Long-term Outcomes of Patients With Recurrent Ovarian Cancer Treated With a Polyvalent Vaccine With Bevacizumab Combination

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Abstract

Background: To characterize the safety, immunogenicity, and outcomes of patients with high-grade serous ovarian cancer (HGSOC) in second or greater remission treated with a polyvalent antigen-KLH plus OPT-821 vaccine construct and bevacizumab.

Methods: Patients with recurrent HGSOC were treated with the vaccine plus bevacizumab at our institution from 01/05/2011-03/20/2012. Follow-up continued until 03/2021. Blood/urine samples were collected. “Responders” had an immunogenic response to ≥3 antigens; “non-responders” to ≤2 antigens.

Results: Twenty-one patients were treated on study. One developed a dose limiting-toxicity (grade 4 fever). Two (10%) experienced bevacizumab-related grade 3 hypertension. Thirteen (68%) and 16 (84%) of 19 responded to ≥3 and ≥2 antigens, respectively (Globo-H, GM2, TF cluster Tn, MUC-1). Four of 21 patients were alive >5 years post-treatment. Responders and non-responders had a median PFS of 4.9 months (95% CI: 2.8-8.1) and 5.0 months (95% CI: 0.7-cannot estimate), respectively; median OS was 30.7 months (95% CI: 16.9-52.0) and 34.2 months (95% CI: 12.8-cannot estimate), respectively. On two-time point analysis (baseline, week 17), increased IL-8 exhibited improved PFS (HR as 10-unit increase, 0.43; p=0.04); increased PDGF exhibited worse OS (HR as 10-unit increase, 1.01; p = 0.02).

Conclusions: This is the longest follow-up of vaccine administration with bevacizumab among patients with ovarian cancer. The vaccine was well tolerated with bevacizumab. Response was not associated with improved survival. On two-time point analysis, increased IL-8 was associated with significant improvement in PFS; increased PDGF with significantly worse OS. For all time point measurements, cytokine levels were not significantly associated with survival.

Trial registration: NCT01223235

Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy due to its typically late stage at presentation and high predilection for metastases and recurrence [1]. Aberrant immunologic responses have been shown to contribute to the invasion and progression of EOC [2]. Greater than 80% of women diagnosed with EOC will relapse following primary treatment [3]. Many patients will have some degree of response to subsequent chemotherapy. Subsequent remissions are of progressively shorter duration, however, until chemotherapy resistance generally develops. This is believed to have a correlation with host immune responses and tumor genomic factors [4, 5]. Because of this, the immune environment remains a key target for therapies in both the maintenance and recurrent settings. The mechanisms to explain the vast differences in downstream ovarian cancer recurrence outcomes among patients with similar histology remains unknown, however, and requires further evaluation.

Previous studies have demonstrated the presence of antitumoral T cells in the ovarian cancer microenvironment [6, 7]. We previously completed a phase I safety and immunogenicity study of the

polyvalent vaccine-KLH conjugate [GM2, Globo-H, Le^y, Tn-MUC1, Tn(c), STn(c) and TF(c)] plus immunological adjuvant QS-21 in patients with epithelial ovarian, fallopian tube, or peritoneal cancer in a first, second, or later complete clinical remission [8]. We subsequently conducted a follow-up phase II randomized, double-blind trial of a polyvalent vaccine-KLH conjugate + OPT-821 versus OPT-821 alone (Gynecologic Oncology Group [GOG]255) [9]. The results from GOG255 demonstrated that vaccine immunotherapy with the addition of an adjuvant was well tolerated, with mild toxicity largely confined to injection site reactions, and greater immunogenicity (compared to vaccine alone) against five of the antigens contained in the vaccine (Globo-H, GM2, MUC1-TN, MUC1, and TF). However, there were no differences in progression-free survival (PFS) or overall survival (OS) between vaccine-KLH conjugate plus OPT-821 versus OPT-821 alone [9]. GOG255 results were published with a median follow-up of 34 months; a later analysis performed in 2018 had a median follow-up of 60 months [9].

The efficacy of anti-angiogenic agents in the treatment of ovarian cancer has also been demonstrated [10, 11]. The AURELIA study (NCT00976911) showed that the addition of bevacizumab to chemotherapy in platinum-resistant ovarian cancer increased the median PFS from 3.4 to 6.7 months [12]. The combination compared with chemotherapy alone also resulted in increased response rates and improved quality of life. In addition to modulation of the tumor vasculature, vascular endothelial growth factor (VEGF) has been shown to suppress dendritic cell maturation and alter antigen presentation. It also exhibits a host of other influences on immune activity, providing a rationale for combining anti-VEGF therapy with vaccination as a source of immune stimulation [13].

