Mechanism of Action of Oregovomab Mab B43.13

The mechanism of action of oregovomab involves infusion of oregovomab at immune stimulatory (2mg per infusion) and not pharmacologic (hundreds of mg per infusion) doses that specifically bind circulating and local CA125. The antibody-antigen complex binds and is taken up by dendritic cells resulting in cross presentation of the peptide fragments of CA125 in the context of class I and II MHC. This indirect immunization to CA125 breaks tolerance, and the generation of a clinically meaningful anti-tumor immune response follows if a favorable immune fostering environment is established through combination therapy with chemotherapy and other immune modulators in a schedule dependent fashion.

Background: The immune system is carefully regulated to protect the body from foreign invaders, including bacteria, viruses, fungi, toxins, parasites, and tumors, while avoiding autoimmune diseases and destruction of the unborn fetus. Immune system protection from tumors is well documented, and functions primarily to prevent the development of tumors through a process called immune surveillance and to destroy, or reject, tumors once they have developed. The very fact that a patient has a tumor means that the immune system has failed in this important function.

A cancer continues to grow because the immune system has developed a passive relationship with the tumor that is referred to as “tolerance”. This state of tolerance is complex and may have one or more different mechanisms. There is good scientific evidence that this state of tolerance can be overcome and that the immune system can be stimulated to destroy the cancer. Oregovomab MAb-B43.13 is designed to stimulate the immune system to destroy ovarian cancers by a unique mechanism. To understand how Oregovomab MAb-B43.13 works, an understanding is needed of how the immune system is regulated and of the mechanism of tolerance.

Antigen specific adaptive immune responses fall into two categories, namely humoral (or antibody) responses, and cellular responses. To initiate an adaptive immune response, T helper cells must be activated. This happens when the antigen is taken up and processed by what are referred to as antigen presenting cells. Several types of cells can function as antigen presenting cells, the most important of which are macrophages and dendritic cells. APC process uptakes antigen by breaking it into fragments, transporting it through intracellular transport pathways to be presented on the APC cell surface in the context of class I and class II MHC (Major Histocompatibility Complex) T cells have specialized receptors (T cell receptors) on them that recognize the combination of specific antigen fragment and MHC antigen.

The activation of the T cell requires two types of signals. The first type of signal occurs when cell-surface molecules on the Antigen Processing Cell (APC) and the T cell interact physically. These interactions include the T cell receptor interacting with the antigen fragment-MHC antigen complex on the antigen presenting cell, CD8 or CD4 on the T cell

interacting with the MHC Class I or Class II molecules on the APC, and co-stimulatory molecules ("danger signals') such as CD28 on the T cell interacting with CD80, CD86 (aka B7) on the APC, among others. The interaction of the antigen presenting cells and T cells induces the secretion of the second set of signals in the form cytokines including IL-1, IL-12, IL-4, Tumor necrosis factor-alpha, and others. Once this happens, the T cell is activated to produce a variety of additional cytokines and to undergo a number of cell divisions. Over a few days, the T cells mature into T helper cells which cause B cells to make IgG antibodies or cause macrophages and killer T cells to become activated and capable of killing cells infected with viruses or cancer cells. Immune dampening second signal molecules such as CTLA4 are also expressed which turn off the specific immune response.

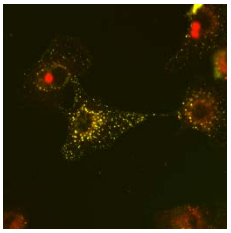
If the T cell receptor interacts with antigen fragment-MHC antigen complex on an antigen presenting cell in the absence of co-stimulation T cell tolerance is induced, the T cell becomes paralyzed and may actually die. In either case, no immune response is observed because no antibody or T killer cells can be measured. The absence of a measurable immune response is called "tolerance". This state of tolerance can occur for several reasons, including having too little or too much antigen in the system.

