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MEETING REPORT

The Summit for Cancer Immunotherapy (Summit4CI), June 26–29, 2016 Halifax, Canada

Maartje C. A. Wouters[1] · Céline M. Laumont[2] · Branson Chen[3] · Seong Jun Han[4,9] · Kathy Matuszewska[5] · Kyle Potts[6] · Jeanette E. Boudreau[7] · Connie. M. Krawczyk[8]

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Abbreviations

CAR	Chimeric antigen receptor
CCIC	Canadian Cancer Immunotherapy Consortium
CRISPR	Clustered regularly interspaced short palindromic repeats
CTLA4	Cytotoxic T lymphocyte-associated protein-4
DC	Dendritic cell
FZD5	Frizzled class receptor 5
HER2	Human epidermal growth factor receptor-2
IFN	Interferon
IL	Interleukin
IRE-1	Inositol-requiring enzyme-1
MEK	Mitogen-activated protein kinase
MHC	Major histocompatibility complex
NK	Natural killer
NKT	Natural killer T
PD-1	Programmed cell death protein-1
PD-L	Programmed death ligand
RAS	RAS-type GTPase family
RGMb	Repulsive guidance molecule b
RNF43	Ring finger protein 43
TAC	T cell antigen coupler
TEX	Tumor-derived exosomes
TIL	Tumor-infiltrating lymphocytes
TIM-3	T cell immunoglobulin and mucin domain 3
TME	Tumor microenvironment
T-Vec	Talimogene laherparepvec
VSV	Vesicular stomatitis virus
XBP1	X-box binding protein-1

Maartje C. A. Wouters, Jeanette E. Boudreau, and Connie. M. Krawczyk contributed equally.

✉ Connie. M. Krawczyk
connie.krawczyk@mcgill.ca

[1] Deeley Research Centre, British Columbia Cancer Agency, 2410 Lee Avenue, Victoria, BC V8R 6V5, Canada

[2] Institute for Research in Immunology and Cancer, Université de Montréal, Montreal, QC, Canada

[3] Department of Laboratory Medicine and Pathobiology, Toronto General Research Institute, University of Toronto, Princess Margaret Cancer Research Tower 2-701, 101 College St, Toronto, ON M5G 1L7, Canada

[4] Campbell Family Institute for Breast Cancer Research, Princess Margaret Cancer Centre, University Health Network, Toronto, Canada

[5] Department of Biomedical Sciences, University of Guelph, 50 Stone Road E., Guelph, ON N1G 2W1, Canada

[6] Department of Oncology, Faculty of Medicine and Dentistry, Cancer Research Institute of Northern Alberta (CRINA), University of Alberta, 116 St. and 85 Ave., Edmonton, AB T6G 2R3, Canada

[7] Departments of Pathology and Microbiology & Immunology, Dalhousie University, Sir Charles Tupper Medical Building, 5850 College Street, Halifax, NS B3H 4R2, Canada

[8] Departments of Microbiology and Immunology and Physiology, Goodman Cancer Research Center, McIntyre Building, Room 705B McGill University, 3655 Promenade Sir William Osler, Montreal, QC H3G 1Y6, Canada

[9] Department of Immunology, University of Toronto, University Health Network, 610 University Ave., Toronto, ON M5G 2M9, Canada

Introduction

In June 2016, the Canadian Cancer Immunotherapy Consortium (CCIC), an association dedicated to the scientific advancement of immunotherapy for cancer, partnered with BioCanRx, a Network of Centre of Excellence program focused on biotherapeutics for cancer treatment, to hold the Summit for Cancer Immunotherapy (Summit4CI) in Halifax, Canada. The synergy of these two organizations—both highly active in the field of cancer biotherapy and supported by the Government of Canada—offered a unique opportunity for multidisciplinary discussions and trainee events. Bringing together researchers, trainees, policy makers, patients, physicians, advocates, and industry partners, this unique event presented a holistic perspective on the past, present, and future of cancer immunotherapy, considering how scientific and logistical advances will translate cutting-edge research into novel therapeutics.