The future direction of ovarian cancer immunotherapies will require the detection of specific biomarkers to predict the effectiveness of different treatment combinations while also minimizing toxicities [14]. The goal of this study is to evaluate both the short-term and delayed immunologic responses, as well as clinical outcomes, in patients with recurrent EOC after receiving a polyvalent vaccine and bevacizumab combination.

Materials And Methods

This study was approved by the Institutional Review Board at Memorial Sloan Kettering Cancer Center (MSK).

Eligibility Criteria

Eligible patients had histologically documented EOC arising in the ovary, fallopian tube, or peritoneum, with stage II-IV disease at diagnosis after second or subsequent clinical remission. Primary treatment must have included cytoreductive surgery and a platinum-based chemotherapy regimen. Eligible patients had relapsed at least once, had completed chemotherapy and/or surgery, and were deemed suitable to enter a period of observation. Patients could have asymptomatic residual measurable disease on computed tomography (CT) scan or be in complete clinical remission. Complete clinical remission was defined as serum CA-125 ≤35 IU/mL, negative physical examination, and no evidence of disease on CT of

the abdomen and pelvis. Patients had to be longer than 4 weeks out from completion of prior cytotoxic chemotherapy. Other requirements included Karnofsky Performance Status (KPS) ≥70; adequate organ function, defined as absolute neutrophil count ≥1000 cells/mm^3; platelets ≥100,000 cells/mm^3; serum creatinine ≤1.5 times the institutional upper limits of normal; and total bilirubin, AST and alkaline phosphatase ≤2.5 times the institutional upper limits of normal. Patients were ineligible if they were of child-bearing potential, had a known autoimmune disease or immune deficiency, a known allergy to seafood, history of myocardial infarction within 6 months, untreated brain metastasis, uncontrolled hypertension, or other standard contraindications to receiving bevacizumab therapy.

Treatment Plan

The administered polyvalent vaccine contained GM2 (30 μg, equivalent to antigen concentration in GM2-KLH conjugate), Globo-H (30 μg), Tn-MUC1 (3 μg) and TF(c) (3 μg), individually conjugated to KLH (600 μg) and mixed with adjuvant OPT-821 (100 μg) in 1.0 mL normal saline as the diluent. The vaccine was administered in 1cc total volume subcutaneously at weeks 1, 2, 3, 7, 11, and 17. Bevacizumab was given via intravenous infusion at 7.5 mg/kg over 20-30 minutes once every 2 weeks, beginning on day 1 of week 1 through week 11, and once every 3 weeks thereafter. Bevacizumab could be continued until disease progression or toxicity.

Vaccine Preparation

The antigen conjugation method and administration protocol was previously referenced in GOG255 [9]. The IND was held by MSK. The following antigens were conjugated to KLH (carrier protein) mixed with OPT-821 adjuvant: Tn glycosylated MUC-1 (Tn-MUC1) was synthesized by Pepceuticals Ltd (Leicester, UK). The Globo-H hexasaccharide-KLH was synthesized and conjugated under good manufacturing practices (GMP) by Optimer Pharmaceuticals Inc. (San Diego, CA). GM2: Ganglioside GM2 was extracted by Matreya Inc. (Philadelphia, PA). It was conjugated to KLH by Althea Technologies (San Diego, CA). TF(c): TF cluster was synthesized by the MSK Organic Synthesis Core. Tn-MUC1-KLH, TF(c)-KLH conjugates were prepared by the MSK Clinical Grade Production (CGP) facility under GMP. OPT-821 is an immunological adjuvant obtained from Optimer Pharmaceuticals Inc. (San Diego, CA). Vaccines and OPT-821 were vialed and released for clinical use by the CGP Core facility.

Dose Adjustment

Dose reduction or delay of vaccine was not permitted. Delays in bevacizumab were permitted in the event of toxicity, with a maximum allowable treatment interruption of 8 weeks. There was no dose reduction of bevacizumab. Toxicity was evaluated according to the National Cancer Institute CTCAE scale version 4 [15]. Patients were to be removed from the study for a vaccine-related dose-limiting toxicity (DLT), defined as grade ≥2 allergic reaction, grade ≥2 autoimmune reaction, grade ≥3 hematologic or non-hematologic toxicity including fever, or grade ≥3 injection site reaction. Any patient with grade 2 or greater toxicity was followed with appropriate studies until results returned to baseline. Patients were removed from the study for progression of disease*, as defined by RECIST [16].*

Evaluation During Study

Pretreatment evaluation included a complete medical history, physical and radiologic examination (CT), vital signs, KPS assessment, and clinical laboratory tests, including hematologic, biochemistry, CA-125, and immunologic testing. Patients had repeat complete blood count (CBC) and comprehensive biochemistry panel at regular intervals, and at the off-study visit. Urine protein was obtained and followed at regular intervals. CT imaging was performed every 3 months while on study, or sooner to evaluate patients if signs or symptoms, blood tests, or physical examination suggested progression of disease. Serologic IgM and IgG antibody responses were measured by ELISA against each antigen at baseline and week 7. The correlative multiplex panels were measured at weeks 1, 17, and off-study.