Mechanism of Action: Oregovomab MAb-B43.13 is a mouse monoclonal antibody that is specific for the cancer antigen CA-125. When Oregovomab MAb-B43.13 is injected intravenously at low doses into a patient, the antibody binds to circulating CA-125 and also at local sites of antigen concentration most typically at the site of a tumor. Complexes are formed between the antibody and the CA-125 antigen (antigen-antibody complexes). These complexes are taken up by antigen presenting cells and at the same time, decrease the amount of CA-125 in the circulation. OncoQuest antibodies including oregovomab have been demonstrated to increase the binding and alter the processing of CA125 for presentation to T cells. The combination of decreased amounts of CA-125 in the circulation and the enhanced presentation of CA-125 on activated antigen presenting cells results in a more efficient stimulation and activation of T helper cells. This would then result in a measurable immune response to CA-125 and the apparent reversal of "tolerance". This has been demonstrated in multiple models both using human donor immune cells in vitro, in animal model systems and in human clinical studies. These are detailed further below.

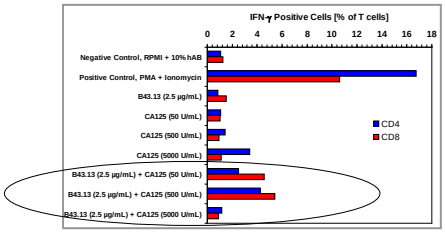
Preclinical Studies: A series of experiments were undertaken to evaluate the potential of Oregovomab MAb-B43.13 to modify the interaction between the tumor-associated antigen (TAA) CA-125 and the immune system, most notably the uptake and processing through APC, to alter the subsequent immune response to the antigen. Initial studies were focused on the uptake of CA-125 antigen in monocytes and dendritic cells. These were followed by binding studies that compared the uptake of CA-125 in the presence and absence of MAb-B43.13 and further binding studies that compared the uptake of the CA-125-MAb-B43.13 complex in the presence and absence of HAMA. Further studies were performed that examined the role that various receptors found on the surface of immature DC play in the uptake of MAb-B43.13, CA-125, and CA-125-MAb-B43.13 complexes. Additionally confocal microscopy experiments examined the intracellular localization of CA-125 either in the presence of absence of MAb-B43.13. Finally a series of T cell activation studies were then undertaken to evaluate T cell activation by CA-125 and CA-125-MAb-B43.13 complexes. The figures below are from a study presented by Schultes et al *AAI Annual Meeting* in as a poster in 2003.

Mechanism of Action 1

- Low dose (2 mg), intravenous administration
- After injection, antibody binds to cancer antigen and forms immune complexes
- The complexes are processed and presented by dendritic cells to activate T cells



CA125-B43.13 Complex
CA125 conjugated with FITC (green) pre-incubated with MAb-B43.13-Cy3 (red) added to day 5 DC for 30 min. before fixation; yellow: co-localized complex

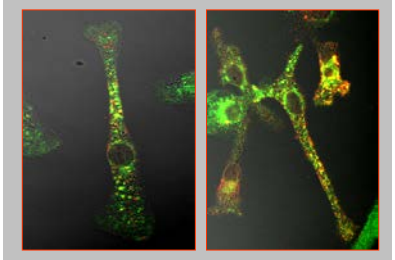


Two rounds of *in vitro* stimulation

Intracellular Trafficking of CA125 and CA125-B43.13 IC

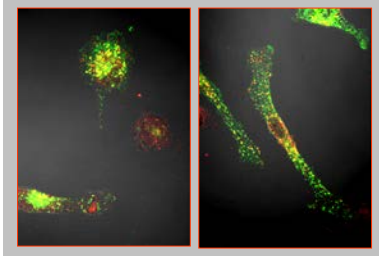
Early Endosomes

CA125 - 1h Pulse



CA125-Cy3 and Transferrin Receptor-FITC

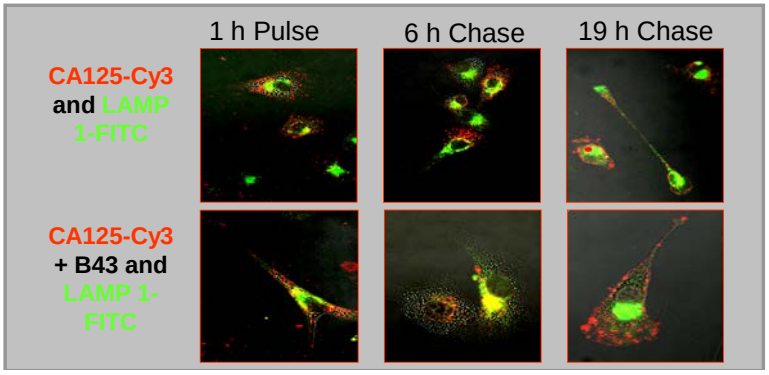
CA125-B43.13 - 1 h Pulse



CA125-Cy3 + B43.13 and Transferrin Receptor-FITC

CA125-B43.13 IC But Not CA125 Co-localize with LAMP-1, A Marker for Late Endosomes

