# CCIC 2012 Talk Summaries

## WEDNESDAY

***Adoptive transfer of gene-modified anti-tumor T cells in hematologic malignancies***

**Dr. Carl June** from the University of Pennsylvania opened the 2012 CCIC with his *Ralph Steinman Memorial Lecture* which discussed the use of CAR (chimeric antigen receptor) T cells with a 4-1BB signaling domain to promote proliferation and tumor recognition. Without 4-1BB, cells only expand for a few days, then crash *in vitro*. Dr. June discussed the use of CARs to target the CD19 marker which is more widely expressed on B cells (than CD20) in patients with CLL. He demonstrated that this treatment could result in killing of cells resistant to chemotherapy and that CAR T cells showed exceptional memory *in vivo* and CAR expression was maintained for 6 months. In fact, 1-2% of CD3 cells continued to express CAR 18 months later - most of which were CD8. *In vivo*, expansion of a polyclonal repertoire of CAR T cells was observed in the absence of IL-2 administration. Dr. June concluded his talk with encouraging data that showed 41/43 patients have CAR T cells after 10 years with CTL having a lifespan of approximately 14 years in humans.

## THURSDAY

***Genetic modification of T cells for immunotherapy of cancer***

**Dr. Gianpietro Dotti** from Baylor College of Medicine, presented his work on CAR engineered T cells which were modified to express IL-15 to enhance their survival. In addition, a caspase-9 suicide gene triggered through a chemical inducer of dimerization (CID) was also added to these engineered T cells. He demonstrated that these CAR T cells migrated to tumor sites and had anti-leukemia effect. *Ex vivo*, Dr. Dotti emphasized that these cells were very dependent on cytokines, but cautioned that IL-2 can cause generation of regulatory T cells (Tregs). Therefore, IL-15 is a much more promising cytokine, despite its toxicity if administered systemically. Dr. Dotti then talked about CID treatment leading to a major depletion of suicide gene-expressing T cells after 30 minutes only. He summarized his talk emphasizing that IL-15 can enhance survival and

anti-tumor effects of CAR T cells, and that proliferation was antigen-dependent and suicide gene treatment successfully controlled graft versus host disease (GVHD) in 4/4 patients.

***Adoptive cell transfer of tumor-infiltrating lymphocytes: from melanoma to metastatic gastrointestinal cancers***

**Dr. Simon Turcotte's** work from NCI/NIH was based on the obtainment of a 20% complete response in melanoma patients with tumor associated lymphocyte (TIL) treatment. Given that the incidence and lethality are high in gastro-intestinal cancers, Dr. Turcotte was interested in transferring knowledge from immunotherapy trials for melanoma to treating GI cancers. He began his talk by stating that mutations which create neoantigens also have T cells specific for them. Specifically, if a mutation occurs in an antigen conferring high HLA affinity, T cells may be capable of responding to tumors. Similar to melanoma, he wanted to ask "can CD8 TILs in GI cancers recognize autologous cancer antigens?" Using 4-1BB as a marker to indicate "better" TILs, Dr. Turcotte concluded his talk indicating that 4-1BB selection allows for the isolation of antigen-reactive T cells regardless of maturation stage because the regulation of 4-1BB expression is more stable probably due to epigenetic control.

***Combination Immunotherapy: Adoptive T cell therapy followed by anti-CTLA4 Ab therapy***

From the University Health Network in Toronto, **Dr. Naoto Hirano** spoke about his work on novel combination immunotherapies. He began with background that T cell persistence is difficult to achieve in patients undergoing adoptive cell transfer. This is because patients must become immunosuppressed and cytokine consumption must be blocked in order for an anti-tumor CTL response with the intrinsic capacity to persist to occur. T cell activation requires a number of stimulating signals from accessory molecules including CD83 and IL-15. Engagement of CD83 allows prolonged expansion of T cells, whereas IL-15 expands long-lived antigen-specific T cells with a central memory phenotype. Dr. Hirano works with K562 artificial APCs (which express HLA, ICAM-1, LFA-3, CD80 and CD83), and he has demonstrated that co-culture allows for long term expansion (.5-1.5 years) of T cells. Dr. Hirano provides evidence that these T