Antibody Response Against Vaccine Antigens

The IgM and IgG antibody responses were measured by ELISA in duplicate against each antigen as described earlier [8]. The criteria for immunogenicity used was based on that of the individual pilot trials: patients must have had IgM/IgG titer >1:40 or an eightfold increase in prevailing antibody titer if present at baseline. These criteria were based on our previous studies [8]. The antibody titer was defined as the highest serum dilution showing an absorbance of ≥0.1 optical density (a measurement of percent transmission). ”Responders” were defined as patients with an immunogenic response to ≥3 antigens; ”Non-Responders” were defined as patients with an immunogenic response to ≤2 antigens.

Multiplex Assays

A multiplex angiogenesis assay panel was performed to include monoclonal antibodies specific for interleukin-8 (IL-8), VEGF, platelet-derived growth factor (PDGF), and fibroblast growth factor-basic (FGF-basic).

Statistical Considerations

The primary endpoint of this pilot trial was safety; the secondary endpoint was to measure patient immunogenicity following administration of the polyvalent vaccine in combination with bevacizumab. No clinically significant systemic toxicity was associated with administration of similar vaccines. Twenty-one patients would be accrued, and if >8 of 21 patients met the criteria for immunogenicity (described above) for three or more antigens, the study would be considered positive [17]. This calculation assumes that the probability of immune response under the null hypothesis (i.e., no activity) is 0.2 versus the alternative hypothesis (i.e., target response probability) of 0.5. Type I and Type II errors were set to 0.1. In prior trials, antibodies were generally present by the completion of the fourth vaccination (week 7).

PFS was measured from the start of vaccine therapy until progression, and OS was measured from the start of vaccine therapy until death or last follow-up (for only one patient). The associations between cytokine measurements between baseline and week 17 or at different time points, and the PFS/OS, were analyzed using time-dependent Cox proportion hazard (PH) models. The PFS/OS by antibody response versus non-response for the five vaccine antigens, as well as the responder versus non-responder

evaluation, was analyzed using landmark analyses with landmark time as months (chosen by the longest interval post vaccination antibody IgG/IgM titers).

Antibodies against the individual antigens were studied by ELISA. All p-values are two-sided, with statistical significance evaluated at the 0.05 alpha level. Ninety-five percent confidence intervals (95% CI) for all parameters of interest were calculated to assess the precision of the obtained estimates. All analyses were performed with the use of R statistical software version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient Characteristics

Twenty-one patients with recurrent high-grade serous ovarian carcinoma were enrolled on the study between 01/05/2011 and 03/20/2012. All 21 patients were included in the safety analysis. Follow-up for survival was continued until 03/2021. Patient characteristics are described in **Table 1**. Patients had a median age of 56 years (range, 49-70 years). Twenty tumors were adnexal in origin, and one was of peritoneal origin. All patients had KPS ranging from 90-100%. The majority of patients (71%) were in complete remission at study entry. Patients were relatively heavily pretreated—38% were in a third, 38% in a fourth, and 14% in a fifth remission. Three patients had a deleterious *BRCA* mutation (2 *BRCA1*, 1 *BRCA2*). One patient was found to have a *TSC2* mutation post-treatment. The median number of systemic treatment regimens administered to patients after this vaccine study was five (range, 0-7). Total systemic treatments after vaccine therapy included platinum-based chemotherapy (23/92, 25%), taxane-based chemotherapy (18/92, 20%), bevacizumab (14/92, 15%), gemcitabine (13/92, 14%), liposomal doxorubicin (12/92, 13%) and poly-adenosine ribose phosphorylase (PARP) inhibitors (8/92, 9%). Thirty-two percent of regimens were combination therapies (30/92, 32%) **(Table 2).**

Adverse Events

One patient experienced a dose-limiting toxicity—a grade 4 fever (40.1°C for 48 h)—following vaccination. Two patients (10%) developed grade 3 hypertension related to bevacizumab; one of these patients had uncontrolled hypertension despite pharmaceutical management and was removed from the study 7 months after the start date due to excessive toxicity. One patient developed grade 3 hyperglycemia, not attributed to the vaccine or bevacizumab. Otherwise, side effects were self-limited; mild fatigue, fever, myalgia, and localized injection site reactions were the most common. **Table 3** depicts the maximum toxicity for treatment-related events with vaccine and bevacizumab. No clinically relevant hematologic abnormalities were noted. There was no clinical or laboratory evidence of autoimmunity. The vaccine was well tolerated overall.