Late Endosomes



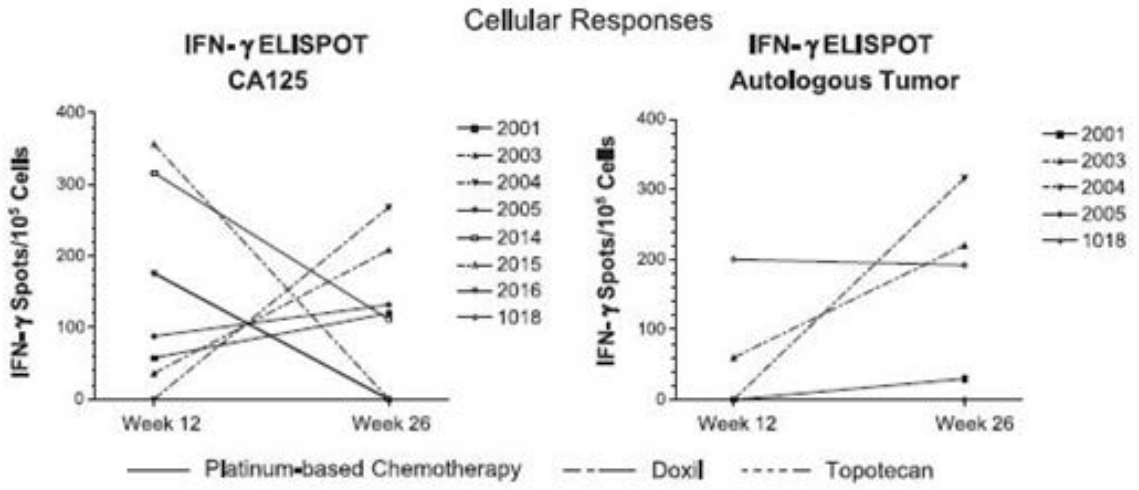
- CA125 is present in early but not in late endosomes. The overall distribution of CA125-Cy3 does not change even after a 19 h chase period
- CA125 given as immune complex is present in early and late endosomes after a 6 h chase

Preclinical Studies for Combination Therapy: OncoQuest researchers have been further studying the mechanism of indirect immunization in combination settings in various preclinical models**.** In 2006 Schultes, Nicodemus and colleagues presented data to the AAI (*J Immunol* 176:S275, 2006. AAI 2006 Abstract #142.13) illustrating the modulation of cytokines and chemokines associated with immune regulation in response to a panel of standard chemotherapeutic agents. Carboplatin and paclitaxel both exhibited properties that could counter immune suppressive mechanisms). Mehla and collaborators (Cancer Immunology and Immunotherapy 2017) published results using the OncoQuest AR20.5 antibody as an indirect immunizer to MUC1 in transgenic animal models in conjunction with chemotherapy and also TLR3 stimulation and PDL-1 pathway inhibition and have again established the ability of combinatorial therapy to induce anti-tumor effects not seen with the individual components in isolation and again establish a CD8 T cell immune component as critical to the observed beneficial anticancer effect. The company will explore the finding clinically in collaboration with Memorial Sloan Cancer Center in an upcoming clinical collaboration in MUC1 associated pancreatic cancer.

Immune responses seen in Clinical Trials:

Anecdotal Observations: Baum et al 1993: When radiolabeled B43.13 antibody was administered to patients with recurrent disease (who were also receiving chemotherapy) without regard to their oncology treatment, clinical benefit was observed anecdotally, although the mechanistic understanding of indirect immunization had not yet been developed. **Noujaim et** al **2001**: Although, role of circulating CA125 related mechanism of action was identified, clinical benefit was attributed to anti-idiotype mechanism (Madiyalakan et al 1995).