Training of highly qualified personnel was a theme and focus throughout the meeting. To foster the next generation of cancer scientists, meeting organizers provided travel awards to 100 trainees and hosted dedicated career development and community outreach sessions. A selection of HQP poster presenters presented their work in plenary in trainee talks and speed poster sessions that offered a highly visible platform from which to demonstrate the cutting-edge research ongoing in Canadian laboratories. Career panels, a "meet the expert" lunch session, and introductions to organizations BioTalent Canada and Let's Talk Science fostered trainees' career development as opportunities for skills development and involvement in the larger community. Finally, the Halifax Public Library hosted a forum about cancer immunotherapy that featured patients, researchers, and clinicians to expand the access of the expertise featured at the Summit4CI to community members.

Kathy Barnard (Save Your Skin Foundation, Vancouver, Canada) opened the Summit with an inspiring firsthand account of her experiences as a melanoma patient and involvement with immunotherapy. She described her journey through traditional treatments and immunotherapies including surgeries, chemotherapy, Proleukin (human recombinant interleukin-2, IL-2), and anti-CTLA4. Barnard's story emphasized the struggles, uncertainty, and hope that a cancer patient experiences. Her cancer treatment "ahead of the curve" has kept pace with research progress and clinical trials, and illustrates how much has been accomplished in the field of cancer immunotherapy.

In an opening night keynote, **Ira Mellman (Genentech, San Francisco, USA)** described how an increasing understanding of immune function is enabling deliberate immunotherapy. For example, identification of IL-2 and IFN-γ as important factors for T cell growth and function led to their clinical use in cancer patients, and identification of immune regulation led to a range of trials using checkpoint inhibitors targeting the programed death receptor-1 (PD-1) and its ligand (PD-L1) and anti-Cytotoxic T Lymphocyte-Associated Protein-4 (CTLA4) which aim to rescue tumor-reactive T cells from inhibition. Although great strides in immunotherapy have been made, much remains to be determined with respect to immune regulation, tumor growth, and immune evasion to foster precision and personalized cancer medicine. Bookending a highly stimulating conference, **Elizabeth Jaffee (The Sydney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, USA)** provided a closing keynote on the challenges facing immunotherapy of pancreatic cancer, a highly immune-exclusionary tumor that has been refractory to chemotherapy and immunotherapy. Reflecting what is likely the future of immunotherapy for many difficult-to-treat cancers, Jaffee is turning to combination treatments and patient-centered approaches.

Cancer immunotherapy is collecting success stories, but much remains to be accomplished before every tumor can be specifically targeted. The Summit4CI appraised the challenges, objectives, ongoing approaches, and next-generation strategies that might make immunotherapy a universally available option for patients with cancer.

The suppressive tumor microenvironment

Interactions between immune, stromal, and tumor cells can establish an immunosuppressive tumor microenvironment (TME), influencing patient prognosis and treatment outcomes. Successful immunotherapy should consider the positive and negative elements predicted by the existing immune landscape of a tumor, with the goal of fostering the anti-tumor immune response and limiting immunosuppression by the TME.

David Brooks (Princess Margaret Cancer Centre, University Health Network, Toronto, Canada) showed that the chronic inflammation present in the TME can drive T cells toward an "exhausted" or dysfunctional state: the combination of type I IFN, IL-10, and PD-1 in the TME can negatively impact generation of a cytotoxic T cell response. **Laurie Glimcher (Dana-Farber Cancer Institute, Boston, USA)** showed that advanced ovarian tumors host a large number of highly immunosuppressive DCs that constitutively express X-box Binding Protein-1 (XBP1), a transcription factor associated with the regulation of immune responses. During a stress response in the endoplasmic reticulum, the Inositol-Requiring Enzyme-1 (IRE-1) transmembrane kinase and endoribonuclease becomes activated and splices XBP1, which acts to induce its canonical target genes of chaperones, disulfide isomerase and glycosylases,

and non-canonical target genes in lipid metabolism. Potential therapeutic applications of IRE-1 or XBP1 inhibition within the tumor-resident DCs to restrict lipogenesis and enhance anti-tumor immune response were successfully explored in preclinical models and may provide new options to improve immunotherapeutic approaches in ovarian cancer.