Immune Response

The predefined definition of immunogenicity to the vaccine was met in 13 of 19 evaluable patients with response to at least 3 of the 5 vaccine antigens (**Table 4)**, and in 16 of 19 patients with response to at least 2 antigens. Two patients were excluded from the immunogenicity analysis due to a lack of post-intervention antibody results. Twelve of 19 patients exhibited an antibody response for GM2; 15/19 for Globo-H; 1/19 for Tn; 12/19 for TF; and 14/19 for MUC1. There was no significant difference between antigen antibody response and cytokine level when segregating by immune responders for each antigen.

Survival Outcomes

The median PFS for the overall cohort was 6.0 months (95% CI: 2.5-7.1 months). The median OS for the overall cohort was 34.6 months (95% CI: 20.1-53). The 5-year OS rate was 23.8% (8.7-43.1%). Among the 4 patients who were alive beyond 5 years, 2 had *BRCA*-associated ovarian cancer (50%). There was no median follow-up time for the cohort, as all patients on trial were followed until their death; one patient who was still alive at her last follow-up date was followed for 112.5 months.

Survival Outcomes by Antibody Response

Responders and non-responders had a median PFS of 4.9 months (95% CI: 2.8-8.1) and 5.0 months (95% CI 0.7-cannot estimate), respectively, and 6-month PFS rates of 30.8% (95% CI: 9.5-55.4%) and 16.7% (95% CI: 0.8-51.7%), respectively **(Table 5).** Responders and non-responders had-a-median OS of 30.7 months (95% CI: 16.9-52.0) and 34.2 months (95% CI: 12.8-cannot estimate), respectively, and 5-year OS rates of 15.4% (95% CI: 2.5-38.8%) and 16.7% (95% CI: 0.8-51.7%), respectively **(Figure 1).**

The median OS for patients with a response to GM2 was 29.1 months, compared with 35.3 months for those without a response (p=0.327). The median OS for patients with a Globo-H response was 35.3 months, compared with 31.9 months for those without a response (p=0.38). The median OS for patients with a Tn response was 33.1 months, compared with 33.0 months for those without a response (p=0.793). The median OS for patients with a TF response was 30.6 months, compared with 42.6 months for those without a response (p=0.156). The median OS for patients with a MUC-1 response was 38.9 months, compared with 30.6 months for those without a response (p=0.94).

Survival Outcomes by Cytokine Measurement

When comparing two time point measurements (baseline and week 17), increased IL-8 levels had a moderately significant association with improved PFS (HR as 10-unit increase: 0.43; 95% CI: 0.19-0.97; p=0.04). Increased levels of PDGF had a moderately significant association with worse OS (HR as 10-unit increase: 1.01; p=0.02). When considering all time point measurements (baseline until progression for PFS or baseline until last measurements for OS), cytokine levels were not significantly associated with survival outcomes (**Table 5**).

Discussion

This study includes the longest prospective follow-up of patients with ovarian cancer who have received vaccine immunotherapy. It is also the first study to report on the safety of polyvalent antigen-KLH plus OPT-821 vaccine construct administered in combination with bevacizumab. The rate of immunogenicity was qualitatively similar to that seen in our prior study, which did not include the addition of bevacizumab. In the current study, 68% and 84% of patients responded to ≥3 and ≥2 antigens, respectively, compared with 89% and 89%, respectively in our prior phase I trial without bevacizumab [8]. Given the potential immune-modulating effects of bevacizumab and the GOG255 study (NCT00857545), which evaluated the vaccine in settings where bevacizumab may have been used, it was necessary to ascertain whether there would be toxicity-related adverse effects due to the addition of bevacizumab. No additional toxicities were observed in this study. Measured cytokines in the multiplex assay likewise showed no changes between baseline and week 17, or when values were considered at each time point until progression of disease or last follow-up. The median PFS from the start of preceding chemotherapy was 13 months (95% CI: 10-20 months), within the reported range of progression in this population, in patients receiving maintenance bevacizumab therapy in the platinum-sensitive setting, as seen in the OCEANS study [18].