Gordon et al 2004: Indirect immunization using low dose antibody as with oregovomab is an effective way to induce a tumor specific immune response. However, as discussed in the introduction to this discussion of mechanism, in order for such induced immunity to amplify to a clinically meaningful magnitude is dependent on the state of immune suppression and in a typical cancer center patients are tolerant of their tumors. The oregovomab study published as Gordon et al Gyn Onc 2004 looked at patients with recurrent disease and dosed them with indirect immunizing oregovomab antibody for 12 weeks prior to initiation of their second line chemotherapy which was administered in the period between week 12 and week 26. The study demonstrated that contrary to expectation the initiation of chemotherapy did not routinely dampen cellular immunity associated with clinical indirect immunization with oregovomab but in the period of the second line treatment resulted in some enhancement of cellular immunity as measured in the peripheral blood. A panel form fig 5 is inserted below.

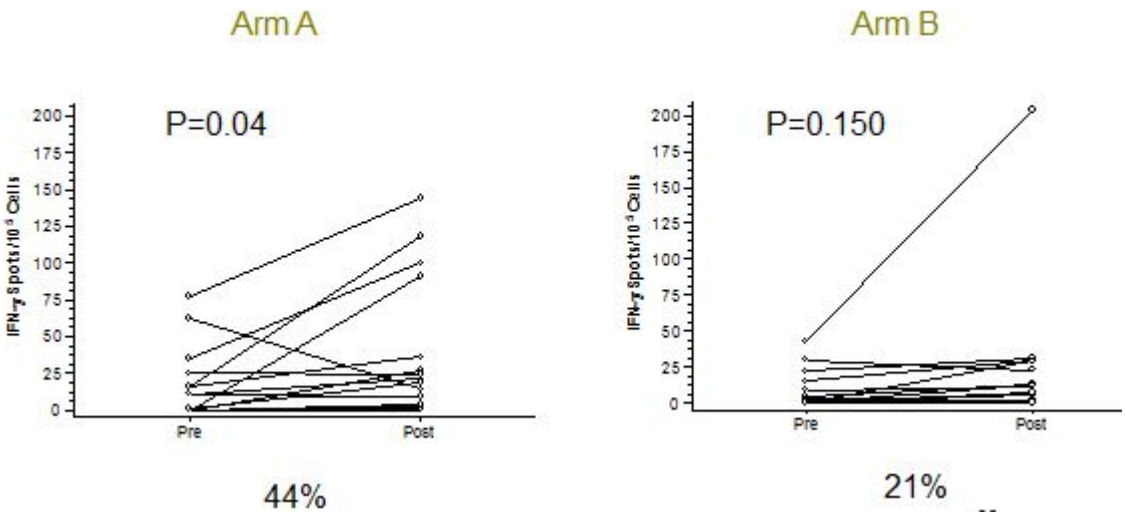


This observation led to detailed preclinical and clinical study of the interaction of the chemotherapy with indirect immunization.

Braly et al 2009: A clinical study to further explore the observations of Gordon *et al* was conducted at the same time as the maintenance monotherapy study with oregovomab that focused on indirect immunization with oregovomab in the front line setting in conjunction with two schedules of administration relative to standard of care IV carboplatin and paclitaxel. This study published as Braly *et al* JIT 2009 found an unexpected influence of simultaneous day infusion of antibody relative to one week delayed infusion of indirect immunizing antibody on the same cycle schedule. Both early antibody response and cellular responses were activated more in the simultaneous day schedule. The early antibody response is reflective of an enhanced state of immune responsiveness in the simultaneous schedule relative to the one week delayed schedule as well as immune responsiveness seen with oregovomab in the maintenance setting. The data strongly suggested schedule of immunization relative to chemotherapy infusion could have a significant impact on clinical and immune response outcomes. In the further translation analysis of samples from the study, specific T cell immunity also was more potently induced using the Arm A simultaneous day schedule on cycles 1, 3, 5 and then cycle 5 plus 12 weeks.