Mast cells, explained **Jean Marshall (Dalhousie University, Halifax, Canada)**, are also key players coordinating inflammation in the TME through production of mediators, including type I IFN. Mast cell infiltration can enable tumor growth by inducing myeloid-derived suppressor cells and tumor-infiltrating lymphocytes (TIL); conversely, through inflammatory mediators, mast cells can also tip the balance of immunity for anti-cancer functions. Production of histamine by mast cells decreases suppressive monocytes and delays tumor growth in preclinical models. In the B16-F10 melanoma model, triggering of toll-like receptor-2 signaling on mast cells leads to increased chemokine expression and recruitment of CD8 T cells and NK cells, which attenuate tumor growth.

Non-cellular components of the TME that may influence the infiltration of immune cells are tumor-derived exosomes (TEX). **Theresa Whiteside (The University of Pittsburgh, Pittsburgh, USA)** explained that TEX dampen immune cell functions by increasing regulatory T cells and myeloid-derived suppressor cells, while decreasing CD8 T cell proliferation. Analysis of TEX obtained from the plasma of patients with acute myeloid leukemia carried immunosuppressive molecules and leukemia blast markers. To induce more sustainable anti-tumor responses with available immunotherapeutics, the immunosuppressive roles of TEX should, therefore, be considered and targeted.

Certainly, inter-patient distinctions exist in the immune environment and surrounding tissues of tumors that may indicate the degree of ongoing suppression or successful anti-tumor immune responses. **Marie-Caroline Dieu-Nosjean (INSERM, Paris, France)** described the positive prognostic impact of tertiary lymphoid structures in the TME of non-small cell lung cancer patients. Composed of T cell-rich areas (i.e., mainly T cells and mature dendritic cells (DCs)) adjacent to a B cell zone (i.e., mainly B cells, follicular dendritic cells, and macrophages), tertiary lymphoid structures act as local ectopic lymph nodes by allowing priming of CD8 T cells directly at the tumor site, resulting in memory CD8 T and type 1 T helper cells, both known to favor tumor cell killing. The B cell zone consists of naïve, memory, and pre-plasma B cells, which were correlated to humoral responses. The presence of DCs and B cells, two antigen-presenting cells in tertiary lymphoid structures, was highly associated with improved prognosis and more T cell infiltrate, showing the importance for these APCs in the TME to favor anti-tumor responses. By understanding the characteristics of a "beneficial" TME, it may be possible to tailor immunotherapeutic approaches to favor anti-tumor immunity over immunosuppression.

Reversing immune inhibition with checkpoint blockade

The success of checkpoint blockades is one of the most important interventions leading to the expansion of cancer immunotherapy and a testament to the suppressive nature of the TME. Still, checkpoint inhibition strategies (i.e., anti-PD-1, anti-CTLA4) are not universally successful, calling for further approaches to understand how cytotoxic immunity can be best supported in the TME. The future of cancer immunotherapy, asserted **Gordon Freeman (Harvard Medical School, Boston, USA)**, will include genomics screening for personalized immunotherapeutic strategies such as neoantigen vaccines. He is using The Cancer Genome Atlas messenger RNA database to gain insights to more precise approaches for checkpoint inhibition therapies. Using this method, he has shown that genetic analysis can identify patient groups with PD-L1 genomic amplification, DNA repair defects, or viral gene expression with higher responses rates to anti-PD1/PDL-1 therapy, and thus, these genetic parameters could be used to stratify patients for personalized treatment.

By performing immunohistochemistry on patient tumors, Mellman's group has defined three distinct phenotypes: inflamed, where tumors are infiltrated with immune cells; excluded infiltrate, in which T cells appear tumor-specific, but are restricted to the stroma surrounding the tumor; and immune desert, where tumors have no immune infiltrate and low expression of major histocompatibility complexes (MHC). They find that immune checkpoint inhibition works best in the inflamed subtype, in the presence of pre-existing T cell responses.

Freeman demonstrated that repulsive guidance molecule family member B (RGMb), is a novel binding partner of PD-L2. Simultaneous blockade of RGMb and PD-1 increased survival in the mouse CT26 colon carcinoma model, indicating an additive benefit of the double blockade. Further experiments demonstrated synergistic potential of PD-1 or PD-L1 monoclonal antibodies in combination therapy with chimeric antigen receptor (CAR) T cells and approaches that promote antigen release, such as radiation, chemotherapy, and oncolytic viral therapies. He found that the T cells from some patients developing resistance to anti-PD-1 therapy express higher levels of the T cell immunoglobulin and mucin domain 3 (TIM-3), raising the possibility that anti-TIM-3 therapy after PD-1 resistance may enable durable anti-tumor immune responses.

