Terminology Criteria for Adverse Events, Version 3.0 (CTCAEv3.0), and the absolute lymphocyte and neutrophil counts each had to be $\geq 500/\mu\text{l}$. Informed written consents for treatment and tests were obtained according to MSKCC Institutional Review Board rules.

Study design

Treatment included 7 subcutaneous injections of the vaccine administered weeks 1–2–3–8–20–32–52 (Figure 1). The vaccine contained 30 μg of GD2L and 30 μg of GD3L, each conjugated to KLH, for all patients, but OPT-821 dosing depended on dosage level and body-surface area. Four OPT-821 dosage levels were planned: 50, 75, 100, and 150 $\mu\text{g}/\text{m}^2$; the dose was “capped” with the maximal dose being 75, 110, 150, or 200 μg at the respective dosage levels.

Oral β -glucan was started week 6 (Figure 1), to allow time for generation of antibodies. Dosage (40 mg/kg/day, 14 days on/14 days off) was identical for all patients and derived

from the phase I MSKCC protocol 05–073 ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00492167) NCT00492167). β -glucan (a botanical extract of sugar polymer consisting of (1→3),(1→6)- β -glycosidic linkage from baker's yeast, manufactured by Biotec Pharmacon ASA Inc., Tromsø, Norway) was supplied as an aqueous solution at 20 mg/ml and stored at room temperature.

Patients continued on study in the absence of relapse (new lesion) and dose-limiting toxicity (DLT). Disease status was assessed every 10–12 weeks by extensive evaluations (see above). Relapse-free survival (RFS) was calculated with the Kaplan-Meier method. Using the CTCAEv3.0, DLT was defined as: grade 2 allergic reaction; grade 2 autoimmune reaction; grade 3 hematologic or non-hematologic toxicity; grade 3 injection site reaction; grade 2 neurotoxicity in patients with no prior neurologic deficit, or worsening of a grade 1 neurotoxicity to grade 2. DLT assessment excluded toxicity related to disease activity, prior therapy, or co-interventions.

Vaccine injections were performed if liver function tests were grade 1 toxicity. For grade 2 or 3 toxicity, injections were deferred until a return to grade 1.

A standard dose-escalation schema was used: escalations were implemented if 0/3 or 1/6 patients at a given dosage level had DLT. At least six patients were studied at the MTD, or at the highest planned level (i.e., 150 mcg/m²) if there were no DLTs.

Materials

GD2 and GD3 were obtained from Matreya Inc. (Philadelphia, PA), with GD2 prepared via extraction of GD1b from rabbit brain followed by treatment with bull testis β -galactosidase,¹⁹ and GD3 extracted from bovine buttermilk.¹⁸ GD2 and GD3 were >95% pure as determined by thin-layer chromatography and immune thin-layer chromatography (ITLC). Clinical grade KLH was acquired from Sigma Chemicals Inc. (St. Louis, MO), and clinical grade OPT-821 from Optimer Pharmaceuticals Inc. (San Diego, CA).

For conjugation of gangliosides to KLH, ozone was used to cleave the ceramide double bond, an aldehyde group was introduced, and this product was coupled to amino groups on KLH by reductive amination as described.¹⁸ The ratio of antigen to KLH in the final conjugates was determined using the resorcinol method for measuring sialic acid and the BioRad dye-binding method. The percent unconjugated antigen in the final vialled conjugates was determined by ITLC using MoAbs against each antigen; <10% unconjugated antigen was permitted. This vaccine was used under an IND held by MSKCC, BBIND 4976.

The GD2-KLH and GD3-KLH were converted to GD2L-KLH and GD3L-KLH by treatment with equal volume of conjugates and glacial acetic acid (vol/vol) x3–4 hours at 37°C and lyophilized, as described.¹⁹ Vaccine was vialled in the MSKCC Clinical Grade Production Facility. Vials were stored at –80°C. GD2L-KLH and GD3L-KLH were vialled together in sterile water containing 33 or 36 μ g of each ganglioside per vial and lyophilized. OPT-821 was vialled in normal saline (NS) at 100 or 200 μ g/0.5 ml, or at 150 μ g/ml.

