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Cancer immunotherapy without frontiers: 2nd Annual Immuno-Oncology Meeting of the Centro de Investigación de Cancer en Sonora (CICS), Ciudad Obregón, Sonora México, Dec 2–4, 2016

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The Centro de Investigación de Cancer en Sonora (CICS, which stands in Spanish for the Sonora Cancer Research Center) is a non-profit organization headquartered in Ciudad Obregón, Sonora, México, with a research and translational development immuno-oncology laboratory in Seattle, WA, USA. CICS, which is devoted to performing clinical relevant research to promote better clinical outcomes for both childhood and adult cancer, organized its 2nd Immuno-Oncology congress that took place in Ciudad Obregón in December 2–4, 2016. This meeting brought together clinicians, scientists, students and postdoctoral fellows from United States, Canada and Mexico with the goal of breaking down international walls and establishing new collaborations. The meeting honored Dr. Jaime De la Garza Salazar, a prominent Mexican medical oncologist who headed the Mexican National Cancer Institute from 1993 to 2003 and pioneered the use of chemotherapy in Mexico. In this review we highlight some of the talks that took place in this Pan-American meeting.

Travis Young (California Institute for Biomedical Research, USA) described a novel platform that affords dose-titratable activity of genetically engineered chimeric antigen receptor (CAR) T cells for adoptive cell therapies (ACT). The state-of-the-art for engineering CAR-T cells uses an antibody single chain variable fragment (scFv). For treatment of B cell malignancies this has been most successful through targeting of CD19, which is also present on healthy B cells. However, this can cause permanent ablation of a patient's B cells as well as dangerous side effects such as cytokine release syndrome (also

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Conflicts of interest

Esteban Celis has filed patent applications based on the use of synthetic peptides and poly-IC combinatorial vaccines. The rights of the patent applications have been transferred to the Moffitt Cancer Center (Tampa, FL). All other authors do not have any conflict of interest.

known as cytokine storm). He described “switchable CAR-T cells” engineered to use an antibody-based “switch” molecule to engage their target in mouse tumor models. The soluble switch bridges a generic switchable CAR-T cell to a particular tumor antigen (e.g., CD19) and allows a fully tunable response through pharmacological titration of the switch. In the clinic, this should provide a safety measure by allowing the cells to be turned off in the case of adverse events and to allow B cells to repopulate after clearance of tumor cells in the treatment of B cell malignancies. Furthermore, the platform can be universal in that the same switchable CAR-T cell may be retargeted to multiple antigens simply by dosing a switch with different specificities for any desired antigen. This obviates the need to redesign a CAR-T cell for each new indication and may allow the retreatment of antigen-loss variants.

Brad Nelson (British Columbia Cancer Agency/BCCA, Victoria, Canada) reviewed basic concepts of ACT and briefly reviewed recent progress with two approaches: Tumor-Infiltrating Lymphocyte (TIL) therapy and CAR T cell therapy. He highlighted the current challenges in the ACT field, including toxicities such as “cytokine release syndrome”; off-tumor side effects, such as the loss of normal B cells with CD19 CART cell therapy; relapse due to antigen loss (e.g. CD19 loss); the paucity of good target antigens for most cancers; and the overall cost and feasibility of ACT. He then introduced the new ACT program being developed at BCCA and described the first three clinical trials they intend to conduct. First, they are working with Rob Holt, John Webb, John Bell, Natasha Kekre, Harry Atkins and others to create a publicly accessible CD19 CAR-T cell vector that can be shared at minimal cost between academic centers in Canada and beyond. Second, in 2017 they are launching a TIL therapy trial for gynecological cancers (i.e. ovary, endometrium, and cervix), which will build on Nelson’s extensive experience in studying TIL in ovarian cancer. Initially, they will use a protocol similar to Steven Rosenberg’s treatment for advanced melanoma. Future improvements will include methods to enrich tumor-reactive T cells from the TIL population, as well as the incorporation of known and novel forms of checkpoint blockade after TIL infusion. Third, Nelson’s team is developing a trial for relapsed follicular lymphoma in which patients will undergo ACT with CD4 and/or CD8 T cells specific for oncogenic driver mutations in their tumor. Finally, he described future directions for the ACT field. To eliminate the need for patients to receive systemic interleukin-2, his team is engineering cytokine receptors that will give clinicians exclusive control over T cells after they are reintroduced into the patient’s bloodstream. They are also engineering oncolytic viruses to express chemokines such as CXCL13, with the goal of rendering the tumor microenvironment more permissive to T cell infiltration and attack.