Samantha Burugu, a trainee from the laboratory of **Torsten Nielsen (University of British Columbia, Vancouver, Canada)**, showed that T cell responsiveness and cancer prognosis were inversely correlated with expression of inhibitory receptors. They examined the expression of targetable immune checkpoint markers PD-1, TIM-3, PD-L1, and Lymphocyte-activation gene-3 on TIL in tumor epithelium or stroma of breast tumors and found that all four markers were significantly associated with high-risk hormone receptor negative for the Human epidermal growth factor receptor-2 ($HER2^+$) and basal-like subtypes of breast cancer. Altogether, this work supports efforts to continue to identify targetable inhibitory receptors, and combined treatment to facilitate durable and ongoing anti-tumor immunity.

Xingxing Zang (Albert Einstein College of Medicine, New York, USA) presented his work on alternative B7 receptor families such as B7H3, B7H4, and B7H7 and their potential as the next targets for immunotherapy. B7H3 and B7H4 were both found to be over-expressed in certain human cancers and its expression was correlated with poor clinical outcomes. Anti-B7H4 therapy leads to an increase in the anti-tumor immune response in mice. B7H7 was shown to inhibit human CD4 and CD8 T cell function, and while it was highly elevated in various human cancers from lung cancer to osteosarcoma, its expression was limited in healthy tissues. Interestingly, transmembrane and immunoglobulin domain containing 2, part of the CD28 family, was one of the receptors for B7H7, further adding to the list of new immunotherapeutic targets.

As the number of checkpoint inhibitors quickly rises and multiple new strategies are underway, leading to more and more patients being treated with these agents with accompanying rising costs, the need for companion diagnostic tests grows. This issue was addressed in a **molecular diagnostics panel** in which the issues involved in getting the diagnostics approved were discussed. The panel agreed there is a need for all Canadian stakeholders, including researchers, Health Canada, insurances, industry, and patients, to have a dialogue about these issues. A pan-Canadian alliance should be formed for improved collaboration to speed up the decision-making process and make sure that the drugs and diagnostics are equally available in all provinces.

Strategies to activate, recruit, and support anti-tumor lymphocytes

T cells and antibodies engineered for anti-cancer impacts are already promising in the clinical setting, and methods to accurately, rapidly, and comprehensively identify tumor antigens will facilitate improvements for targeted immunotherapy. While prevalence and frequencies of mutations in some proteins are evident, many tumors have a unique molecular profile including the expression of tumor antigens that reflects the distinct ontogeny of a patient's tumor. Activating, recruiting, and supporting T cell function within the tumor has been challenging, owing to the immunosuppressive nature of the TME and the relative paucity of pre-existing effector cells with specificity for tumor antigens.

Yuki Kagoya, trainee in **Naoto Hirano's laboratory (The Princess Margaret Cancer Center, University Health Network, Toronto, Canada)**, described the difficulties in obtaining sufficient numbers of stem cell-like and central memory T cells to use in engineered adoptive cell therapies. To solve this problem, he is treating CD3-activated T cells with the Bromodomain and extra-terminal protein inhibitor, JQ1, and reports that CAR T cells expanded in this condition demonstrate greater persistence and anti-tumor effects in tumor-bearing mice.

Christopher Helsen, a post-doctoral fellow in **Jonathan Bramson's lab (McMaster University, Hamilton, Canada)**, discussed genetically engineered T cell antigen coupler (TAC) receptors. Similar to CAR T cells, TAC receptors redirect T cell killing toward a tumor antigen and recruit the T cell receptor, but TACs provide the additional advantage of recruiting co-stimulatory signals via incorporation of CD4. TAC T cells demonstrated cytotoxicity *in vitro* against 4 cell lines, and in preclinical in vivo models of ovarian and breast cancer without evidence of dose-limiting toxicity.