cells express CD27, CD28, CCR7, but lack CTLA-4, PD1 and FOXP3 – indicative of a memory phenotype, and he shows that these T cells persist *in vivo* for up to 4 months (0.2%). In a phase I clinical trial for melanoma, Dr. Hirano used K562 artificial APCs pulsed with MART-1 peptide to generate an antigen-specific CTL response that successfully traffic to the tumor site and persist without prior lymphodepletion. Switching gears slightly, Dr. Hirano spoke about anti-CTLA-4 (Ipilimumab) therapy which promotes the expansion of infused antigen-specific CTL as memory T cells. He presents data that indicate that higher dose of anti-CTLA-4 at 10mg/ml results in a better response than the FDA-approved 3mg/ml which has very weak and limited effect. Dr. Hirano concludes his talk by indicating that anti-CTLA-4 can improve objective response to tumors.

***Rapamycin treatment endows CAR-engineered CD8$^+$ effector T cells with memory-like properties resulting in enhanced in vivo engraftment***

**Dr. Joanne Hammill** from McMaster University discussed the use of a second generation Her2-CAR T cell (with CD3zeta and CD28 domains with a CD8 hinge) and how this could boost CAR engraftment in adoptive cell therapy. Briefly, she talked about how T cell activation through TCR and cytokine receptors activates PI3K/mTOR signaling which leads to increased proliferation, glycolysis, and inhibiting CD62L expression. Rapamycin, a specific inhibitor of mTOR, is able to regulate T cell fate by promoting the memory phenotype – which are the more preferred T cell phenotype in adoptive cell therapy. Dr. Hammill described that rapamycin and IL-2 increases T cell viability compared to IL-2 alone, and is able to increase CD62L expression. In addition, Rapamycin also increases Eomes, T-bet, granzyme B, TNF-a, Myc, and decreases IFN-g and CPT1a. Memory-like Her-2 CAR T cells constitute a better population for ACT. Rapamycin treatment results in better engraftment of T cells in tumors in mice, and also increases the percentage of CAR T cells in blood and in tumor draining LN. Furthermore it delays tumor progression and increases survival in mice.

***Preclinial evaluation of oncolytic Maraba virus vaccines in murine and simian models***

**Dr. Jonathan Pol** from McMaster University spoke about the clinical potential of oncolytic viruses since they specifically infect, replicate and kill tumor cells. Specifically,

Maraba virus can be used as an oncolytic vaccine, and his work was done in a murine and simian model. He described the use of a MaraV-MAGE-A3 vaccine with a prime/boost protocol. However, MAGE peptides are expressed in testis, so this vaccine was difficult to test on male mice and simians. Dr. Pol concluded that MaraV infects B cells in the spleen mainly in mice and that MaraV is capable of infecting all tested tumors.

***The MHC I immunopeptidome of cancer cells***

**Dr. Claude Perreault** from the Université de Montréal began his talk by describing the immense number of peptides in a given cell population. He states that more than half of peptides are increased in immunoproteasome (IP) cells, and 15-20% of cells have unique peptides due to IP. MIP (MHC I peptide repertoire) of thymocytes and DC are different with 40% being unique to thymocytes. He explains that all cells therefore possess different antigens. 25% of MIP are differentially expressed on tumor cells, most of which are regulated at the translational level and consistently expressed at the mRNA level. Dr. Perreault explains that more abundantly presented peptides are not the result of transcription, translation, or degradation, but rather because of DRiPs (defective ribosomal products). Furthermore, rapamycin increases the amount of polyubiquitinated proteins which may constitute DRiPs. MIP repertoire conveys a preview of perturbed metabolic pathways in the affected cells - 13% of MIP are derived from low expression proteins. The MIP transcriptome is enriched in microRNA response element containing regions, which are preferential sources for MHC I peptides. Dr. Perreault concludes his talk by presenting  MiHA cannot be detected by mass spectrometry because compared to databases which do not take into account mutations and polymorphisms. Moreover, IP helps eliminate oxidized proteins which could help confer a survival advantage to tumor cells, considering many tumor cells increase expression of IP subunits.