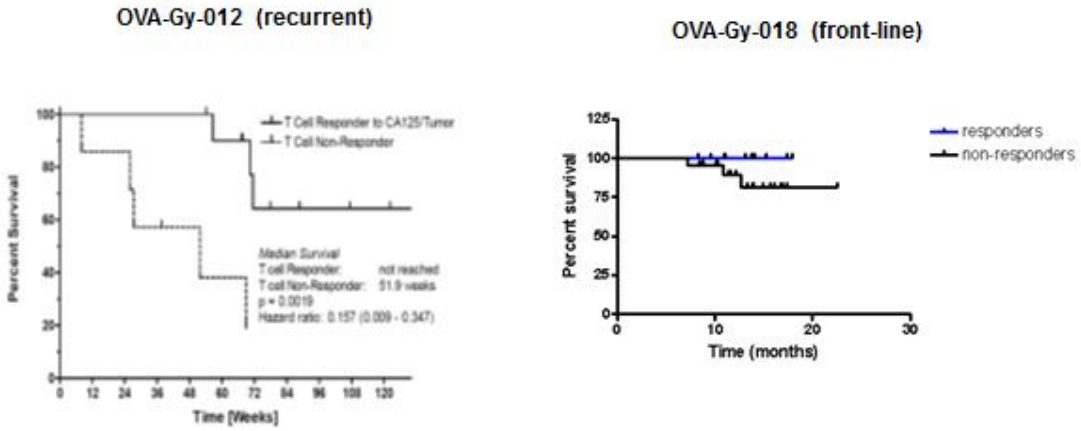
Cellular Immune Response Tracks Humoral Immune Response

CA125 Specific ELISPOT assay

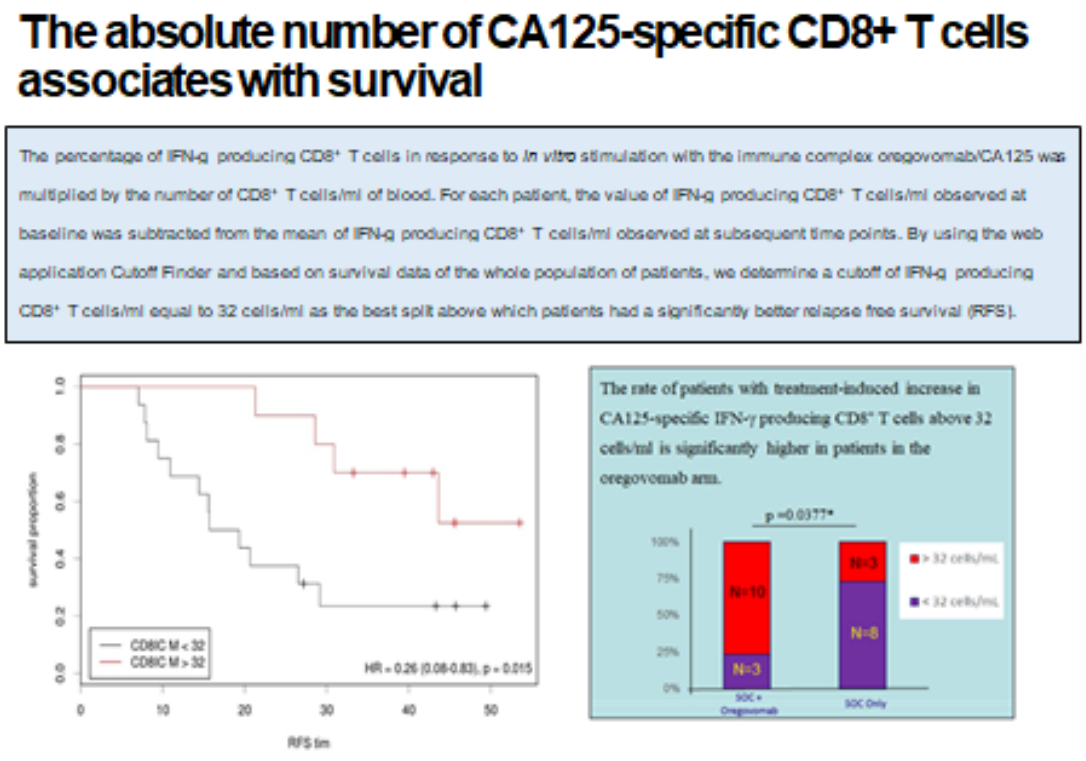


As in other studies in the program, induced T cell immunity correlated best with improved clinical outcomes and suggested that generation of effective immunity was a schedule dependent event. The figure below shows data from both Gordon 2004 and Braly 2009 correlating induction of antigen specific CD4 and CD8 IFNγ positive cellular immunity with favorable survival outcomes.