Ideally, T cells will respond to antigens specific to the tumor, but because tumors are derived from healthy "self" tissues, potently responsive T cells are often edited out of the patient's repertoire. **Victor Engelhard (The University of Virginia School of Medicine, Charlottesville, USA)** explained that an accelerated rate of phosphorylation in the tumor, compared with healthy tissues, led to increased biogenesis of tumor antigens in the form of phosphopeptides, and suggested that their identification could reveal novel, targetable antigens.

Similar to T cell-based strategies, the identification of novel tumor neoantigens for antibody targeting may be key to developing new antibodies for cancer. **Jason Moffat (University of Toronto, Toronto, Canada)** described high-resolution CRISPR screens to identify genes for tumor "fitness" using a panel of human cell lines. His group identified a ring finger protein-43 (RNF43) mutation prevalent in multiple cancers, including pancreatic cancer. RNF43 encodes an E3 ubiquitin ligase that negatively regulates Wnt signaling. They showed that FZD5, a frizzled class receptor, is required for the growth of RNF43-mutant pancreatic cancer cells. To block RNF43-mediated growth, they synthesized an anti-FZD5 antibody which successfully

regulated RNF43-mutant patient-derived xenografts and pancreatic cancer cell lines *in vitro* and fosters improved survival in in vivo models.

Combining genomics and immunopeptidomics, **Lélia Delamarre (Genentech, San Francisco, USA)** has evaluated the bioinformatics methods to select mutant MHC I peptides and found that the level of expression of the mutant gene together with MHC I binding affinity prediction help identifying MHC I peptide candidates. They further identified features that were likely to predict the ability of T cells to respond to the peptide; peptides with mutations sticking out of the MHC I groove were more likely to stimulate T cell responses. Similarly, work of trainee presenter **Hillary Pearson** from **Claude Perreault's laboratory (IRIC, Université de Montréal, Montreal, Canada)** demonstrated that the immunopeptidome of Epstein-Barr virus positive B lymphoblastic cell lines is selective: only ~10% of the exome can be presented on MHC. They found this to be attributable to gene expression levels, protein abundance and length, as well as a preference to proteasomal degradation resulting from disordered regions and ubiquitylation of lysines. Aggregating these and other features in a linear regression model, using RNA-sequencing data as input, accurately predicted MHC-associated peptides.

John Babcook (Zymeworks Vancouver, Canada) described structure-guided protein engineering for therapies aimed at immune modulation. The ZymeLink™ drug-conjugate platform specifically delivers cytotoxic payloads to tumor cells exhibiting target antigens. In preclinical studies, this approach has demonstrated anti-tumor efficacy and tolerability extending the therapeutic window. Additional drug conjugates are currently being explored for efficacy and safety to bring forward into the clinic.

Oncolytic virus engineering for cancer treatment

Session chair, **Brian Lichty (McMaster University and Turnstone Biologics, Hamilton, Canada)**, discussed past approaches, current challenges, and future avenues to improve the success of oncolytic viruses. BioCanRx Scientific Director and oncolytic virus pioneer, **John Bell (The Ottawa Hospital, University of Ottawa, Ottawa, Canada)**, described oncolytic viruses as a multifaceted platform for cancer treatment, owing to their anti-vascular and cytolytic capacities. Mutations within the tumor that enable resistance to interferon signaling, a mechanism by which viral infection is often controlled, allow selective replication of oncolytic viruses in the tumor and open the possibility to deliver specific and complementary therapeutic agents. For these viruses, entry into the tumor may be facilitated by their infection of the tumor's endothelial vasculature, explained Bell. Vascular endothelial growth factor, upregulated by the tumor to facilitate angiogenesis and cancer growth, stimulates the activity of the transcriptional repressor, Blimp-1, which suppress the type I IFN response, allowing selective and ongoing growth of the virus.

Features of the host and tumor might hinder successful, ongoing oncolysis, including the existence of pre-existing immunity against the oncolytic virus. One approach to overcome this challenge, taken by Bell and Lichty, is to use Maraba virus, for which pre-existing immunity is not expected in the human population. After priming an immune response against tumor-associated antigens using recombinant adenovirus, Lichty is administering Maraba virus encoding the same tumor-associated antigen to induce potent boost of the memory T cell response. This prime-boost strategy has shown a good safety profile in non-human primate preclinical trials and is now the subject of a clinical trial in patients with advanced or metastatic solid tumors.