Four patients (19%) were alive more than 5 years after the study intervention; and 3 (14%) were alive after more than 6 years. One patient treated on study in second remission was still alive at her most recent follow-up, 9 years after the start of the trial. Patients with an antibody response against Globo-H compared to those without a response had a median OS of 35.3 versus 31.9 months, respectively; patients with response against MUC-1 compared to those without had an OS of 38.9 versus 30.6 months, respectively. These findings were not significant, which could be attributed to the small sample size. We did not identify predictors of survival. Although we observed antibody responses to the vaccine antigens, we were not able to conclude that such responses induced protective immune responses. Unfortunately, our correlative studies did not include T-cell response profiling.

Ovarian cancer is a notoriously aggressive malignancy in part due to its tumor microenvironment and interaction with host immunity. Previous studies have suggested that FGF and FGF-1 play a crucial role in the progression of ovarian cancer. A 2017 study by Sun et al. found that FGF increased cellular proliferation, migration, and invasion while also regulating protein kinase pathways, resulting in epithelial-to-mesenchymal transition [1]. Similarly, PDGF has also been implicated in the progression of ovarian cancer cells. Matei et al. conducted one of the first studies to demonstrate a strong correlation between PDGF expression and VEGF in EOC; in PDGFR-expressing immortalized ovarian cancer cells, PDGF stimulated the expression of VEGF in association with the PI3K/Akt pathway [19]. IL-8 has also been shown to be elevated in ovarian cyst fluid, ascites, serum, and tumor tissue in ovarian cancer. Wang et al. demonstrated that IL-8 secretion by ovarian cancer cells increases proliferation through alteration of Cyclin D1/B1 proteins and invasion, angiogenic potential, adhesion, and invasion by correlating with MMP-2/MMP-9 activity and expression [20]. Globo-H, a glycosphingolipid of the globo series and a sugar terminus mimicking the antigen H determinant, has also been associated with EOC stem cells, angiogenesis, and an immunosuppressor through Notch signaling [21]. Lastly, microbial glycans, TF and Tn, which are widely expressed in adenocarcinomas, have been shown to likely play a role in self versus

altered-self immunoreactivity [22]. Given these previous findings, antibodies against different cancer-associated antigens are desired targets when generating therapeutic cancer vaccines.

The current study has several limitations. This was a small sample size of 21 women diagnosed with recurrent EOC. It is not possible to ascertain whether individuals with *BRCA* mutations had prolonged survival due to immunoreactivity following the vaccine or disease characteristics secondary to their BRCA mutation status. While our study evaluated associations and trend levels, causality cannot be determined.

Since the inception of this study utilizing antibodies as the primary effectors, the field of immune modulation has continued to expand with the use of checkpoint inhibitors, as well as a variety of other agents, such as OX40 and ICOS. In addition, the evidence base suggesting synergy between bevacizumab and other immunomodulators continues to grow [13]. Given the putative mechanisms of bevacizumab with respect to the immune response, further study with vaccines or other mechanisms prompting T-cell effector proliferation or an integrated response (rather than antibodies alone, as seen in the vaccination here) is critical.

This study includes the longest reported follow-up of patients with ovarian cancer undergoing vaccine therapy. Bevacizumab and polyvalent-KLH vaccine can be safely administered together, with retention of vaccine immunogenicity. Increased levels of IL-8 were associated with a moderately significant improvement in PFS; increased levels of PDGF were associated with a moderately significant improvement in OS. When considering all time point measurements, cytokine levels were not significantly associated with survival outcomes.

Declarations

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References

1. Sun Y, Fan X, Zhang Q, Shi X, Xu G, Zou C. Cancer-associated fibroblasts secrete FGF-1 to promote ovarian proliferation, migration, and invasion through the activation of FGF-1/FGFR4 signaling. Tumour Biol. 2017 Jul;39(7).

2. Li, S., et al., Cancer immunotherapy via targeted TGF-β signalling blockade in T(H) cells. Nature, 2020. **587**(7832): p. 121-125.

3. Dood, R.L., et al., Defining Survivorship Trajectories Across Patients With Solid Tumors: An Evidence-Based Approach. JAMA Oncology, 2018. **4**(11): p. 1519-1526.

4. Markman, M., et al., Duration of Response to Second-Line, Platinum-Based Chemotherapy for Ovarian Cancer: Implications for Patient Management and Clinical Trial Design. J Clin Oncol, 2004. **22**(15): p. 3120-3125.

5. Anderson, K.S., et al., p53 autoantibodies as potential detection and prognostic biomarkers in serous ovarian cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 2010. **19**(3): p. 859-868.