Vaccine preparation

On the day of vaccination, the prescribed dose +10% of OPT-821 was diluted with NS to a final volume of 1.1 or 1.2 ml for the 33 or 36 µg vials of GD2L-KLH and GD3L-KLH, respectively. The resulting solution was refrigerated at 4°C ×1 hour. For administration, 1 ml (containing 30 µg of GD2L-KLH and of GD3L-KLH) was drawn into a syringe and injected subcutaneously in a single site.

Serology

Anti-GD2 and anti-GD3 antibodies were detected by enzyme-linked immunosorbent assay (ELISA). In brief, 20 ng/well of GD2 or GD3 was coated on CDGH rows of 96-well microtiter plates and air-dried overnight. Blocking step was performed using 200 µl/well of 0.5% bovine serum albumin (BSA) in 1× phosphate-buffered solution (PBS) ×1 hour at room temperature, and washed with 1×PBS. Patient serum diluted in 1:10 and 1:30 in 0.5% BSA in duplicate was added to the designated wells with and without GD2/GD3 at 50 µl/well. For anti-GD2 assay, anti-GD2 antibody Hu3F8-IgG1 was used to generate a standard curve, at concentrations ranging from 1.23-to-100 ng/ml. Both standards and samples were incubated ×2.5 hours at 37°C. After washing with PBS, peroxidase-conjugated mouse anti-human IgG1 (γ specific) at 1:1000 dilution was added at 100 µl/well. Upon incubation at 4°C ×1 hour and washing with PBS, color reaction with chromogen o-phenylenediamine and substrate hydrogen peroxide was added to the plates ×30 minutes at room temperature in the dark. Reaction was stopped using sulfuric acid, and optical density read using ELISA plate reader at 490nm. For GD2 assay, test sera were quantified in ng/ml based on the standard curve; positivity was defined as an increase of at least 10 ng/ml over pretreatment levels. For GD3 assay, sera were defined as positive when titer at 1:10 was greater than 0.1 O.D. after background subtraction.

MRD detection

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used as described^{13,29,30} to assess MRD in heparinized BM aspirates pooled from four sites (2–2.5 ml/site, from bilateral posterior and anterior iliac crests). The MRD marker panel included cyclin D1 (*CCND1*), GD2 synthase (*B4GALNT1*), ISL LIM homeobox 1 (*ISL1*) and paired-like homeobox 2b (*PHOX2B*). β 2 microglobulin (*β 2M*) was used as the endogenous control, and NB cell line NMB7 as the positive control. Each sample was quantified using the comparative CT method as fold-difference relative to NMB7. All gene expression assays were from Applied Biosystems: *CCND1*: Hs00277039_m1; *B4GALNT1*: Hs00155195_m1; *ISL1*: Hs00158126_m1; *PHOX2B*: Hs00243679_m1; *β 2M*: 4326319E. For each marker, positivity was defined as greater than the upper limit of normal. All samples were run in duplicates. MRD panel positivity was defined as any one of 4 markers being positive, and negativity as all 4 markers being negative.

RESULTS

Patient characteristics

The study was completed with 15 patients (enrolled July 2009–November 2010) because there was no DLT and OPT-821 dosing reached that used in adults (Table 1). At enrollment, patients were 3.3–16.7 (median 8.1) years old. All patients had stage 4 NB, 11 were in 2nd and four in 3rd CR/VGPR. Five (33%) patients had *MYCN*-amplified NB. The time from diagnosis to first relapse was <12 months (n=2), 12–18 months (n=5), 19–24 months (n=4), and >24 months (n=4). Retrieval therapy before study enrollment included local control with radiotherapy alone (n=7), radiotherapy plus surgery (n=7), strongly myelosuppressive chemotherapy (n=8),^{5–8} and non-immunosuppressive treatments such as irinotecan-temozolomide (n=9)^{3,4} and anti-GD2 MoAb¹³ (n=5); no patient underwent stem-cell transplantation as part of retrieval. These retrieval treatments were for relapses that were localized in 10 patients, including soft tissue (n=7) or osteomedullary by MIBG and MRI (n=3), and widespread in five patients, including BM by histology and MIBG scan (n=2), multifocal MIBG osteomedullary with (n=1) or without (n=1) soft tissue, and retroperitoneal nodes plus brain (n=1).