Although reduced antiviral responses that foster cancer growth enable selective replication of oncolytic viruses, some tumor cells maintain intact defense mechanisms against viral infection and are therefore resistant to oncolysis. **Kensuke Hirasawa (Memorial University, St. John's, Canada)** observed that the RAS-type GTPase family and Mitogen-activated protein kinase (RAS/MEK) pathway inhibits the function of cellular antiviral protein Interferon regulatory factor-1 through post-translational sumoylation. Tumor cells overexpressing Interferon regulatory factor-1 also resist vesicular stomatitis virus (VSV) infection, highlighting the potential of increased RAS/MEK for improved oncolytic virus delivery. Therefore, reducing the antiviral capabilities of a tumor by reducing type I IFN signaling may facilitate improved oncolysis by VSV. In this vein, **Ramya Krishnan**, a member of the laboratory of **Jean-Simon Diallo (The Ottawa Hospital Research Institute, Ottawa, Canada)**, described a stable analog of Viral Sensitizer-1, a small molecule that inhibits IFN-β signaling. The analog, which does not affect VSV replication in normal cells, enhances viral replication in VSV-resistant tumors and enhances survival in a murine model of colon carcinoma.

A complementary approach to modifying the TME using an oncolytic virus is to silence key suppressive components in the tumor. **Victoria Jennings** presented her work from Bell's laboratory cloning known and synthetic miRNAs to target suppressive factors into rhabdoviruses. In a murine metastatic melanoma model, this virus increased tumor control and enhanced anti-tumor T cell responses. **Swami Murugappan (Amgen, Thousand Oaks, USA)** presented the work with talimogene laherparepvec (T-Vec), an oncolytic herpesvirus modified for reduced neurovirulence and expression of granulocyte–monocyte colony stimulating

factor, which is proposed to work by causing oncolysis of injected tumor and potentially inducing tumor-directed systemic immune responses. T-Vec has demonstrated the ability to induce durable anti-tumor responses in patients with unresectable stage IIIB–IV melanoma in a large randomized Phase 3 study and is the first FDA-approved oncolytic virus therapy. To further enhance melanoma control, T-Vec treatment is now being clinically tested in combination with checkpoint inhibition.

Combination therapies to boost therapeutic efficacy

Traditionally, the concept of combination therapy involved administration of multiple drugs to challenge chemo-sensitive cells as well as those resistant to monotherapy. In immunotherapy, this concept is evolving to include innovative ways of enhancing antigen presentation, boosting immune cell activation, localizing reactive leukocytes to the tumor, and reversing immunosuppression. Throughout the Summit4CI, many investigators described combination therapies aimed at boosting immune responses. Although the number of combinatorial approaches is rapidly increasing, they have in common a goal of relieving immunosuppression while simultaneously supporting anti-tumor reactivity.

Brent Johnston (Dalhousie University, Halifax, Canada) has demonstrated an important role for NKT cells in the prevention of breast cancer and melanoma metastasis, and is now aiming to support NKT cell reactivity in the therapeutic setting. Surgical excision of breast cancer with adjuvant NKT cell activation via administration of α-galactosylceramide-loaded DCs supports improved control of metastases. Using C-X-C motif chemokine ligand-16-sufficient and deficient DCs, Johnston's group revealed a critical contribution of the chemokine receptor C-X-C motif chemokine receptor-6 on the surface of NKT cells for activation of anti-tumor responses. By coupling chemotherapy or oncolytic virotherapy with NKT cell activation, they are now demonstrating how the inflammation and antigen release associated with these debulking techniques reenforces the anti-cancer impact of NKT cells.

The pancreatic cancer microenvironment is highly exclusionary to both drug and immune access and immunosuppressive; therefore, strategies to activate T cells against tumor antigens and support their infiltration and function in the tumor are needed. To this end, Jaffee is exploring combination therapies for pancreatic cancer. She described a series of clinical trials, including neoadjuvant "GVAX" vaccines, where a whole tumor vaccine is combined with anti-PD-1 or anti-CTLA4 to support T cell activation and function in the tumor or GVAX administration prior to surgical tumor excision. Using highly parametric multiplex immunohistochemical analyses, Jaffee has now demonstrated how tumors respond and change as a result of immune therapy. In a typical "immune desert" cancer mouse model, vaccination leads to recruitment of immune effectors, dissolution of the tumor stroma, and improved outcomes. Reasoning that immune recruitment is therefore a biomarker for successful treatment, Jaffee advocates for repetitive tumor biopsies to measure patient progress and design patient-centric approaches to treatment.