Favorable Clinical Outcome Correlates to generation of CA-125 specific T-cell Response

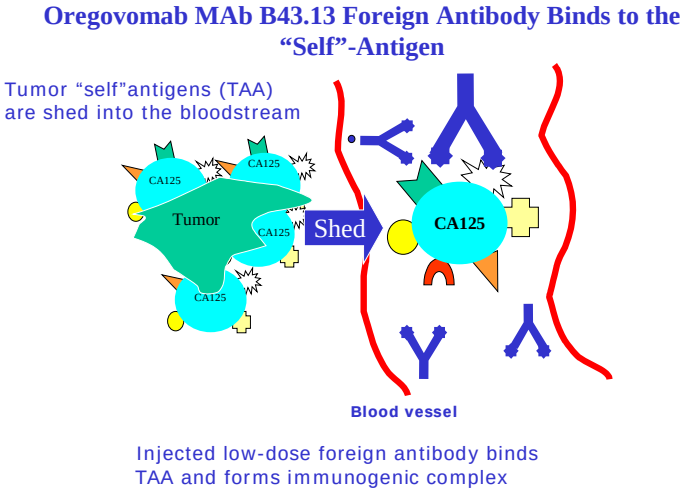


Ferrandina et al and Battaglia et al 2017: The conduct of OncoQuest clinical trial QPT-ORE-002 as reported by Ferrandina et al ASCO proceedings 2017 and Battaglia ESGO Proceedings 2017 have demonstrated that scheduled indirect immunization with oregovomab in conjunction with front line carboplatin paclitaxel results in the expected induced cellular immunity as seen previously and that cellular immunity was again associated with favorable outcomes, but more importantly the treatment effect of the combination treatment was without precedent in its ability to improve both progression free survival (PFS) as well as survival (OS) in this randomized phase II study of 97 patients. The study had been powered to study the technically complex ELISPOT assay to see if it could be marginally improved over its performance in the Braly 2009 report and thus potentially serve as a surrogate assay for individual patient evaluation for clinical development of OncoQuest antibodies in the continuing study of combinations. The assays proved technically inconsistent when expanded to an international multi-center model, but, more importantly, the effect of the combination therapy resulted in a dramatic clinical effect using the optimized schedule that was readily established with a highly statistically significant clinical treatment effect in the 97 patient randomized sample size. The effect will be reproduced in a confirmatory study using commercial grade antibody.

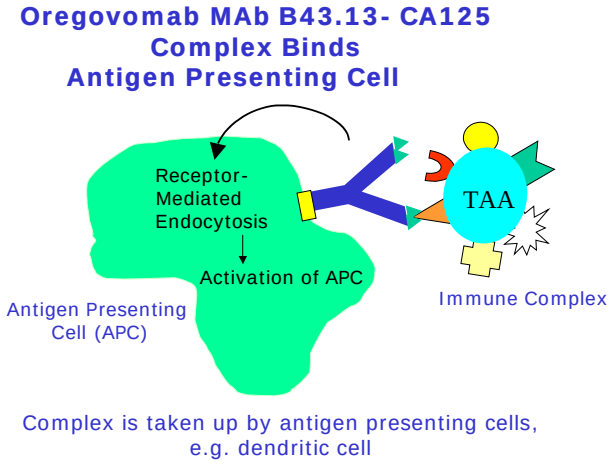


Appendix - Summary

The current understanding of the mechanism of action of Oregovomab MAb B43.13 is outlined below:

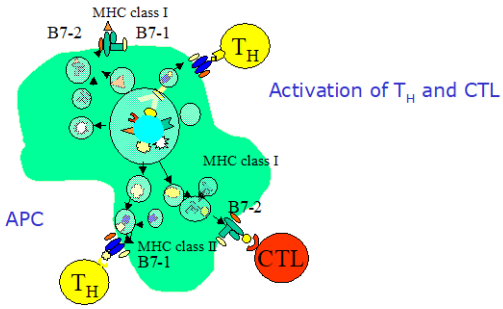


- The key event is the formation of a complex between intravenously administered Oregovomab MAb-B43.13 and the circulation tumor-associated antigen CA-125. Complex formation was confirmed in human PK studies (Noujaim *et al.*, *Cancer Biother & Radiopharm* (2001)16:187). As the antibody also concentrates at the site of tumor addional antigen binding and altered antigen processing is expected to occur in the local tissues associated with tumor as well.