Survival and toxicity

13/15 patients received all seven protocol-prescribed injections of vaccine, including 12 patients who remain relapse-free at 21+to-36+ (median 29+) months and one who had a focal relapse (supraclavicular node) at 21 months. Two patients had early focal relapses (2.3 and 4.6 months). All three relapses were among patients receiving 100–150 µg/m² of OPT-821, including one with *MYCN*-amplified and two with *MYCN*-nonamplified NB. RFS was 87±9% at 12 and 80±10% at 24 months (Figure 2). Overall survival was 93±6% (follow-up >25 months). One relapse-free survivor received a cycle of chemotherapy because of radiologic findings deemed suspicious for soft tissue relapse of his *MYCN*-amplified NB; laparotomy revealed no evidence of NB so patient was allowed to resume the vaccine/β-glucan immunotherapy.

Treatment was well tolerated, with no DLT, delays in administration of vaccine, co-interventions, or neurologic sequelae. There was no relation between OPT-821 dosage and side effects. Injections were painful and elicited grade 1 local reactions in 12 patients (varying from 1–4 injections per patient) and a grade 2 local reaction in one patient (dose level 1, with injection #7); these side effects subsided within 24 hours. No other grade 2 toxicities of protocol treatment were noted. One patient had a grade 3 elevation in liver function enzymes on day 5 after injection #2 (dose level 1). The only other grade 3–4 toxicities – all self-limited and attributable to prior therapy or intercurrent events – were concurrent hypocalcemia and hypokalemia (one patient), lymphopenia (one patient), and neutropenia (one patient).

Serological response

Patient serum for assessment of anti-GD2 and anti-GD3 titers was collected before vaccine injection #1 and after the subsequent injections. Seropositive response was defined as negative titer at baseline, and positive titer anytime, whereas seronegative response was

defined as negative titer at baseline and all subsequent time points tested. 3/15 patients had seronegative response. The rest of them became seropositive against GD2 alone (n=2), GD3 alone (n=3), or both (n=7) (Table 1). Anti-GD2 seropositivity was documented after cycles #2 (n=1), #3 (n=2), #4 (n=5), and #7 (n=1). Anti-GD3 seropositivity was documented after cycles #3 (n=2), #4 (n=2), #6 (n=2), and #7 (n=4). Seronegativity did not correlate with relapse.

MRD response

At study enrollment, all 15 patients had CR in BM by histology. Response with respect to MRD was assessed by comparing BM collected at baseline before vaccine injection #1 versus after the last injection. Of 11 patients who were MRD positive at baseline, six became MRD negative, four remained MRD positive, and one patient in continual remission was not re-tested for MRD because there was no available sample (Table 1). Four patients were MRD negative at baseline and remained MRD negative. MRD nonresponse did not correlate with relapse.

DISCUSSION

This phase I trial in patients with HR-NB in 2nd CR/VGPR assessed the toxicity of escalating dosages of the immunological adjuvant OPT-821 in a bivalent vaccine containing GD2L and GD3L, each covalently attached to the immunological carrier protein KLH. The patients also took the oral immunostimulant β -glucan. Because 13/15 study patients received all seven injections of the protocol, the study's primary objective regarding safe dosing of OPT-821 was achieved. Treatments were devoid of acute toxicities aside from local reactions immediately post-injection. No delayed toxicities developed, alleviating concerns about neurotoxicity given GD2/GD3 expression on peripheral nerves and in brain. Prolonged oral intake of β -glucan by the children in this study was uncomplicated, consistent with the absence of major side effects in preclinical animal models and in human adults.