To support T cell infiltration, **Tak Mak (University Health Network, Toronto, Canada)** has successfully applied a different combination strategy: vaccination with IL-7 treatment, to simultaneously target adhesion and chemokines. He additionally showed that Fc receptor fusion treatment with anti-PD-1 displays synergistic anti-tumor immune responses to increase CD8 T cell infiltration. He described how the use of anti-PD-1 in conjunction with the polio-like kinase 4 inhibitor, CFI-400945, to simultaneously target spindle assembly and centriole duplication checkpoint in aneuploidy, resulted in cancer genomic instability and better tumor control compared with either therapy alone. Combining therapies in this way is allowing simultaneous adjustment of the TME to foster improved cancer control by existing immune mechanisms.

In some patients, the pre-existing anti-tumor immune response is present but insufficient to eliminate the tumor. To improve cancer control, increasing the titer of anti-cancer T cells will be important. While it is possible to expand tumor-specific T cells *ex vivo*, re-inoculation to the patient is practically challenging. The risk of a cytokine storm and the requirement to support *ex vivo*-derived effectors limits the number of cells that can be injected. Therefore, activation of effector T cells *in situ* may be a more effective approach to generating effective anti-tumor T cell responses. **Esteban Celis (Augusta University, Augusta, USA)** evaluated the impact of vaccine designs on the cytotoxic activity of T cells. To induce major T cell responses, peptide-based vaccines need to mimic a systemic viral infection. Besides using TLR agonists as adjuvants, increasing the amphiphilicity of immunogenic peptides allows self-association, which mimics the structure of viral particles that are better uptaken by DCs and promote improved T cell responses. The utility of this was shown in a preclinical setting by combining anti-CD40, Polyinosinic:polycytidylic acid, and bis-(3′–5′)-Cyclic dimeric guanosine monophosphate to synergistically increase the immune responses to hgp100 TriVax vaccination.

In a different strategy to support a strong anti-cancer T cell response, **Yonghong Wan (McMaster University, Hamilton, Canada)** transfers a small pool of *in vitro*-differentiated central memory T cells with anti-cancer potential, followed by a boost with an oncolytic rhabdovirus

encoding their epitope to treat established solid tumors. In addition to the tumor debulking mediated by oncolysis, this approach supports effective expansion of transferred T cells in the periphery and rapid recruitment into the tumor. In collaboration with Bramson, the Wan lab is now testing whether this approach can similarly induce in vivo expansion of CAR T cells to extend the success of CAR T therapy beyond certain blood cancers.

Genomic and epigenetic approaches to personalized immunotherapy

Tumors are heterogeneic, dynamic, and adaptive ecosystems that do not uniformly respond to treatment. Improved outcomes may be facilitated by studying the specific mutations and epigenetic changes present in a patient's tumor. **Steven Jones (BC Cancer Agency, Vancouver, Canada)** described the Personalized Oncogenomic Program, which aims to leverage next-generation sequencing data to identify the most precise treatment options for individual patients. For each tumor sample, dysregulated genes as well as those bearing genomic aberrations, such as fusions, copy number variations, or somatic point mutations, are identified and further analyzed with a manually curated and continuously expanding knowledge base. Using these data, a team of scientists and physicians annotate alterations to make patient- and mutation-specific treatment recommendations.

Computational models can predict treatment outcomes and inform the best combinations and timing of treatments. In his models, **Alexander Anderson (The Moffitt Cancer Center, Tampa, USA)** is considering molecular, cellular, and organism features to dissect and predict tumor progression and immunogenicity. His models incorporate real and simplified data to understand adaptive, immune, and metabolic parameters that change in response to treatment. For instance, he established a model of tumor-immune interactions by modeling features of PD-L1 and T lymphocytes and demonstrated that localized expression of PD-L1 on tumor margins facilitate their invasion.