***Receptor tyrosine kinase signaling establishes breast cancer immunosuppression***

**Dr. Josie Ursini-Siegel** from McGill University began her talk by asking, “How do RTK regulate tumor progression?” Briefly, ShcA is an adaptor protein that interacts with 80% of RTK. She explains that removal of ShcA binding site results in restoration of ShcA binding site in tumors, and restoring ShcA binding site results in restoration of tumorigenesis associated with RTK. Dr. Ursini-Siegel emphasized that 40% of ShcA

regulated genes are from the immune response, and that ShcA null tumors show a Th2 response. She shows that ShcA signaling induces an early immunosuppressive state in breast cancer progression during initial establishment of immunosuppression, and high ShcA expression in tumors correlates with low CD8 T cell infiltration. Dr. Ursini-Siegel concludes her talk suggesting the clinical significance of ShcA as a biomarker for stratifying breast cancer patients.

***B7-H4 modulates anti-tumor responses by limiting immunosuppression in the tumor microenvironment***

**Dr. Joanne Leung** from Institut de recherches cliniques de Montreal, began her talk by describing the relatively unknown B7 family member, B7-H4. She described B7-H4 mRNA to be ubiquitously expressed, however at the protein level, it is specific to B cells and APCs and the B7-H4 ligand remains unidentified. She notes that B7-H4 is highly overexpressed in multiple human cancers and is associated with cancer progression as it is correlated with a decreased infiltration of TILs. Moreover, B7-H4 can negatively regulate the expansion of neutrophils as well as the adaptive immune response. In a 4T1 transplant model, Dr. Leung works with B7-H4 KO mice which have tumors that express greater amounts of anti-tumor transcripts, iNOS, arginase, IL-10, and simultaneously decreased transcripts associated with a T cell response, namely T-bet. She notes that opposing pro- and anti-tumor factors are induced in the absence of B7-H4, and B7-H4 KO tumors grow more slowly and recruit more T cells. Dr. Leung concluded her talk by emphasizing that the absence of B7-H4 allows MDSC to better inhibit anti-tumor response via T cells. B7-H4 significantly enhances immunoediting.

***IL-15 influences spontaneous breast tumor formation and metastasis: immunomodulation within the tumor***

From McMaster University, **Dr. Amy Gillgrass** spoke about how IL-15 is able to promote the proliferation, survival, and activation of NK cells which contribute to the anti-tumor response. She describes that IL-15 can control the proliferation and survival of central memory CD8 T cells without T cell help and can rescue anergic CD8 T cells. Dr. Gillgrass' work primarily uses the spontaneous breast cancer model MMTV-PyMT, IL-15 transgenics (under the control of the MHC I promoter) and IL-15 knockouts. She

presents data which describe PyMT/IL-15 KO mice to have decreased NK cells and CD8 memory T cells, while PyMT/IL-15 transgenics have enhanced survival, decreased tumor metastasis to the lungs, and enhanced infiltration of NK (NK1.1, CD69, CD27) and CD8 cells at the tumor site. She describes the transgenics exhibiting more CD8 and but not CD4 T cells in tumors, and the CD8 phenotype is more of a central memory phenotype with higher production of IFN-γ and TNF-α, but decreased PD-1 compared to knockouts. Finally, in experiments where she depleted NK with NK1.1 antibodies in IL-15 transgenics, Dr. Gillgrass was able to revert their phenotypes back to wildtype, demonstrating the importance of these cells.

