From Sara Kost, First year M.Sc. student (Supervisor: Dr. Brad Nelson), Dept. of Biochemsitry/Microbiology, University of Victoria, and Deeley Research Centre, BC Cancer Agency:

Dr Glenn Dranoff from the Dana-Farber Cancer Institute, the keynote speaker, gave a talk entitled "Mechanisms of protective tumor immunity". He started out by talking about different kinds of immunocancer vaccines. He then went on to talk about the status of GM-CSF for cancer immunotherapy and how it has two roles: tolerance and protective immunity. The former is mediated through apoptosis and Tregs whereas the latter is facilitated by an enhancement of antigen presenting cells. The most interesting part of his talk was when he explained PLGAs, engineered polymer scaffolds. These porous disks can hold cells and upon implantation into animals they can facilitate a slow release of treatment over time. I find this to be an incredibly interesting technology and could possibly have various other applications. He went on to explain how the dual role of GM-CSF described above can help to explain the limited success with GM-CSF as an antitumor immunotherapy until now. He finished his talk by talking about a triple knockout mouse model that he used to show that the loss of murine homeostasis leads to an increase in tumors and that lung cancer could be a good target for immunotherapy.

Dr. Willem Overwijk from MD Anderson gave a talk entitled "Understanding the limited efficacy of clinical cancer vaccines: of T cell sinks and graveyards". He began by talking about peptide cancer vaccines that are composed of the peptide gp100 and IFA, which is a water/oil emulsion. He talked about how there was a great initial response when this vaccine was given, but the secondary response after boosting was often weak or lacking completely. He performed quite interesting experiments to identify the migration patterns of the T cells. He found that they were localized to the vaccine site and not making their way into the tumor. He thus concludes that he is seeing an increase in cell number, just at the wrong site. He found that a diminished response could have been due to overexposure to the antigen and that if a water-based vaccine was used instead the T cells would migrate to the tumor. The tumor environment represents a low exposure to antigen and thus the T cells live longer when the water-based vaccine is given. He also notes the need for adjuvant to activate the T cells. The highlight of his talk was the pictures of the locations of the T cells, be it in the tumors or at the vaccine site.

Dr. Pere Santamaria from the University of Calgary gave a talk entitled "Autoregulatory T cell memory". He began by talking about how type 1 diabetes can be used as a model system for studying the initiation of an immune response. He talked about how dendritic cells are able to activate both high avidity CD8 T cells with effector functions and low avidity T cells. The low avidity T cells form memory T cells and serve as a feedback response by killing the DCs. This stops the activation of too many effector T cells. A really interesting technology that Dr. Santamaria was exploiting was the use of nanoparticles for imaging. He found that the use of these nanoparticles leads to death of activated and naive high avidity T cells but rapidly expanded the low avidity T cells. He demonstrated how nanoparticles can be used to reverse diabetes. The nanoparticles were able to expand the natural phenomenon initiated by nature but they alone could not initiate the response. He mentioned really interesting applications of this technology since it works for any epitope and never activates high avidity T cells; it only gets rid of them.

Dr. Jianda Yuan from the Memorial Sloan-Kettering Cancer Centre gave a talk entitled "Antigen specific immunity after CTLA-4 blockade: investigating the role of prior vaccination". He started by talking about

ways to improve DNA cancer vaccines by combining the vaccine with ipilimumab. Ipilimumab is able to block the inactivation of T cells by CTLA-4. He found that NY-ESO-1 antigen specific CD8 T cell responses are correlated with better survival induced by the CTLA-4 blockade. He also found that vaccinating with ipilimumab boosts the immune response to enhanced specificities, providing evidence for the benefits of combined therapy. Large amounts of T cells were seen when CTLA-4 was blocked. Initial regression of a tumor is seen when ipilimumab is given a long with an anti-CTLA, but this tumor did relapse. He concludes that blockade of CTL4 leads to better survival and that the ipilimumab combination is promising but clinical trials will need to be done. The highlight of his talk was when he talked about how combination therapy with ipilimumab could possibly be used as an effective immunotherapy due to its observed clinical benefits.

Dr Kunle Odunsi from the Roswell Park Cancer Institute gave a talk entitled "Co-ordinate negative regulation of human ovarian tumor immunity by PD-1 and LAG-3". He starts by talking about how two signals are needed to activate a T cell. A partial signal can be seen leading to downstream effects and negative signals are mediated by PD-1. PD-1 is upregulated for activation of a T cell, and downregulates effector effects. PD-1 or PD-L are thus good targets for immunotherapy. LAG-3 is also important since it has homology to CD4, interacts with MHC class II, and reduces influx of calcium leading to decreases in activation. Interestingly, he found that ovarian cancer patients with high infiltration of CD8s and T cells that express NY-ESO-1 do better. There are thus vaccines for NY-ESO-1 currently in clinical trials. Vaccination with NY-ESO-1 boosted the number of NY-ESO-1 specific T cells that showed the effector memory phenotype within the tumors. These cells are able to produce INF-γ. He found that when there were high amounts of LAG-3 present there was low INF-γ production. He finished his talk with an explanation about IL-10 activating both PD-1 and LAG-3.