With a different approach, **Barbara Seliger (Martin Luther University, Halle-Wittenberg, Germany)** demonstrated that, besides transcriptional suppression, expression of classical MHC class I genes is reduced through the downregulation of genes involved in the antigen presentation pathway. This silencing, which occurs via altered miRNA expression, results from the overexpression of various oncogenic pathways in cancer, such as HER2 or the BRAF proto-oncogene. As a consequence, she suggested that cytokines, such as IFN-γ, or therapeutics targeting signaling proteins, transcriptional regulators, and microRNAs might improve antigen presentation and T cell recognition of such tumors.

Although molecular tumor analysis is relatively new, the multifaceted approaches applied to understand the unique features of a patients' tumor let to better knowledge of the molecular changes that allow it to grow, evolve, and evade treatment, fostering more precise approaches to therapy.

Treatment monitoring and novel techniques for in vivo imaging

New imaging technologies are shedding light on the anticancer response and allow us now to follow oncolytic viruses in vivo. Using a novel intravital imaging technique, **Victor Naumenko**, trainee in **Craig Jenne** and **Douglas Mahoney's laboratory (The University of Calgary, Calgary, Canada)**, showed that a second dose of VSV improves outcomes without infecting tumor cells. Instead, the benefit of a second dose results from activation of neutrophils, recruited to the tumor microenvironment by monocytes activated by the first dose of VSV.

In vivo imaging may soon be able to monitor ongoing responses in patients. An effective imaging method for monitoring therapy responses in cancer patients was presented by Corby Fink, a PhD Candidate in **Gregory Dekaban's laboratory (Robarts Research Institute, London, Canada)**. He has designed an autologous APC-based cancer vaccine using patient-specific 19F-labeled peripheral blood mononuclear cells.

Preclinical science and health outcomes research: clinical trial design

Designing a clinical trial requires consideration of all stakeholders involved, including patients, researchers, the industry, governmental or academic partners funding the project, policymakers, and clinical staff. Foundational steps involve collating information from all available publications to identify promising approaches in narrative reviews or systematic reviews. **Dean Fergusson (The Ottawa Hospital Research Institute, Ottawa, Canada)** asserted that conducting empirical and systematic review of the literature is essential to resolve conflicting evidence or opinions, answer outstanding questions, or explore the impacts of a given intervention on patients or patient subpopulations. Rapidly growing, the field of cancer immunotherapy will benefit from systematic reviews and can take cues from other fields where such knowledge synthesis is more prominent. **Jonathan Kimmelman (McGill University, Montreal, Canada)** showed that rational attempts to forecast outcomes will enable better management of "operational

uncertainties" such as risk and benefit, equipment needs, and the likelihood that study outcomes will be more broadly generalizable, while still addressing novel scientific questions. **Katherine Bonter (The Personalized Cancer Immunotherapy Program, Hôpital Maisonneuve-Rosemont, Montreal, Canada)** is using systematic reviews to study trends in clinical trial design. Clinical trials in immunotherapy have grown 162% over the last decade, with academic/clinical institutions sponsoring the greatest proportion, and the majority based in the USA, China, Japan, and the Netherlands. Industry involvement is increasing, especially in sponsorship of targeted therapies, and may be underestimated based on limitations to assessing industrial contributions. These approaches make the data accessible to all stakeholders.

Conclusions

The current and future advances in cancer immunotherapy embrace combinatorial approaches to target diverse arms of the immune system and tumorigenesis to precisely treat patients, representing an exciting beginning of the new era of immunotherapy. Computational approaches are enabling faster and more high-throughput analysis of data and information than ever before. This Summit4CI brought together researchers, stakeholders, clinicians, and the next generation of researchers to make strides toward the continued forward progress in cancer immunotherapy. We look forward to more exciting progress and interactions at the BioCanRx Summit for Cancer Immunotherapy in Ottawa, Ontario (June 25–28, 2017) and the 10th Annual Canadian Cancer Immunotherapy Consortium meeting in Montreal, Quebec (December 13–15, 2017).

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest. Connie Krawczyk participated in the organization of this meeting as coordinator of knowledge dissemination and outreach.