***BLp-25: ongoing development of a MUC-1 vaccine in Non Small Cell Lung Cancer***

**Dr. Denis Soulières** from the Université de Montréal described his work and his results from a randomized phase IIB trial of Stimuvax (vaccine targeting MUC-1). He begins by describing MUC-1 as an antigen that is expressed in many cancer types and is associated with metastatic potential and poor prognosis. The extra-cellular domain can be shredded and detected in the plasma. Also in cancer, Dr. Soulières describes that there is a hypoglycosylation process that results in a more exposed core of the protein. Stimuvax is a vaccine with a lipoprotein of 25 amino acids (BLP25) which is contained in a liposome. Dr. Soulières presented the results of the trial, describing that there was no difference with or without Stimuvax, only slight differences in locoregional disease. There was, however, a very low rate of adverse events and almost no severe one. No antibodies could be detected in the patients analyzed. Switching gears, Dr. Soulières also talked about another trial: MAGE3 vaccine, EGF vaccine and finally Lucanix (a mix of different cell types transfected with a TGF-β anti-sense to stimulate an immune response against a variety of antigens).

***Vaccines with Cancer/Testis Antigens***

From the Ludwig Institute for Cancer Research at memorial, **Dr. Sasha Gnjatic** reminds the audience of several known target antigens – namely NY-ESO1, p53 and MAGE. Dr. Gnjatic poses the question, “Why do patients with anti-NY-ESO1 response still have tumors?” Indeed, there is tolerance associated with this antigen. If this was combined with Ipilimumab (Anti-CTLA-4), Dr. Gnjatic described an increase in patient survival in

NY-ESO1+ patients and that antibodies could be generated with protein and overlapping peptide immunization. Long overlapping peptides (LOLP) are multi-epitopic, cross-presented on any HLA allele, and naturally-processed. These are all advantages that small peptides do not present. Dr. Gnjatic used LOLP together with TLR3 ligand (PolyIC) and showed a dramatic increase in antibody titers to NY-ESO1. TLR3 stimulation resulted in the most efficient induction of antibodies, CD4, and CD8 simultaneously. In experiments where Tregs were depleted by CD25-depletion preceding vaccination, immune response was promoted but unaffected if done following vaccination. In conclusion, Dr. Gnjatic described Montanide (IFA) may not be an optimal adjuvant because it may attract immune cells to the site of injection rather than to tumors.

***Ups and downs of small antigenic peptides for CD8 T cell vaccination against cancer***

**Dr. Daniel Speiser** from Ludwig Center for Cancer Research described his work using Montanide (IFA) combined with VLP or long peptide vaccination is able to induce higher T cell numbers, mostly effector memory cells. Short peptide vaccines, on the other hand, are best for mobilizing CD8 T cells and do not depend on cross-presentation. They may, however, tolerize T cells and are not targeted for DCs. Dr. Speiser suggested that the best adjuvant combination for small peptides is IFA and CpG. Exhausted TILs maintain their capacity to exert cytotoxic effects but eventually lose it. Inhibitory receptors, such as BTLA, 2B4, PD-1, TIM-3, LAG-3, are highly expressed on the surface of TILs. Dr. Speiser argues that all of these inhibitory markers may in fact be good targets for immunotherapy to reduce tolerance. Other inhibitory receptors include CD160 and KLRG-1.

FRIDAY

***Regulation of innate and adaptive immunity within the tumor microenvironment***

**Dr. Thomas Gajewski** from the University of Chicago began his talk by describing a microarray analysis demonstrating that expression of CXCL19, CCL21 and TCRα within the tumor correlated with better cancer outcome. The innate immunity with the secretion of chemokines seemed important for efficient recruitment of specific T cells. Using a model with implanted tumors, Dr. Gajewski demonstrated that an immune response