From Lisa LeShane, Master's Candidate in Biomedical Science (Supervisor: Dr. Sheila Drover), Memorial University of Newfoundland and Labrador:

Dr. Bernard Fox, of Providence Health and Services, focused his talk on "translating and monitoring combination immunotherapy". In particular, Dr. Fox guided us through his previous studies that focused on vaccinations in lymphopenic environments and whether this caused an improvement of therapeutic efficacy. Several of his studies involved lymphopenic subjects (either mice or patients who had underwent chemotherapy), and reconstituting the lymphocytes within the host, followed by inoculation with a tumor vaccine. Based upon a model that used vaccinated lymphopenic mice injected with a GM CSF modified melanoma cell line and reconstituted with naïve spleen cells, his group found that tumor vaccine draining lymph nodes of the reconstituted lymphopenic mice illustrated an increase in activated T cells. However, if the reconstituted lymphocytes were derived from the spleen on a tumor bearing D5 mouse, then the antitumor effect was lost. Depleting the tumor bearing D5 mice spleen cells of CD25+ Treg cells led to recovery of the antitumor behavior. The Fox lab is currently expanding their research from mice into clinical studies with patients that have been made lymphopenic through chemotherapy treatments. These patients are then reconstituted with either PBMCs or CD25+ Treg depleted PBMCS and vaccinated with autologous tumor and GM CSF. Preliminary data from these clinical trials have collected thus far.

Dr. Yonghong Wan presented work from his studies within McMaster University and his presentation was titled "Potentiation of oncolytic vaccine therapy by histone deacetylase inhibition". His session began with an outline of the challenges for therapeutic cancer vaccinations. He underlined the importance of the level of functionality of CD8+ cytolytic T cell (CTL) response and alterations in the tumor environment that can improve the CTL response. The Wan lab is currently using oncolytic vectors to boost cancer vaccines. They are using viruses that are either naturally mutated or engineered to destroy tumors. Further manipulation of oncolytic viruses has the potential to achieve transient viral oncolysis and de-bulk the tumor mass. As a consequence of viral infection in the tumor, there will be a corresponding increase in tumor infiltrating lymphocytes (TILs) and induction of epitope spreading could occur. The Wan lab is using Vesicular Somatitis Virus or VSV, an easily manipulated virus for the oncolytic studies. Evidence for VSV medicated tumor destruction could be observed in B16 melanoma. Within a few days of the infection with the virus, the tumor size decreased. However, there was no association or benefit observed with respect to recurrence free survival. The study continued by treating intracranal B16F10 models with Ad-HDCT and VSV and monitoring the CD8 T cell frequency in the PBMC. When VSV was administered with Ad-HDCT, a significant increase in CD8 T cells was observed both with the tumor infiltrating population and the peripheral blood population. To try and improve upon this combination therapy, either SAHA or MS275 was administered with VSV and Ad-HDCT. Combination therapy with MS275 induced lymphonia. Among the lymphocyte populations that were depleted, the FOXP3+ Treg cells recovered the quickest. Antigen specific secondary CD8 T cell responses remained intact despite the lymphonic conditions. Dr. Wan concluded his talk by summarizing the results of his studies; MS275 can enhance efficacy, decrease the Treg cells, preserve antigen specific responses, increase tumor immunogenicity and prolong oncolytic activity.

Dr. Mark Cattral, of the Sunnybrook Research Institute, presented work concerning "Tumor Conditioning of Dendritic Cells." His introduction focused on the nature of inflammatory infiltrate and the profound impact the infiltrate can have on tumor immunity. Many tumor antigens are processed and presented to the host through dendritic cells. In lymphoid tissues, dendritic cells can interact with naïve T cells and cause differentiation into cytolytic CD8 T cells (CTL) specific for tumor antigens. However, in the majority of cases, there are insufficient numbers of CTLs or the CTLs can be ineffectual within a tumor microenvironment. With this knowledge, Dr. Cattral sought to answer the following questions during his talk; where do dendritic cells originate in cancer and what happens to them within a tumor, and do tumor dendritic cells affect immune responses and be used to enhance cancer immunotherapy. Dr. Cattral then explained a current model of dendritic ontogeny; in particular he noted that pre cDCs can exist in tumors and express CD3, GR1, and FLT3 among other markers. To determine the behavior of these pre cDCs within a tumor environment, an adoptive transfer was carried out. Tumor bearing mice were injected with pre cDCs. It was found that the pre cDCs in the tumor expressed GR1 at a frequency of 20%. However, the pre cDCs found in the spleen and the lung did not express GR1. Therefore, it would appear, that GR1 maybe be a marker for tumor specific pre cDCs. Subsequent studies found that 20 – 50% of pre cDCs in the tumor expressed GR1 and that percentage increased with metastases. GR1+ cDCs appear to be functionally defective as there was no up regulation of MHC Class II or CD86. Next, the development of GR1+ cDCs was analyzed and it was noted that in the absence of IL-6, there was a reduced frequency of GR1+ cDCs. Further, the absence of IL-6

seemed to augment efficacy of adoptive T cell therapy, and correlate to lower tumor growth and increased recurrence free survival. The possibility of tumor cell antigen presentation to T cells or DC cross presentation was investigated next. CD11c DTR mice were treated with diphtheria toxin and it appeared that cDCs were stimulating the CTL proliferation. Dr. Cattral concluded by implicating that in situ proliferation is a key determinant of CTL frequency within a tumor.

