

Regulatory T cells in ovarian cancer: biology and therapeutic potential

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Abstract

Tumors express tumor-associated antigens (TAAs) and thus should be the object of immune attack. Nonetheless, spontaneous clearance of established tumors is rare. Initial thinking was that lack of TAA-specific immunity was largely passive: tumors did not present enough TAAs, or antigen-presenting cells did not have sufficient stimulatory capacity. Attempts were made to boost TAA-specific immunity using optimal antigen-presenting cells or by growing TAA-specific effector T cells *ex vivo* followed by adoptive transfer. These approaches met with some success in mice, and showed some early clinical efficacy in human trials, although long-term efficacy remains to be established, and logistical problems are considerable. These studies established that experimentally induced TAA-specific immunity is a rational and potentially efficacious cancer treatment. Nonetheless, recent work by us and others demonstrates that lack of naturally induced TAA-specific immunity is not simply passive, and clearly demonstrates that *tumors actively hinder TAA-specific immunity through induction of TAA-specific tolerance*, mediated in part by regulatory T cells (Tregs), the focus of our group. Means to revert tolerance represent novel anti-cancer therapeutic stratagems. We discuss Tregs in this regard in human ovarian cancer and present evidence that depleting Tregs in human cancer using denileukin difitox (Ontak) improves immunity and may be therapeutic.

Ovarian cancer is the fifth leading cause of cancer deaths in American women. It usually presents as advanced disease, making cure unlikely. In the US in 2005, there will be an estimated 22,200 new cases with 16,210 related deaths. Approximately 167,002 women are alive in the US with ovarian cancer. Current treatment for advanced-stage epithelial ovarian cancer is surgery plus combination chemotherapy but is rarely curative, with five-year survival under 20%. There is no effective therapy for relapsed or metastatic disease failing such therapy. Thus, effective new therapies are urgently needed. Our group focuses on novel immune-based strategies. Our prior published works suggest dysfunctional dendritic cells, aberrant T cell costimulation and Tregs as plausible explanations for past failures of immune-based therapy and points to novel immune-based strategies to treat ovarian cancer.

Most TAAs are self-antigens, and therefore subject to control by peripheral tolerance. Thus, exaggerated self-tolerance may be a critical mediator of suppressed TAA-specific immunity. CD4+CD25+ Tregs are key mediators of peripheral tolerance. Therefore, engendering strong anti-tumor immunity likely involves breaking Treg-mediated peripheral tolerance to TAAs. Treg depletion in tumor-bearing mice improves immune-mediated tumor clearance, improves TAA-specific immunity and enhances tumor immune therapy. We recently

demonstrated that CD4+CD25+ Tregs inhibit TAA-specific immunity, allow tumor growth in the presence of TAA-specific immunity and predict poor survival in human ovarian cancer.

Our human studies and this prior work in mice predicted that depleting Tregs would improve TAA-specific immunity in human cancer, and could be therapeutic. Ontak (denileukin difitox), is interleukin (IL)-2 genetically fused to diphtheria toxin. It is FDA-approved to treat CD4+CD25+ cutaneous T cell leukemia/ lymphoma. We hypothesized that Ontak would also deplete CD4+CD25+ Tregs.

We undertook a phase I/II dose-escalation trial of a single Ontak infusion to test this concept. Seven patients with advanced epithelial cancers received a single intravenous Ontak infusion at 9 $\mu\text{g}/\text{kg}$ ($n = 3$) or 12 $\mu\text{g}/\text{kg}$ ($n = 4$). Patients received no cytotoxic drugs, radiation therapy or immune-modulating agents for at least 30 days prior to study. Ontak was well-tolerated. Blood was studied before and one week after treatment, except as noted. Results for 9 or 12 $\mu\text{g}/\text{kg}$ were similar and thus pooled for analysis.