6. Zhang, L., et al., Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med, 2003. **348**(3): p. 203-13.

7. Coukos G, Tanyi J, Kandalaft LE. Opportunities in immunotherapy of ovarian cancer. Ann Oncol. 2016 Apr;27 Suppl 1(Suppl 1):i11-i15. .

8. Sabbatini, P.J., et al., Pilot study of a heptavalent vaccine-keyhole limpet hemocyanin conjugate plus QS21 in patients with epithelial ovarian, fallopian tube, or peritoneal cancer. Clin Cancer Res, 2007. **13**(14): p. 4170-7.

9. O'Cearbhaill RE, Deng W, Chen LM, Lucci JA 3rd, Behbakht K, Spirtos NM, Muller CY, Benigno BB, Powell MA, Berry E, Tewari KS, Hanjani P, Lankes HA, Aghajanian C, Sabbatini PJ. A phase II randomized, double-blind trial of a polyvalent Vaccine-KLH conjugate (NSC 748933 IND# 14384) + OPT-821 versus OPT-821 in patients with epithelial ovarian, fallopian tube, or peritoneal cancer who are in second or third complete remission: An NRG Oncology/GOG study. Gynecol Oncol. 2019 Dec;155(3):393-399.

10. Burger, R.A., et al., Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a Gynecologic Oncology Group Study. J Clin Oncol, 2007. **25**(33): p. 5165-71.

11. Garcia, A.A., et al., Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the California, Chicago, and Princess Margaret Hospital phase II consortia. J Clin Oncol, 2008. **26**(1): p. 76-82.

12. Pujade-Lauraine, E., et al., Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial. J Clin Oncol, 2014. **32**(13): p. 1302-8.

13. Li, Y.L., H. Zhao, and X.B. Ren, Relationship of VEGF/VEGFR with immune and cancer cells: staggering or forward? Cancer Biol Med, 2016. **13**(2): p. 206-14.

14. Odunsi K. Immunotherapy in ovarian cancer. Ann Oncol. 2017 Nov 1;28(suppl_8):viii1-viii7. doi: 10.1093/annonc/mdx444.

15. Institute, N.C., Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0. U.S. Department of Health and Human Services, 2009.

16. Eisenhauer, E.A., et al., New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer, 2009. **45**(2): p. 228-47.

17. Yao, T.J., C.B. Begg, and P.O. Livingston, Optimal sample size for a series of pilot trials of new agents. Biometrics, 1996. **52**(3): p. 992-1001.

18. Aghajanian, C., et al., OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol, 2012. **30**(17): p. 2039-45.

19. Matei D, Kelich S, Cao L, Menning N, Emerson RE, Rao J, Jeng MH, Sledge GW. PDGF BB induces VEGF secretion in ovarian cancer. Cancer Biol Ther. 2007 Dec;6(12).

20. Wang Y, Xu RC, Zhang XL, Niu XL, Qu Y, Li LZ, Meng XY. Interleukin-8 secretion by ovarian cancer cells increases anchorage-independent growth, proliferation, angiogenic potential, adhesion and invasion. Cytokine. 2012 Jul;59(1):145-55.

21. Pochechueva, T., Alam, S., Schötzau, A. et al. Naturally occurring anti-glycan antibodies binding to Globo H-expressing cells identify ovarian cancer patients. J Ovarian Res 10, 8 (2017).

22. Smorodin EP, Sergeyev BL. The level of IgG antibodies reactive to TF, Tn and alpha-Gal polyacrylamide-glycoconjugates in breast cancer patients: relation to survival. Exp Oncol. 2016 Jun;38(2):117-21.

Tables

Table 1: Baseline patient demographics (N=21)

Variable	No. of Patients
Age, median (range), years	56 (49-70)
FIGO stage at diagnosis	
≤II	1 (4.8%)
III	14 (67%)
IV	6 (29%)
Cancer type	
Ovarian	18 (86%)
Primary peritoneal	1 (4.8%)
Fallopian tube	2 (9.5%)
Karnofsky Performance Status, median (range)	90 (90-100)
Remission at Start of Study	
Second	2 (9.5%)
Third	8 (38%)
Fourth	8 (38%)
Fifth	3 (14%)
Complete	15 (71%)
Partial	6 (29%)
Platinum sensitivity status	
Platinum sensitive	18 (86%)
Platinum resistant	3 (14%)
Penultimate treatment prior to vaccine*	
Platinum	10 (48%)
Taxane	3 (14%)
Gemcitabine	8 (38%)
Bevacizumab	4 (19%)
Cyclophosphamide	1 (4.8%)
Pegylated liposomal doxorubicin	5 (24%)
Pemetrexed	1 (4.8%)