Although the absence of toxicity with the bivalent vaccine and β -glucan appears well-established, anti-NB efficacy should be interpreted with great caution. The study patients achieved excellent RFS (Figure 2), in contrast to dismal predictions based on the international experience with relapsed HR-NB,^{31–33} and without myeloablative therapy with autologous stem-cell transplantation, which is widely used as consolidation of first or subsequent remissions. Yet the contributory role of, for example, β -glucan to RFS is uncertain, and major confounding factors included patient selection and the proximity and complexity of therapy before vaccine treatment. Nevertheless, 12/15 patients mounted an anti-disialoganglioside immune response, and BM MRD response (from positive to negative) occurred in 60% of evaluable patients. All three patients who progressed had CR of BM MRD. It is of note that two patients had isolated progression (one in a neck node and one in left ichium) with continual marrow remission for 17 months and 24+ months. The third patient progressed with a new paraspinal soft tissue mass with only one of six marrow samples turning positive, and had negative marrow aspirates and biopsies (total of 60 samples) alive and well at 41+ months since. The inability of BM MRD to detect extramedullary isolated relapse was analyzed in a recent publication, showing early negative

MRD in BM after immunotherapy was less predictive of late or non-marrow relapse among patients with high-risk stage 4 NB.³⁴

Vaccine therapy in NB has previously focused on T-cell immunity. Syngeneic or allogeneic NB transfected with interleukin-2 can stimulate anti-NB IgG.³⁵ Allogeneic NB transfected with both interleukin-2 and lymphotactin has induced major responses in patients.^{36,37} Dendritic cells expanded in vitro and “pulsed” with crude cell lysates or tumor RNA have also been tested in children.^{38,39} Unfortunately, to date, specific CTLs (e.g., against survivin) have not been shown to home to NB in patients.⁴⁰ Furthermore T-cell immunity has been limited by the state of immunosuppression following dose-intensive chemotherapy. In the current study, the successful induction of anti-GD2 and anti-GD3 antibodies using lactone-KLH in the presence of OPT-821 in patients despite prior history of immunosuppressive chemotherapy is encouraging.

In preclinical models^{41,42} and clinical trials,^{11–13} immunotherapy using anti-GD2 or anti-GD3 MoAbs has shown activity against NB and melanoma. Benefit is optimal in the adjuvant setting, with ablation of micrometastasis.⁴³ The anti-neoplastic mechanisms include opsonization, antibody-dependent cellular cytotoxicity (ADCC), and complement-mediated cytotoxicity (CMC). The ADCC involves leukocytes of all types, including neutrophils, macrophages, and natural killer cells. Our bivalent vaccine is constructed with the purpose of inducing host anti-ganglioside antibodies that can replicate the anti-neoplastic effects of exogenously administered MoAbs.

Various strategies have undergone clinical testing to induce or augment immune-mediated attack against cancer.⁴⁴ Few clinical trials, however, have used humoral vaccines against solid tumors in children, especially with ADCC and CMC as the principal underlying immuno-cytotoxic mechanisms.⁴⁵ Also, an anti-neoplastic role for myeloid cells (e.g., neutrophils) has received scant attention.²⁷ A treatment program combining an anti-NB vaccine with an agent that enhances granulocyte cytotoxicity (i.e., β -glucan) represents a novel strategy to maximize this myeloid effect in the emerging field of immunotherapy.

The setting of this protocol is an ideal model system to assess anticancer vaccine therapy: patients with a low disease burden, but at great risk for relapse; target antigens highly expressed on the cancerous but not normal tissues; immunological adjuvants (KLH and OPT-821) to optimize induction of antibodies against the target antigens (GD2 and GD3); and a nontoxic oral agent (β -glucan) to enhance effector cytotoxicity. The basis for emphasis on multivalent vaccines is tumor-cell heterogeneity, variability of the immune response among patients, and the correlation between the concentration of tumor-selective antibody and effector mechanisms (opsonization, ADCC, and CMC). In the adjuvant setting, prolonged presence of anti-neoplastic antibodies should be more efficient against MRD. Maintenance of antibodies over months or years is more readily achieved with vaccines than MoAbs where repeated administrations are necessary and human anti-mouse or anti-human blocking antibodies can develop. We speculate that the fluctuations of anti-GD2 titer in vaccinated patients could represent multiple idiotypes within an idiotypic network while unimodal anti-GD2 response could represent ineffective idiotypes associated with an

unfavorable outcome.^{46–48} Alternatively, these fluctuations could represent random oscillations of unknown significance.