Mean blood CD3+CD4+CD25+ T cell prevalence was elevated at 25.3%, but dropped significantly ($P = 0.025$) to 17.7% after Ontak. Mean blood CD3+CD4+CD25+ cell concentration simultaneously fell from 123/ mm^3 to 63/ mm^3 ($P = 0.025$). Mean prevalence (0.95% to 3.0%) and concentration (8/ mm^3 to 27/ mm^3) of blood CD3+ T cells expressing the Ki-67 proliferation antigen increased following Ontak ($P < 0.03$ for each). Mean blood interferon- γ +CD3+ T cell prevalence (21.0% to 36.5%; $P = 0.046$) and concentration (173/ mm^3 to 264/ mm^3 ; $P = 0.05$) increased after Ontak. Interferon- γ +CD3+CD8+ T cell prevalence (21% to 37%) and concentration (10/ mm^3 to 23/ mm^3) increased ($P < 0.05$ for each) following Ontak and remained elevated for > 28 days. These data are consistent with prolonged immunologic improvement following CD3+CD4+CD25+ Treg depletion.

Strong *FOXP3* message expression in CD3+CD4+CD25+ T cells was greatly reduced one week after Ontak. Non-selective T cell depletion cannot explain decreased *FOXP3* message because mean total CD3+ T cells (1030/ mm^3 before, versus 900/ mm^3 after) and mean CD3+CD8+ T cells (450/ mm^3 , comprising 48% of all T cells before, versus 423/ mm^3 , comprising 50% of all T cells after) were not significantly altered. The prevalence and concentration of blood B cells and monocytes were not significantly altered by Ontak, consistent with selective depletion of CD3+CD4+CD25+FOXP3+ T cells.

CD3+CD4+CD25+ T cells before Ontak were up to 4.2-fold more potent in suppressing T cell proliferation compared to cells obtained up to 30 days after, consistent with depletion of functional CD3+CD4+CD25+ Tregs. These data also demonstrate prolonged functional Treg depletion, further supported by increased interferon- γ +CD8+ T cell prevalence

and reduced CD3+CD4+CD25+ cell *FOXP3* message up to 28 days after Ontak. Three patients received additional Ontak on a weekly schedule, which eventually depleted interferon- γ +CD8+ T cells. Thus, we chose monthly dosing for further studies.

Patient 4 in this dose-escalation trial had stage IV ovarian cancer. She received 6 additional, weekly Ontak infusions at 12 μ g/kg 39 days after her initial infusion in an IRB-approved amendment. Blood CA-125 dropped from 121 unit/ml to 17 unit/ml (normal, 0-35). A PET/CAT fusion scan demonstrated reduction or complete resolution of all lymphatic, visceral and bony metastases except for a left groin mass which increased. Biopsy demonstrated anaplastic ovarian carcinoma, distinct from the original poorly differentiated histology. This mass did not respond to local irradiation. She refused further treatment and died 7 months later, 13 months after her first Ontak treatment. At death her CA-125 was normal and she had no detectable disease outside the left groin mass. On this basis, we initiated a Phase II trial of Ontak in ovarian cancer, the updated results of which will be presented and discussed.

Most cancer immunotherapy focuses on augmenting numbers and function of essential immune cells such as T lymphocytes and dendritic cells. Our data demonstrate that depleting dysfunctional Tregs is a promising strategy that may work alone, but which also has potential to improve current active immunotherapies, whose successes in cancer treatment have thus far been modest. Ontak represents the first agent effective for human Treg depletion to test such concepts. Depletion of CD4+CD25+ Tregs is necessary, but not sufficient to induce autoimmune phenomena, and we have not observed this problem to date. Nonetheless, potential for inducing pathologic autoimmunity requires further study. Repeated Ontak also appears to deplete effector T cells, which requires further study.

Our ongoing clinical trial demonstrates that Treg depletion is feasible in human cancer using Ontak, and is associated with improved immunity with an appropriate dose and schedule. Current data confirm that a single intravenous dose of Ontak at 12 μ g/kg reduces phenotypic and functional CD4+CD25+ blood Tregs, paving the way for studies of clinical efficacy alone or in combination with additional treatments. Means to overcome active tumor-mediated immune subversion may finally allow the realization of the full benefits of immune-based therapy for cancer.

Acknowledgements

This work was supported by The Ovarian Cancer Research Fund, CA100425, CA105207, CA092562, CA100227, and the Tulane Endowment. Thanks to Ligand Pharmaceuticals for providing Ontak. Thanks to Tracey Todd, Shuang Wei, Ben Daniel, Michael Brumlik and Pete Mottram for excellent technical assistance.