In a separate talk, **David Kroege**r, a fellow with Brad Nelson discussed the prognostic significance of tumor infiltrating lymphocytes in human high-grade serous ovarian cancer (HGSC). Although the presence of CD8 T cells within the tumor microenvironment is associated with better outcomes in most solid tumor types, the significance of other lymphocyte types remains controversial. Using multicolor immunohistochemistry Kroege

r found that patients with tumors exhibiting infiltration by multiple different lymphocyte types survived the longest. This included a group of “elite” patients whose tumors contained CD4 and CD8 T cells, CD20+ B cells, as well as CD38+ plasma cells. This group of patients

showed a remarkable (~60%) overall survival at 10 years. Thus, it appears that survival is greatest when both the cellular and antibody components of the immune system are engaged in antitumor immunity. Emerging evidence has linked mutation load (i.e., neoantigens) to clinical response to immunotherapy. However analysis of The Cancer Genome Atlas (TCGA) data showed no association between lymphocyte infiltration and expression of neoantigens in HGSC. However, cancer-testis (CT) antigens, such as NY-ESO-1, MAGE, and SSX antigens were strongly associated with the presence of plasma cells in tumors. Kroeger concluded that CT antigens might be important targets for immunotherapy particularly in tumor types such as HGSC that exhibit low mutational burden.

Esteban Celis (Georgia Cancer Center, Augusta University USA) described his group's peptide vaccination strategy aimed at inducing vast CD8 and CD4 antitumor T cell responses. This strategy follows the example of how immune responses take place during acute viral infections, which are known to generate massive numbers of antigen specific T cells in a short time period. The vaccine strategy utilized synthetic peptide constructs containing T cell epitopes that are optimized for MHC binding (heteroclitic epitopes) and modified to increase the overall amphiphilicity in order to self-associate into nanoparticles that resemble viruses. These peptides are mixed with poly-IC and are administered systemically (i.v. or i.m.) in order to disseminate the vaccine components throughout the lymphoid organs to improve the recruitment of antigen-specific T cells to the response. Celis' group has found that poly-IC functions as a potent T cell adjuvant due to its unique ability to stimulate 2 distinct pathogen recognition receptors, endosomal toll-like receptor 3 (TLR3) and cytoplasmic MDA-5. He explained that in order to generate huge numbers of antigen-reactive T cells, the vaccines first stimulate a TLR3-mediated response, which primes the naïve T cells in an IL-12 dependent manner. Subsequently, the vaccine boost (5–12 days after the prime) stimulates MDA5, generating a type-I interferon response that induces the vast expansion of the T cells. He hypothesizes that the boost mimics the situation when T cells encounter antigen in non-professional APCs that produce type-I interferon as a consequence of infection. Formulation of poly-IC with poly-lysine and carboxy-methylcellulose (poly-ICLC) facilitates the escape of poly-IC from the endosomal compartments into the cytoplasm allowing MDA-5 activation. The potency of these peptide vaccines can be further enhanced by the use of agonistic anti-CD40 antibodies or IL-2/anti-IL-2 complexes. Experiments in several mouse tumor models demonstrates that these peptide vaccines have the ability to eradicate established tumors, but in some instances PD-1 blockade needs to be included in the vaccination protocol.

Suzanne Ostrand-Rosenberg (University of Maryland Baltimore County, USA) described two novel cancer immunotherapeutics designed to simultaneously activate tumor-reactive T cells and neutralize PD1-mediated pathway immune suppression. One therapeutic is a bi-specific T cell engager (BiTE) that consists of the variable regions of anti-CD3 and anti-PD-L1 monoclonal antibodies. In vitro studies showed BiTE mobilized CD4 and CD8 T cells from healthy donors and cancer patients rendered them cytotoxic for multiple PD-L1⁺ cancer cells. She also described a soluble version of the costimulatory molecule CD80 (sCD80) as a cancer immunotherapeutic. In vivo studies demonstrated that treatment with sCD80 significantly delayed progression of large established mouse tumors. The last

segment of her talk focused on myeloid-derived suppressor cells (MDSC). MDSC are a significant obstacle to many cancer immunotherapies and various strategies are being explored to neutralize their function. She presented studies showing that the Nrf2 gene is critical for promoting MDSC survival in the inflammatory and hypoxic tumor environment, and suggested that modulation of this gene may provide an avenue for reducing MDSC survival.

