

Esophageal Cancer Translational Research Initiative Planning Workshop Report

February 17, 2016

8:30am – 4:00pm

Location: OICR | West Tower Boardroom 5-20/21

Attendees

Invitees:

| | |
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| Samuel Asfaha | University of Western Ontario |
| Maria Cirocco | St. Michael's Hospital |
| Jay Conner | Mt. Sinai Hospital |
| Ralph DaCosta | Princess Margaret Cancer Centre |
| Gail Darling | Toronto General Hospital |
| Mathieu Derouet | University Health Network (UHN) |
| Craig Earle | Institute for Clinical Evaluative Sciences |
| Elena Elimova | Princess Margaret Cancer Centre |
| Lorenzo Ferri | McGill University |
| Christian Finley | McMaster University |
| Oliver Fisher | Gastroesophageal Cancer Program, St Vincent's Centre for Applied Medical Research, Sydney, Australia |
| Stuart Foster | Sunnybrook Research Institute |
| Tony Godfrey | Boston University |
| Wenlei Jiang | Ontario Cancer Institute/UHN |
| Jules Lin | University of Michigan |
| Virginia Litle | Boston University |
| Geoffrey Liu | Cancer Cancer Trials Group/Princess Margaret Cancer Centre |
| Norman Marcon | St. Michael's Hospital |
| Wayne Phillips | Peter MacCallum Cancer Centre (Melbourne, Australia) |
| Robert Riddell | Mt. Sinai Hospital |
| Brendon Stiles | Weill Cornell Medical College |
| Christopher Teshima | St. Michael's Hospital, University of Toronto |
| Robert Weersink | UHN |
| Rebecca Wong | Princess Margaret Cancer Centre |
| Jon Yeung | University of Toronto |

OICR & FACIT

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| John Bartlett | Director, Transformative Pathology Program, OICR |
| Kelly Battistuzzi | Coordinator, Scientific Secretariat, OICR |
| Rob Campos | Head, Research Operations, OICR |
| Jeff Courtney | Chief Commercial Officer, FACIT |
| Irina Kalatskaya | Project Manager, Informatics and Bio-computing Program, OICR |
| Paul Krzyzanowski | Post-doctoral Fellow, Informatics and Bio-computing Program, OICR |
| David O'Neill | Vice-President, Business Development, FACIT |
| Nicole Onetto | Deputy Director and Chief Scientific Officer, OICR |

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| Teresa Petrocelli | Director, Scientific Secretariat, OICR |
| Lincoln Stein | Director, Informatics and Bio-computing Program, OICR |
| Rebecca Tamarchak | Director, Strategic Planning and Outreach, OICR |
| Jessica Vaisica | Sponsored Awards Officer and Scientific Officer for the workshop, OICR |
| Brent Zanke | Executive in Residence, FACIT |

Please note that this is a summary of the workshop prepared by the organizers. For more details please contact the TRI workshop leaders:

- Lincoln Stein: lincoln.stein@gmail.com
- Gail Darling: gail.darling@uhn.ca

1. Opening remarks (*Lincoln Stein*)

Attendees were welcomed and thanked in advance for their participation. The workshop is intended to bring together researchers in esophageal cancer to discuss opportunities to develop translational researcher projects based on ongoing research activities and other assets. The workshop is intended to function as a day-long brainstorming session in which the talks inspire discussion and point to new ideas. The group should use the day to find opportunities for integrative/collaborative research projects that can ultimately act to improve the clinical management of the disease. Ideas for projects should lead to near-term impact on the diagnosis and management of esophageal cancer. Ultimately, we hope to fund a mix of 2-4 projects that can be bundled together to address various aspects of the disease/disease management and at least one clinical trial-based project.