ensues within 5 days. Interestingly, IFNα/β-receptor-deficient mice fail to accumulate DCs in tumors, which are critical for T cell priming. He reminds his audience that TLR ligands, ATP and DNA, can trigger IFN-β in DCs, and that tumor DNA alone is sufficient to cause IFN-β production by DC in a p204, STING, and IRF3-dependent manner. Dr. Gajewski shows that DCs can then upregulate the expression of CD40 and CD86 in addition to the production of chemokines that orchestrate T cell infiltration into the tumor. Inflamed tumors are high in IDO, PDL-1, Tregs, and anergic T cells, creating a regulatory environment. Tumors in CD8- or IFN-γ- KO mice have no IDO or PDL-1, or Tregs (CD8 KO only), and these mechanisms are regulated by chemokines. Finally, Dr. Gajewski concludes his talk by speaking about anti-PD1 therapy in melanoma, and how it has demonstrated a striking 30% response in patients. Other plausible targets include DGK (ERG2) which is active in anergic T cells, LAG-3 and CRTAM which are upregulated in PD-1-positive CD8 T cells.

***The meaning of life through death: a MFG-E8 story***

From the Université de Montréal, **Dr. Jean-François Cailhier** spoke about immunogenic cell death as an important trigger for the immune system. He compares the release of ATP from autophagy as being able to induce macrophage recruitment, whereas apoptosis is non-immunogenic. He points out that chemotherapy is able to cause HMGB1 release, and is a process that induces high apoptosis in tumors and stroma. Macrophage TGF-β is able to direct macrophage polarization either towards the pro-inflammatory, M1 or the anti-inflammatory M2 phenotype. Dr. Cailhier describes that IL-6, IL-10, and phagocytosis of apoptotic cells can polarize macrophages to the M2 phenotype which are found within the tumor as anti-inflammatory TAMs. MFG-E8 is a phosphatidylserine opsonizing molecule which allows integrin-dependent phagocytosis by macrophages, therefore important for opsonization of apoptotic cells. However, MFG-E8 is not released as a consequence of necrosis. Apoptotic-conditioned media produces more anti-inflammatory cytokines and less pro-inflammatory molecules, however, MFG-E8 depletion reverses this effect. Dr. Cailhier described the importance of STAT3 phosphorylation for MFG-E8 release to mediate anti-inflammation, and he concludes that MFG-E8 could be an inflammatory switch promoting conversion from M1 to M2 macrophages.

*Activation of NF-kB in cancer cells*

**Dr. Yves St-Pierre** from INRS Armand-Frappier began his talk by discussing galectin binding to glycoproteins and its expression in myoepithelia cells. Galectin-7 is differentially expressed in breast cancer patients: it is highly expressed in Her-2 and basal-like phenotypes but not in low grade luminal A or B. Basal-like breast cancers have no Her-2 or hormone receptors and are highly metastatic. Interestingly, Galectin-7 transfection renders cells more metastatic - 53% of tumor cells expressing Galectin-7 will migrate. Dr. St-Pierre prompted the audience, "Why highly expressed in tumors?" The expression of Galectin-7 is activated by TNF-α and there is a NF-κB binding site in the Galectin-7 promoter. Deletion of this binding site impedes Galectin-7 expression. The model Dr. St-Pierre suggests is that TNF induces Galectin-7 through the NF-kB pathway, and Galectin-7 is able to prevent apoptosis. He concludes with data showing that Galectin-7 modulates Bcl-2 expression by sequestering it to repress apoptosis, and that Galectin-7 is involved in resistance to chemotherapy by interacting with Bcl-2.

