Phase I Study of Intrapерitoneal (IP) Denileukin Diftitox (ONTAK) in Patients with Advanced Ovarian Cancer (OC)

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INTRODUCTION

- OC is the leading cause of death from gynecologic cancers and the 5 year survival rate is < 20% in patients with advanced stage disease.[1]
- OC is immunogenic and associated with multiple mechanisms of tumor-induced immunosuppression.
- CD4+CD25+Foxp3+ regulatory T cells (Tregs) which induce T cell suppression are increased in the ascites and peripheral blood (PB) of OC patients and associated with a poor prognosis.[2-4]
- ONTAK, a diphtheria/IL-2 R fusion protein depletes PB Tregs in humans when given IV and enhances vaccine-induced immunity.[5]
- Our previous studies have shown high levels of tumor ascites in neo-tg mice to be associated with immune suppression; and depletion of Tregs using ONTAK resulted in enhanced tumor antigen-specific immunity and tumor regression.[6]
- We questioned whether IP infusion of ONTAK was safe and able to reverse immune suppression in the peritoneum by depleting local Tregs.

PATIENTS AND METHODS

Patient Population: A phase 1 dose escalation study designed to establish the MTD of IP ONTAK and assess its effect on Tregs in patients with OC was approved by the UW IRB and FDA. Patients with advanced-stage refractory OC were eligible for study if they: (1) had completed standard treatment with primary debulking surgery and platinum-based/paclitaxel chemotherapy, and (2) were unable to achieve 1st CR with 1st or 2nd line chemotherapy or had disease relapse after achieving 2nd CR. Three dose levels of ONTAK were investigated with subjects enrolled sequentially to Dose Level 1 (5μg/kg) first, then Dose Level 2 (15μg/kg), then Dose Level 3 (30μg/kg). A treatment cycle consisted of IP ONTAK on Days 1-3 every 14 days for total of 4 cycles. Toxicity was evaluated and graded per CTCAE v3.0, at baseline and Days 1-3, 8, 14 of each cycle. Treg analysis occurred at baseline, after cycle 2 and 4, and CA125 was checked serially to 10 subjects with the following characteristics were enrolled median age 56 (range 38-73), median months from diagnosis 9 (range 1.5-11), and number of chemotherapy regimens at 3 (range 2-7). Data is provided on 9 subjects (3 at Dose Level 1 and 6 at Dose Level 2) who received treatment.

Evaluation of T regulatory cells: Treg levels in PB and/or ascites (TIL) were measured using RT-PCR where FoxP3 expression was standardized to CD4 expression. Briefly, total RNA is extracted from PB and/or TIL, using RNeasyPCR kit (Ambion, TX). RNA integrity is tested using an Agilent Bioanalyzer (Foster City, CA). cDNA is generated from 5 μg of RNA by SuperScript III reverse transcriptase (Invitrogen, San Diego, CA) with oligo-dT as primers per manufacturer’s protocol. 5 μl of 1:40 diluted cDNA is used as template for RT-PCR analysis. The primers and probes (FAM-MGB) for FOXP3 and CD4 are purchased from Applied Biosystems (Foster City, CA). RT-PCR is performed in 384 well thin-wall PCR plates using ABI Prism 7900HT under the following conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec. and a combined annealing/extension step at 60°C for 1 min. Data analysis is performed using SDS 2.1 (Applied Biosystems). The relative levels of Tregs are analyzed using the comparative CT method, where the average FoxP3 CT value of treated animals is normalized using the average CD4 gene CT value. Comparative CT value is used to calculate any fold of change values since it reflects the relative amount of Tregs among total CD4+ T cells.

Statistical methods: Descriptive statistics (numbers and percentages) for patient demographics, adverse events, and diagnostics were calculated. All analyses were performed using Stata 10.0 (StataCorp, College Station, TX), GraphPad Prism 5.01 (GraphPad Software, Inc., San Diego, CA), or Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA).

RESULTS

- 3 subjects completed all 4 cycles of low-dose (5μg/kg) IP ONTAK without DLT.
- 4/6 subjects completed all 4 cycles of intermediate dose (15μg/kg) IP ONTAK without DLT.
- 1 patient had DLT (Grade 3 esophagitis)
- 1 patient withdrew prior to starting ONTAK
- Toxicity in Dose Levels 1 & 2 was primarily Grade 1/2 hypoalbuminemia and abdominal bloating/pain.
- Majority of patients had decreased peripheral blood Tregs after IP ONTAK per RT-PCR analysis.
- Tregs in TIL were decreased in patients treated with low and intermediate dose ONTAK per RT-PCR analysis.
- CA125 initially decreased in majority of subjects in Dose Level 2 (intermediate dose) and then increased by end of ONTAK treatment.
- 4 of 8 (50%) subjects with disease progression per RECIST at end of study had significant lymphadenopathy without increased visceral involvement.

CONCLUSIONS

- IP ONTAK at 5-15 μg/kg is well-tolerated in heavily pre-treated subjects with refractory OC.
- IP ONTAK results in decreased Tregs at site of tumor (ascites).
- Predominant lymph node enlargement without increase in visceral disease after IP ONTAK suggest acute inflammatory changes.
- Enrolment to Dose Level 3 (25 mcg/kg) continues.

REFERENCES


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