- CA-125 complexed with Oregovomab MAb-B43.13 is taken up uptake by dendritic cell much better than the CA-125 antigen alone (Schultes *et al.*, *Proceedings AACR* (2001) 42:276; AltaRex Report RT-PRE-011).

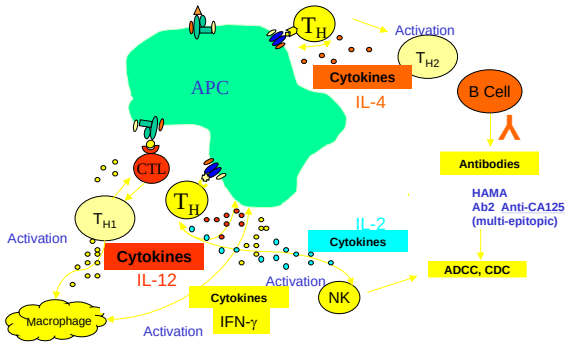
Altered Processing and Activation of Antigen Presenting Cell



Complex is processed and presented on MHC class I and II, B7-1 and B7-2 are up-regulated as a result of complex internalization

- The xenotypic nature of Oregovomab MAb-B43.13 aids in triggering the release of cytokines (Noujaim *et al.*, *Cancer Biother. & Radiopharm* (2001)16:187).
- Dendritic cells are capable of presenting antigen-derived peptides from extracellular proteins on MHC class I and II molecules. This mechanism of 'cross-priming' is very effective when the antigen is presented in complex with a specific antibody (Regnault *et al.*, *J Exp Med* (1999)189:371; Manca *et al.*, *J Immunol* (1998)140:2893; Berlyn *et al.*, *Clin Immunol* (2001)101:276).
- Intracellular cytokine staining and *in vitro* antigen presenting assays have demonstrated that MAb-B43.13-CA-125 immune complexes are vastly superior to CA-125 alone in stimulating IFN-γ secreting CD4+ and CD8+ T cells (Schultes *et al.*, *Proceedings AACR* (2001)42:276).

Induction of T Helper Cells and CTL



Activation of multiple arms of the immune system

- Injection of Oregovomab MAb-B43.13 can also lead the generation of human-anti-CA-125 antibodies. These antibodies recognize several epitopes on CA-125 and are of a T cell dependent isotype suggesting recognition of the entire immune complex by the patients'

immune system (Noujaim *et al.*, *Cancer Biother & Radiopharm* (2001)16:187). Induced anti-CA125 antibodies illustrate that the immune response indirectly triggered by oregovomab has specificity that generalizes beyond the specificity fo the antibody itself.

- Injection of Oregovomab MAb-B43.13 leads to the induction of CA-125-specific T cells (Noujaim *et al.*, *Cancer Biother & Radiopharm* (2001)16:187).
- CA-125-specific CTL can directly kill tumor cells. In this context, activated MHC class I (and II) restricted T cells have been demonstrated in patients after treatment with Oregovomab MAb-B43.13, which respond with IFN-γ production upon stimulation with CA-125 and autologous tumor (Schultes *et al.*, *Proceedings AACR* (2001) 42:276, and Gordon *et a*l., Gynecologic Oncology (2004) 94:340-351).
- The magnitude and quality of induced T cell immunity to CA125 or autologous tumor is influenced by the timing of Oregovomab MAb-B43.13 infusion relative to other cancer treatments. The combination of carboplatin and paclitaxel can be immune enhancing. (Braly *et al.*, *J Immunother* (2009) 32:54-65), and this combination results in a substantial improvement in clinical outcomes associated with induced cellular immunity and enhanced immune reactivity in the optimal schedule of indirect immunization with oregovomab and carboplatin paclitaxel chemotherapy..
- TLR stimulation in association with Oregovomab MAb-B43.13 and antigen has been found to augment induced immunity in preclinical models (Nicodemus *et al.*, *Am J Obstet Gynecol* 2010).
- PD-1 pathway blockade can enhance the effect of induced immunity with Oncoquest indirect immunizing antibody and this effect is further augmented by TLR3 stimulation (Mehla CII 2017)

In summary, multiple effector arms of the immune system have been shown to be activated by Oregovomab MAb-B43.13 injection into patients, and these effects are supported by animal models and human autologous cell *in vitro* antigen processing and immune response models.

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