Elizabeth Repasky and graduate student **Mark Bucsek** (Roswell Park Cancer Institute, USA) each gave separate talks highlighting the role of newly recognized players in the regulation of the immune phenotype of tumors: sympathetic nerves, adrenergic stress and the neurotransmitter, norepinephrine. Mice are exquisitely sensitive to ambient temperature and under conditions of even mild cold stress they increase heat production via adaptive thermogenesis, a process which depends upon sympathetic nerves and norepinephrine signaling through β -adrenergic receptors (β -AR). While researching the impact of commonly used mildly sub-thermoneutral housing temperature, they found enhanced baseline tumor growth rates in several murine tumor models as compared to those observed in mice housed at a thermoneutral temperatures. The data presented showed that treatment of mice exhibiting increased adrenergic stress (due to cool housing temperatures) with the pan- β -blocker, propranolol slowed tumor growth compared to non-treated mice. Pharmacologically blocking β -AR signaling using propranolol resulted in tumors with a significant increase in frequency of effector CD8 T cells with a corresponding decrease in suppressive Tregs. The suppressive role of β -AR signaling in immune cells was confirmed by showing that stress induced temperature had no suppressive impact in mice deficient in β_2 -AR receptors (isoform found on immune cells). They showed that while anti-PD-1 checkpoint blockade had minimal antitumor efficacy when used as a monotherapy in mice housed under standard cool conditions, combination with propranolol increased the efficacy. Understanding the degree to which immunosuppression is regulated by adrenergic stress, may reveal additional novel combinations using FDA approved, safe, and readily available β -blockers which could improve immunotherapy in patients as well as helping to identify biomarkers of therapeutic resistance.

Arturo Suplee (Sonora Cancer Research Center, CICS Mexico) talked about his ongoing studies to assess the expression of CD3, CD8, PD-L1 and T-BET in ovarian cancer and their potential use as biomarkers. Current clinical trials in ovarian cancer with immunotherapy have failed in part due to the lack of companion biomarkers that will help identify patients that will benefit from such therapies. He has compared the tissue expression levels of these markers by immunohistochemistry in three subtypes of ovarian cancer. He did not find evidence suggesting differing expressions of CD8, CD3, PD-L1, or T-BET across clear cell, endometrioid and high-grade serous subtypes. The lack of difference suggests that these subtypes of ovarian cancer could be treated similarly with immune based therapies such PD-1 blockade.

Alejandro Camacho-Hernandez (Sonora Cancer Research Center, CICS Mexico) talked about their use of Metformin, an oral hypoglycemic agent to evaluate its potential anti-tumorigenic effect. He mentioned some potential mechanisms for the ability of metformin to suppress cancer growth that are being explored: 1) activation of LKB1/AMPK pathway; 2)

induction of cell cycle arrest and/or apoptosis; 3) inhibition of protein synthesis; 4) reduction of circulating insulin levels; and 5) activation of immune system and eradication of cancer cells.

Yasmin Thanavala (Roswell Park Cancer Institute, USA) discussed the ability of the anti-angiogenic drug sorafenib to reduce the burden of immunosuppressive cells in patients with advanced hepatocellular carcinoma (HCC). Sorafenib is a small molecule inhibitor of multiple tyrosine protein kinases, including VEGF receptors (VEGFR), which are also expressed on immunosuppressive cells. She tested the hypothesis that sorafenib could reduce the burden of immunosuppressive cell types in HCC patients, and as a consequence, invigorate anti-tumor effector T cell responses. She presented data demonstrating the association of PD-1 decreased T cell expression with improved overall survival (OS) of patients following sorafenib therapy. Additional findings included improvement in OS that was observed in patients exhibiting a greater decrease in the number of T regulatory cells (Tregs) and the ratio of effector cells to Tregs was significantly increased following sorafenib therapy. The studies from the Thanavala group suggest that inhibiting the VEGFR signaling pathway may potentially be a useful adjunct in combination with immunotherapeutic approaches to enhance the therapeutic efficacy of immune-based strategies against advanced malignancies.

Juan Pablo Marquez (Sonora Cancer Research Center, CICS Seattle WA USA) delivered two talks. The first one was an educational workshop “Immuno-oncology for clinicians” and in the second one he talked about the use of immunomodulatory drugs in combination with immunotherapy in pancreatic cancer. He mentioned that immunomodulatory antibodies require a better trial design as we can have progressions if we have activation of Tregs and Th2 cells rather than pseudoprogressions (immune infiltration of effector Th1 and CD8 cells). He remarked that we urgently need validated biomarkers to rationally prescribe anti-blockade antibodies (CTLA-4, PD-1, PD-L1), in order to avoid clinical and economical toxicity. He presented data on how some drugs could be repurposed as potential immunomodulatory cancer agents to improve clinical outcomes in pancreatic ductal adenocarcinoma (PDAC). Patients were treated with bortezomib, meloxicam and erdosteine to evaluate if this combination at low doses could promote CD8 and Th1 cell responses and decrease pro-tumor cytokines such as IL-6, IL-8 and IL-18. Future studies will combine peptide vaccines and immunomodulatory repurposed drugs.

Arvind Thakur (AccuTheranostics, Inc., USA) described the development and technical validation of a chemosensitivity (ChemoFit™) test. This test uses the patient’s own tumor cells to identify chemotherapy drugs have the most potential for treating the patient’s cancer. Drug effect on the viability of tumor cells is assessed by flow cytometry using multiple fluorophores and a chemo-resistance/sensitivity drug profile is generated to assist oncologist in their treatment management decision.