2. Background (*Nicole Onetto*)

- OICR Strategic Plan 2016-2021: overview
- Translational Research Initiatives

OICR's 5-year strategic plan (2016-2021) was reviewed, including the Institute's mission and goals. Important highlights include the aims to: advance Ontario's best cancer research to improve cancer care and treatment; perform cutting-edge translational cancer research, enhance Ontario's global leadership in cancer research; partner with the Ontario cancer community; and drive adoption and/or commercialization of cancer innovations in Ontario. Emphasis was placed on OICR's community outreach efforts, intended to forge collaborations and to move the most promising ideas to the clinic for impact.

The Institute's concept of Translational Research Initiatives (TRIs) was discussed, which require expertise, funding, and approaches to move findings and technologies to the clinic. Workshop participants were encouraged to consider leveraging networks supported by OICR (e.g., Global Alliance for Genomics & Health, Ontario Tumour Bank, Canadian Cancer Clinical Trials Network) as well as OICR's Technology Programs which will play a role in providing expertise and access to technologies to the Ontario research community, and in supporting OICR's strategic initiatives.

Structure-wise, TRIs represent large scale, multi-disciplinary collaborations between laboratory and clinical scientists, to advance Ontario assets and improve cancer patient outcomes. They should be focused on a clinical need that builds on assets or innovations in an area of leadership for Ontario. TRIs will include 2-5 projects, with at least one mandatory clinical trial which must be started in the first 2 years of a TRI. A budget of up to \$10 M over 4 years may be requested for a TRI, with the clinical trial budget comprising at least \$2 M over the 4 years (if less than \$2 M, then the total budget will be reduced accordingly). Additional supplemental funding should be sought at the start and during the course of the TRI in order to support all TRI activities.

TRI workshops are intended to support development of Letters of Intent (LOIs) for TRIs. TRI's will be led by two co-leaders (preferably not from the same institution), one scientific and one clinical.

The timing for TRI development was described as follows:

- TRI workshops - until March 2016
- Declaration of LOI interest – by April 15, 2016
- TRI LOI submission – May 2, 2016
- TRI LOI selection – July 15, 2016
- TRI FR submission – October 31, 2016
- TRI FR international review panel – February 2017
- TRI FR communication – March 2017
- Funding begins – April 2017.

All guidelines about the TRI process are available by contacting OICR's Scientific Secretariat at scientificsecretariat@oicr.on.ca.

3. Esophageal Cancer: Challenges in Detection and Management (*Gail Darling*)

Dr. Darling provided an overview of the progression and current state of esophageal cancer to set the stage for project discussions. She notes that 2,200 Canadians will be diagnosed with esophageal cancer this year and 2,100 will die (95%), which is second only to pancreatic cancer. In western countries, squamous cancers have been on the decline, while adenocarcinomas have been increasing. If we are able to detect the cancer early, the survival rate is somewhat improved. The main risk factor for developing esophageal cancer is Barrett's esophagus (BE). In the clinic, most patients with esophageal cancer present with symptoms of trouble/painful swallowing and weight loss, but this is indicative of advanced disease (70% with stage 3 or 4). The standard treatment for these patients is esophagectomy (surgery). The 5-year survival rate is <10%, so surgery alone isn't a good enough treatment option.

Dr. Darling discussed the anatomy of the esophagus noting that there is a unique anatomical problem that allows these cancers to move into the lymphatic system very quickly, early on in the disease. She noted that we must be able to better detect the early stages of the disease in order to develop more successful treatment and management strategies.

4. Early Diagnosis and Management of Early Disease

Barrett's: can we do better? (*Norman Marcon*)

Dr. Marcon provided an overview of what we know about the development of Barrett's esophagus (BE) and its development into esophageal cancer. He noted that in the development of BE, the columnar epithelium replaces the stratified squamous epithelium. There are roughly 350,000 cases of BE, but only 1,500 resulting cancers. The transition from BE to cancer evolves over a long period of time – if we're able to identify those BE patients at higher risk of transitioning to cancer, we'll be able to better treat the disease. He also noted that despite screening for BE and surveillance programs, 94% of esophageal cancer is diagnosed in patients without a prior diagnosis of BE. Most of these patients have advanced, fatal disease, as the early lesions (dysplasia) are silent. He reviewed some of the tools available for detection/management (e.g., multi-biopsy forceps, capsules, endoscopic treatments,

surgery, etc.), but noted that we can't yet prevent the development of BE, dysplasia, and cancer and we need biomarkers to aid in earlier detection/patient stratification. He suggested the development of a centre of excellence which includes endoscopists, pathologists, surgeons, researchers, tissues banks, and translational endoscopy units to help tackle the challenges ahead.