Topotecan	1 (4.8%)
Histologic subtype	
High-grade serous carcinoma	21(100%)
Germline mutation status	
Wild type	18 (85.7%)
***BRCA1* variant**	2 (9.5%)
***BRCA2* variant**	1 (4.8%)

FIGO, International Federation of Gynecology and Obstetrics

*Given in combination for select patients

Table 2: Patient outcomes following vaccine therapy

Patient	Subsequent systemic regimens following vaccine	OS from diagnosis (months)	OS from trial (months)
1	4	97	53
2	0	119	14
3	5	135	59
4	6	131	112
5	7	122	52
6	6	85	27
7	5	131	88
8	7	62	20
9	5	109	73
10	7	127	52
11	6	72	29
12	5	26	34
13	1	56	18
14	1	60	32
15	2	52	32
16	3	36	15
17	7	59	36
18	2	31	9
19	4	80	43
20	7	85	62
21	2	37	19

OS, overall survival

Table 3: Patients per maximum toxicity grade for treatment-related adverse events with vaccine and bevacizumab (N=21)

Treatment-Related Adverse Events	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)
Leukopenia	2 (9.5%)	0	0
Anemia	1 (4.8%)	0	0
Hyperglycemia	0	1 (4.8%)	0
Amylase	2 (9.5%)	0	0
Bilirubin	1 (4.8%)	0	0
Fever	2 (9.5%)	0	1 (4.8%)
Hypertension	0	2 (9.5%)	0
Diarrhea	1 (4.8%)	0	0
Nausea/anorexia	1 (4.8%)	0	0
Injection site reaction	3 (14%)	0	0
Headache	1 (4.8%)	0	0
Epistaxis/nasal congestion	1 (4.8%)	0	0

Table 4: Time-dependent analysis for immunology response and response summary

Time-dependent analysis for immunology response (includes either IgM or IgG)

	HR (95%CI)	Cox PH p-value	Response* No. of patients	Non-response* No. of patients
GM2	0.96 (0.37 - 2.45)	0.925	12	7
Globo-H	0.69 (0.23 - 2.03)	0.496	15	4
Tn	0.78 (0.10 - 6.06)	0.816	1	18
TF	2.33 (0.80 - 6.79)	0.123	12	7
MUC-1	0.89 (0.33 - 2.39)	0.816	14	5
Response summary				
	No. of patients			
Response to 5 vaccine antigens	0 (0%)			
Response to 4 vaccine antigens	6 (32%)			
Response to 3 vaccine antigens	7 (37%)			
Response to 2 vaccine antigens	3 (16%)			
Response to 1 vaccine antigen	3 (16%)			

*(N=19, 2 patients removed given lack of serologic testing)

** Cytokines include Globo-H, GM2, MUC1-TN, MUC-1, and TF).

Table 5. Overall survival and progression-free survival by antibody response status and time-dependent cytokine measurements

Overall survival by antibody response status

Variable	n	Deaths	Median OS	5-Year OS Rate	HR	p-value
Non-Responder	6	5	34.2 (95% CI: 12.8-NE)	16.7% (95% CI: 0.8-51.7%)	1.0	0.62
Responder	13	13	30.7 (95% CI: 16.9-52)	15.4% (95% CI: 2.5-38.8%)	1.31 (95% CI: 0.46-3.70)	

Progression-free survival by antibody response status

Variable	n	Progression	Median PFS	6-Month PFS Rate	HR	p-value
Non-Responder	6	6	5.0 (95% CI: 0.7-NE)	16.7% (95% CI: 0.8-51.7%)	1.0	0.84
Responder	13	13	4.9 (95% CI: 2.8-8.1)	30.8% (95% CI: 9.5-55.4%)	0.9 (95% CI: 0.34-2.43)	

Overall survival by time-dependent cytokine measurements

Variable	Comparing Baseline and Week 17		All Cytokine Values	
	HR (in 10 unit) (95% CI)	Cox PH p-value	HR (in 10 unit) (95% CI)	Cox PH p-value
FGFb	0.99 (0.92-1.07)	0.782	1.01 (0.96-1.07)	0.687
IL_8	0.62 (0.27-1.38)	0.24	1.02 (0.89-1.17)	0.782
PDGF-BB	1.01 (1-1.02)	0.02	1 (1-1.01)	0.1
VEGF	1.02 (0.97-1.08)	0.479	1.01 (0.98-1.05)	0.413