Dr. Weiping Zou, of the University of Michigan, presented a lecture focusing on "Major immunosuppressive elements and their impact on tumor immunotherapy". His introduction focused on Treg cells, as defined as CD4+CD25+FOXP3+. These regulatory cells migrate to the bone marrow, lymph nodes, inflammatory tissues and tumor areas due to chemokine receptors present and their respective ligand location. Regulatory cells can target antigen-presenting cells (APCs) and render them ineffective via CTLA4. Another section of his introduction focused on suppressive APCs markers, B7-H1+ and B7-H4+. B7 family members can be found on APC and tumor cells. B7-H1+ is also known as PDL1 and binds to CD80 or PD1. However, there is no known receptor for B7-H4. The importance for B7-H1 is highlighted through immune evasion and in the tumor microenvironment lots of tumors cells express B7-H1. They mediate suppression through exhaustion, IL-10, anergy and apoptosis. Further, B7-H1 tumor cells avoid CTL mediated killing. Less is understood about B7-H4. Myeloid APCs may express B7-H4 and it seems that IL-6 and IL-10 stimulate B7-H4 expression, whereas GM-CSF and IL-4 suppress B7-H4 expression. Next, Dr. Zou discussed the major immunosuppressive elements and their impact with respect to the pathology between cancer and immune cells in the microenvironment. He also discussed how this network of cells could protect the tumor and tumor associated antigens (TAA) with regards to active tolerance in the microenvironment. He mentioned cancer stem cells, or the concept of the ability of cancer cells to renew themselves and feature particular markers: CD133, CD24, CD44 and ALDH. The cellular and molecular mechanisms for controlling cancer stem cells are unknown, however, there is some evidence to suggest Tregs target the cancer stem cells and promote tumorgenesis. Dr. Zou began to conclude his talk by discussing the mutated and environmental stimuli and their effects on suppressed immunity. He discussed a three-signal oncogenesis model composed of genetic signaling, extrinsic signaling and immunosuppressive signaling.

Dr. John Stagg, of L'Universite de Montreal, discussed "Targeting CD73 rescues adaptive anti-tumor immunity". Dr. Stagg began with an introduction to CD73. CD73 generates adenosine and adenosine can lead to immunosuppression. CD73 is ubiquitously expressed and can be induced due to hypoxia or increased levels of type I interferon. CD73 is also found on FOXP3 Treg cells. The first question that Dr. Stagg investigates was could CD73 be targeted for cancer treatment? His study involved ER negative breast cancer cells, partially because ER negative tumors express high CD73. The study observed in vitro tumor growth in 4T1.3 mice with or without knock out of CD73. With a partial knock of CD73 (40%), it was observed that tumors were growing slower. The next set of experiments used SCID and wild type mice and with anti-CD73 treatment. Following the treatment, wild type mice were observed to have smaller tumors. It was also found that using anti CD73 would also inhibit spontaneous 4T1.3 lung metastases in both wild type and SCID mice. Therefore, it appears that CD73 has some metastases promotion independent of immunosuppression. To test this theory, chemotaxis experiments were conducted. Activation of A2B adenosine receptors subsequently led to 4T1.3 cell chemotaxis in vitro

and metastasis in vivo. Dr. Stagg next sought to explain the effect of CD73 on host cells, in particular FOXP3 Treg cells. CD73 knock our mice were used and were found to be resistant to tumor growth of ovalbumin expressing MC38 colon cancer, EG-7 lymphona, AT-3 mammary tumors and B16F10 melanoma. The immune escape as a consequence of depleted CD73 was dependent on antigen specific CD8+ T cells and increase antigen specific IFN-γ. To understand the effect that CD73 plays on Treg cells, DEREG mice (depleted of Tregs) were injected with either wild type or CD73 knockout Tregs. Next, these mice were challenged with MC38-ova cells and it was found that CD73 expression on FOXP3+ Treg (wildtype) mediated tumor growth and the CD73 knock out Treg infused mice were found to have no tumor growth. CD73 knock out mice were also protected from pulmonary metastasis of B16F10 melanoma after injection. It was also found that the prometastatic effect of host derived CD73 was dependent on CD73 expression on non-hematopoietic stem cells. He concluded his talk by highlighting the targeting of CD73 on multiple subsets (tumor, Treg, non hematopoietic cells) and how CD73 maybe a new target therapy for triple negative breast tumors. The next steps will involve combining anti-CD73 treatments with chemotherapy.