*Indoleamine 2,3-dioxygenase, Tregs and cancer*

**Dr. David Munn** from the Medical College of Georgia began his talk about tolerance in tumors and tumor-draining LN and how this is acquired, active and dominant. He emphasizes that suppression is highly local and that the molecule IDO is counter-regulatory and is induced by inflammation. While IDO is able to suppress both the innate and adaptive immune response, it was important to note that IDO is a natural endogenous molecule, and is made in response to inflammation as a regulator molecule. A striking example is fetal rejection that occurs when IDO is blocked. Dr. Munn described apoptotic cells being potent inducers of IDO in the spleen, and being important for tolerance to self antigens as a result from cell death. IDO KO mice develop antibodies against self antigens derived from apoptotic cells, whereas IDO overexpression allows haplo-unmatched graft acceptance long term. In AML, high IDO expressers do poorly. IDO can be found in tumor cells and host cells, and can also be expressed in pDCs. Dr. Munn presents data that show high IDO correlates with poor prognosis in melanoma cohorts. He next describes the self-amplifying tolerogenic loop set in the tumor draining LN and

the tumor. IDO sends signals to Tregs which turn on CTLA-4 expression and leads to higher IDO expression – a vicious feedback loop that drives a tolerogenic state. IDO produces kynurenine and the metabolite tryptophan which leads to Treg signaling and activation. Degradation of tryptophan leads to local deprivation in amino acids sensed by GCN-2 and contributes to immunosuppression. Tumor draining LN Tregs are potent at suppressing but those from other LN are not. Dr. Munn argues that the presence of activated CD8 T cells is required which affects APCs and leads to Treg development and amplification. The FOXO3α transcription factor downstream of B7 ligation to CTLA-4 leads to IDO expression and suppression. CD40/CD40L interaction leads to blocking of IDO by the production of IL-6 and self-amplifies Th1 and immunogenicity. The combination of vaccines with IDO inhibitor 1-MT leads to tumor suppression, and IDO blockade is synergistic with CD40 agonist antibody. Dr. Munn described the utility of 1-MT which has gone through two phase 1 trials and is now in phase 2 trials. Interestingly, 1-MT treatment of patients with Ipilimumab results in hypophysitis due to hyperinflammation. Dr. Munn concludes his talk explaining that high expression of IDO in the tumor environment seems to be a predictor of patients who will better respond to Ipilimumab and that CD40/CD40L and IL-6 together can block IDO.

***Immune Homeostasis: to kill or be killed***

Concluding the 2012 CCIC was a talk by **Dr. Tak Mak** from the University Health Network. He began his talk by reminding the audience that T cells that have engaged antigen have the potential to expand every 5 hours, and that eventually, this response must cease. Indeed, cell death occurs in several ways. Knocking out Fas in T cells, Dr. Mak demonstrated that this leads to lymphopenia, and because germinal center B cells undergo high proliferation and are prone to mutations, B cells must be highly sensitive to apoptosis by Fas. Another crucial mediator of cell death is cytochrome c. Without cytochrome c ligation to Apaf-1, T cells and memory T cells fail to die or contract. Dr. Mak also spoke about viral persistence by PD-1 and IL-10. Switching gears slightly to cell survival, Dr. Mak presented data describing IL-7 treatment with LCMV promotes virus clearance – where vaccination and IL-7 treatment profoundly increases survival of mice. He notes that IL-7 decreases TNF-α and TGF-β levels, and renders T cells

refractory to Treg inhibition by affecting PD-1 expression. Furthermore, IL-7 promotes IL-22 production which protects the liver from liver damage (assayed by ALT/AST). Finally, IL-7 also promotes thymic output. In another example, Dr. Mak described TSLP KO mice which shut off neutrophil elastase causing tissue damage resembling Rheumatoid Arthritis. TSLP is a molecule that secretes SLP1, which inhibits neutrophil elastase and reduces tissue damage. He then speaks about TNF and its ability to control infections. Nfil3 (a gene involved in leukemia development) is a basic leucine zipper transcription factor that selectively reduces NK cells when it is knocked out. iRhom2 activates TACE and protects from TNF at huge doses. Dr. Mak concludes his talk with a final example of cell homeostasis regulator, 2MeO-E2, which is an immunosuppressant and inhibits B and T cell proliferation. By shutting off B and T cell calcium flux, this molecule is able to prevent mast cell degranulation.