Progression-free survival by time-dependent cytokine measurements

Variable	Comparing Baseline and Week 17		All Cytokine Values	
	HR (in 10 unit) (95% CI)	Cox PH p-value	HR (in 10 unit) (95% CI)	Cox PH p-value
FGFb	0.98 (0.91-1.05)	0.581	0.98 (0.92-1.05)	0.522
IL-8	0.43 (0.19-0.97)	0.043	0.66 (0.32-1.38)	0.272
PDGF-BB	1.00 (0.99-1.01)	0.814	0.99 (0.98-1.01)	0.836
VEGF	0.99 (0.95-1.03)	0.795	0.98 (0.94-1.03)	0.562

OS, overall survival; PFS, progression-free survival; NE, not estimable

p-value was obtained by applying permutation log-rank test with 5000 permutation times

OS was measured from the start of study intervention until date of death or last follow-up

Figures

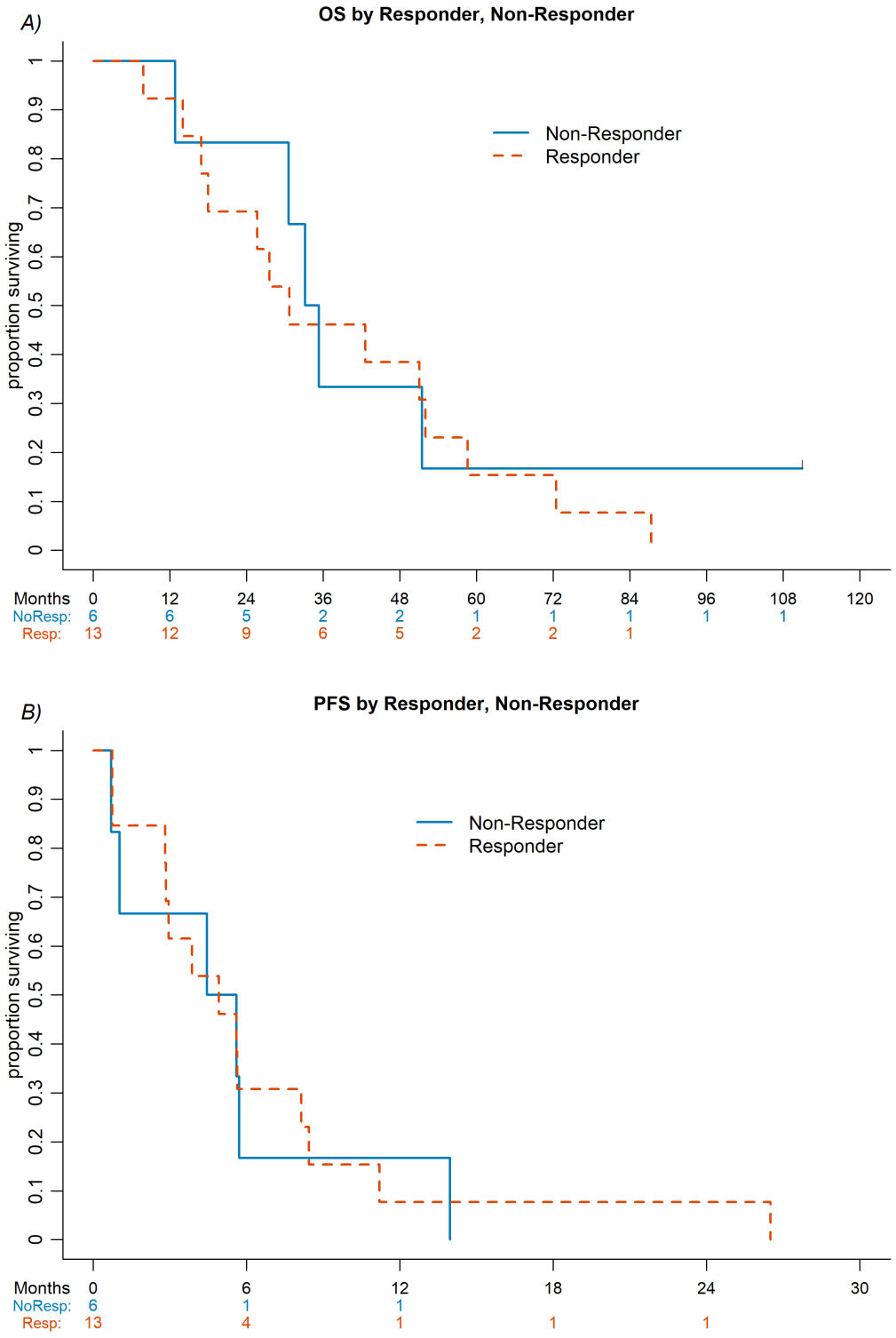

Figure 1

Overall (A) and progression-free (B) survival curves by responder status