Jaime De la Garza-Salazar (Instituto Nacional de Cancerología de México) gave a talk on inflammatory breast cancer (IBC) and mentioned that this rare disease is the most aggressive type of breast tumors representing only 5% of all the breast cancer. Diagnosis is made through clinical observation, but histologic confirmation is needed where lymphocyte

infiltration to the breast skin confirms diagnosis. Most patients have been through menopause when receive the diagnosis. Current therapy for IBC relies in a combination of neo-adjuvant chemotherapy, surgery, radiotherapy and target therapy. Anthracycline is currently the pillar for systemic therapy. Novel therapies being explored for IBC include checkpoint blockade and VEGF and EGFR inhibitors.

Erik Ramos (Sonora Cancer Research Center CICS Seattle WA USA) talked about immunotherapeutic targets for pancreatic ductal adenocarcinoma (PDAC). He discussed the current limitation of chemotherapy and active immunotherapy treatment options and the obstacles given by the microenvironment in relation to optimal treatment delivery and recognition of malignant cells by the immune system. He is working in a preventive cancer vaccine for PDAC to reduce the disease by targeting pancreatic intraepithelial neoplasia (PanIN). The approach is to educate the immune system to recognize proteins that are overexpressed in stages of premalignant lesions (PanIN-1 to PanIN-3) and to target high-risk individuals and patients with chronic inflammatory pancreatic conditions. He described how this would reduce the disease progression by stimulating the correct immune response and destroy PanIN at the proper time with a multi-peptide vaccine to block simultaneously several oncogenic drivers biologically important in pre-malignant pancreatic cells. He is currently assessing humoral (IgG and IgA) immune responses to the proteins overexpressed in PanIN. Subsequently, he will proceed to identify the most immunogenic regions of potentially candidate proteins capable of stimulating Th1 responses to develop peptide vaccines.

Dolores Gallardo-Rincón (Instituto Nacional de Cancerología de México) gave a detailed talk regarding the patterns of ovarian cancer recurrences. She mentioned that high-grade papillary serous is the most common diagnosed type of ovarian cancer. Most patients are diagnosed in advanced stages IIIc or IV and 75% of patients will present a recurrence. Basal low levels of serum albumin (< 3g/dl) are associated with recurrence in 80% of patients. In contrast, levels > 4g/dl are associated with only 27% rate of recurrence. Checkpoint blockade inhibitors are being explored as an immunotherapy approach for ovarian cancer.

Since the meeting had a strong learning component to disseminate current knowledge about the use of cancer immunotherapy to clinical community several educational talks were given. **Luis Oñate-Ocaña** (Instituto Nacional de Cancerología de México) talked about developing TIL therapy for colorectal cancer. **Pedro Lucero-Díaz** (Sonora Cancer Research Center, CICS Mexico) spoke about immunogenic chemotherapy and radiotherapy. **Alberto Durazo-Acuña** (Sonora Cancer Research Center, CICS Mexico) spoke about the need and challenges of developing immunotherapy for neuro tumors such as astrocytic glioma grade IV, otherwise referred as glioblastoma. **Jorge Martinez-Tlahuel** (Instituto Nacional de Cancerología de México) talked about the adverse events that are related to cancer immunotherapy. **Jose Matute-Briceño** (Sonora Cancer Research Center, CICS México) spoke on the clinical importance of studying the immune infiltration patterns in solid tumors.

The consensus of the participants and the audience was that the main goal of bringing together basic and translational scientists in the field of cancer immunotherapy from Mexico, Canada and the USA was a success for the development of new strong collaborative efforts

that should be able to overcome any geographical, physical or political barriers that could stand on the way of helping cancer patients. The meeting ended with the announcement that the 3rd Annual Immuno-Oncology Meeting of the Centro de Investigación de Cancer en Sonora, CICS México will take place in San Diego in October 2017.

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Abbreviations

ACT	Adoptive T cell therapy
β-AR	β-adrenergic receptor
BiTE	Bi-specific T cell engager
CAR	Chimeric antigen receptor
CICS	Centro de Investigación de Cancer en Sonora
CT	Cancer-testes
HCC	Hepatocellular carcinoma
HGSC	High-grade serous ovarian cancer
IBF	Inflammatory breast cancer
MDA5	Melanoma Differentiation-Associated protein 5
MDSC	Myeloid derived suppressor cell
OS	Overall survival
PanIN	Pancreatic intraepithelial neoplasia
PDAC	Pancreatic ductal adenocarcinoma
Poly-IC	Polyinosinic polycytidylic acid
scFv	Single chain variable fragment
TCGA	The cancer genome atlas
TIL	Tumor-infiltrating lymphocyte
TLR3	Toll-like receptor 3
Treg	T regulatory cell