In conclusion, the bivalent-vaccine/ β -glucan immunotherapy program engenders no major toxicity, even in heavily prior-treated children, and is readily transportable to multiple institutions. Patients have no neuropathic pain, unlike patients receiving intravenous anti-GD2 or anti-GD3 MoAbs. Serological and MRD responses were encouraging. Multiple factors may account for the excellent RFS. This limited experience provides support for pursuing an aggressive curative strategy against relapsed HR-NB. In addition, unlike most pediatric phase I studies, this one could have a major impact on treatment of high-risk NB. Thus, a phase II trial is already in progress, using the phase I study's maximum OPT-821 dosage (150 $\mu\text{g}/\text{m}^2$); the results might support vaccine-glucan as adjuvant after completion of the standard upfront HR-NB multi-modality program. The latter now includes anti-GD2 MoAb¹² but additional non-toxic therapy for MRD is eagerly sought, to help further improve outcome.

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CONFLICTS OF INTEREST

GR is a paid consultant and co-founder of MabVax Therapeutics Inc., which has licensed the KLH-conjugate vaccines from MSKCC. In addition, GR has stock options in Optimer, the company that manufactures OPT-821. GR's role in this trial was limited to preparation of the vaccine in the Clinical Grade Production facility at MSKCC.

β -glucan was licensed to Biotec Pharmacon by MSKCC, and NKC was named as one of the inventors.

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STATEMENT OF TRANSLATIONAL RELEVANCE

In preclinical models and clinical trials, immunotherapy using anti-GD2 or anti-GD3 monoclonal antibodies (MoAbs) has proven beneficial against high-risk neuroblastoma (HR-NB) and melanoma. This article presents results of a phase I study of a vaccine designed to induce host anti-ganglioside antibodies that can replicate the anti-neoplastic activities of intravenously-administered MoAbs. Patients also received β -glucan which synergizes with anti-GD2/GD3 MoAbs. The vaccine/ β -glucan treatment was well tolerated, and serological and minimal-residual-disease (MRD) responses were encouraging. Multiple factors may account for the excellent relapse-free survival despite prior relapse which historically conferred a dismal prognosis. This phase I experience could have a major impact on treatment of HR-NB. Thus, a phase II trial is already in progress; the results might support vaccine/ β -glucan as adjuvant after completion of the standard upfront HR-NB multi-modality program. The latter now includes anti-GD2 MoAb but additional non-toxic therapy for MRD is eagerly sought, to help further improve outcome.

Vac = GD2-GD3 vaccine containing OPT-821

Glucan = beta-glucan from yeast

Figure 1.
Protocol treatment schema.

Figure 2.
Relapse-free survival (red) and overall survival (black) of the 15 study patients from the start of vaccine.

Figure 3.

Antibody response against GD2 in ng/ml (y-axis) after each vaccine cycle number (x-axis) for individual patients who turned seropositive, grouped according to the dose level of OPT-821 (50, 75, 100 and 150 $\mu\text{g}/\text{m}^2$). At 100 $\mu\text{g}/\text{m}^2$ dose level, one patient (dash) had unusually high antibody titer plotted on the secondary y-axis. Patients who did not mount any anti-GD2 antibody response were not included in these plots (1 of 3 patients at 75 $\mu\text{g}/\text{m}^2$ dose level and 4 of 6 patients at 150 $\mu\text{g}/\text{m}^2$ dose level). The three patients who progressed were marked with black arrows.

Table 1

Patients treated with bivalent vaccine and β -glucan

Patient #	Dose level*	MYCN ampli-fied?	Time to 1 st relapse	Sites of relapse	Treatment of relapse	Time from relapse to vaccine	MRD in BM pre/post	Serologic response [§] to: GD2 GD3	Outcome (Time from 1 st vaccine)
Patients in 2nd CR									
1	1	no	11m	tibia	CPT-TMZ(x12), RT, Thalidomide-Celecoxib	26m	+/-	- + (7)	RFS (48m)
2	1	no	15m	brain, abdomen	GTR, CPT, RT, CPT-TMZ(x2), IT-3F8, CIT, oral TMZ(x5), CRA, Ritux-Cyclo	34m	+/n.d.	+ (3) + (7)	RFS (47m)
3	2	no	17m	abdomen	GTR, CPT-TMZ(x5), RT, Lenalidomide-Celecoxib	7m	+/+	+ (4) + (3)	RFS (46m)
4	2	no	23m	abdomen	GTR, CTV(x2), CIT, RT, 3F8, CRA	25m	-/-	- + (4)	RFS (45m)
5	2	no	23m	mandible, skull	ICE, RT, CPT-TMZ(x10), CRA	23m	+/-	+ (4) + (7)	RFS (44m)
6	4	yes	59m	pelvic nodes	GTR, CTV(x3), RT, CPT-TMZ, 3F8, CRA	15m	-/-	- + (6)	RFS (39m)
7	4	no	22m	thorax, bones	CTV(x2), GTR, CIT, CPT-TMZ(x9), RT, Ritux-Cyclo, 3F8, CRA	40m	-/-	- -	RFS (39m)
8	4	yes	30m	femur, BM	CTV(x2), RT, CPT-TMZ(x5), CRA, ABT-751 (x3 yrs)	63m	+/+	+ (4) + (6)	RFS (35m)
9	4	no	8m	paraspinal soft tissue, rib	CAV(x3), P/E(x2), GTR, CTV, RT, 3F8, CRA	19m	+/-	- -	RFS (33m)
10	4	no	12m	skull	CPT-TMZ(x7), RT, Ritux-Cyclo	6m	+/+	- -	RFS (33m)
11	4	yes	26m	sphenoid, BM	CTV(x2), ICE, RT, Bevacizumab-CPT-TMZ(x5)	9m	+/-	+ (4) + (3)	relapse (ischium; 2m). DoD (26m)
Patients in 3rd CR									
12	1	yes	15m	thorax	GTR, RT, CPT/TMZ(x5), 3F8, CRA, Thalidomide-Celecoxib	15m	-/-	+ (3) + (7)	RFS (48m)
13	3	no	47m	thorax	Cyclo-Topo(x8), Lenalidomide-Celecoxib	15m	+/-	+ (2) -	relapse (BM, paravertebral; 5m); alive in CR (42m)
14	3	yes	21m	tibia	CCV(x2), RT, Cyclo-Topo(x2), Ritux, Lenalidomide-Celecoxib	10m	+/+	+ (4) + (4)	RFS (40m)
15	3	no	17m	R supra-clavicular	¹³¹ I-MIBG, RT	3m	+/-	+ (7) -	relapse (neck; 21m); alive in CR (42m)

BM, bone marrow; CAV, high-dose Cyclo-doxorubicin-vincristine; CCV, high-dose Cyclo-CPT-vincristine;⁷ CIT, high-dose carboplatin-CPT-TMZ;⁶ CPT, irinotecan; CR, complete remission; CRA, 13-*cis*-retinoic acid; CTV, high-dose Cyclo-Topo-vincristine;⁵ Cyclo, cyclophosphamide; DoD, died of disease; GTR, gross total resection; ICE, high-dose ifosfamide-carboplatin-etoposide;⁸ IT-3F8, intrathecal ¹³¹I-3F8;⁴ MRD, minimal residual disease; n.d., not done; P/E, cisplatin-etoposide; RFS, relapse-free survival; Ritux, rituximab; RT, local radiotherapy; TMZ, temozolomide; Topo, topotecan

* OPT-821 dose level 1=50 $\mu\text{g}/\text{m}^2$, dose level 2=75 $\mu\text{g}/\text{m}^2$, dose level 3=100 $\mu\text{g}/\text{m}^2$, dose level 4=150 $\mu\text{g}/\text{m}^2$

[§]Number of prior vaccine injections on this protocol is in parentheses