During Q&A, Dr. Marcon was asked about the availability of predictors of the likelihood that a patient with BE will progress. He replied that there are none so far. He was also asked about the incidence of dysplasia in patients with EAC resections in Ontario, and replied that it has been difficult to obtain accurate numbers from Cancer Care Ontario due to how the reporting is grouped.

Prospects for a sponge-cytology based genetic signature to detect patients at high risk of esophageal adenocarcinoma (*Lincoln Stein*)

Dr. Stein provided additional information regarding the incidence of gastroesophageal reflux disease (GERD) and Barrett's Esophagus (BE), the major risk factor for esophageal adenocarcinoma (EAC). He then introduced an encapsulated sponge (EsophaCap) which can be used to assess the cytology of the esophagus. This encapsulated sponge is swallowed, expands, and is then pulled back up to scrape the esophagus and facilitate cell collection for analysis. Sponge cytology coupled with immuno-histochemistry has fairly good sensitivity for BE (70-90%), but has poor success in identifying high-grade dysplasia/carcinoma. The Stein group is coupling sponge cytology with sequencing, with the aim of developing a sequence-based assay capable of distinguishing non-dysplastic BE from dysplasia/EAC.

The first phase of this work was exome sequencing of EAC/normal pairs to create a signature. An AmpliSeq panel is currently in production. Stage 2 of this work is to look at matched biopsy samples. This portion of the project is ongoing, but results to date indicate that almost all genes that contain mutations in EAC are also mutated in BE, but at different frequencies. A proof-of-principle classifier, developed using machine learning, shows promise for distinguishing BE from EAC based on the mutation frequencies in a series of genes.

During Q&A, Dr. Stein was asked how this work compares to other sponge collection and signature discovery projects, and he responded that the work was competitive, but that he would be very open to collaboration in order to maximize the number of specimens available.

Characterization of copy number changes in esophageal cytology samples via FISH and FACS (*Tony Godfrey*)

Dr. Godfrey introduced the concept that as the disease progresses, so does the mutational load. As such, we should be able to identify a disease progression signature. He further explained that DNA copy number differences are clearly different between BE and EAC. EAC samples show deletions and amplifications that are not present in BE. His group has been using fluorescent *in situ* hybridization (FISH) on the EsophaCap cytology samples. Using FISH on its own would necessitate counting hundreds of cells/nuclei for each different fluorophore employed; they have therefore decided to couple the FISH experiments with flow cytometry. This technique allows for the use of 10 different markers and various probe choices.

MLPA and Oncomine – high throughput copy number variation (CNV) analysis from formalin-fixed, paraffin-embedded (FFPE) tissues (*John Bartlett*)

Dr. Bartlett discussed some approaches his group has been using in breast cancer that might be useful for the esophageal cancer group. They are working to integrate genomic signatures from comparative genomic hybridization, targeted sequencing and RNAseq. Specifically, the group is looking to compare multiplex ligation dependent probe amplification (MLPA) to the ThermoFisher company's OncoPrint platform with the intent of choosing the best approach for identifying CNVs in tumour samples. The gold standard, FISH, will be used to measure each technique's accuracy. Preliminary results on breast cancer samples indicate that MLPA is more consistent with FISH results than is OncoPrint.

During Q&A, Dr. Bartlett explained how MLPA works, and indicates that tissues must be macro- or microdissected to ensure sufficient purity for accurate CNV assessment. He indicated that cells collected via the sponge method would likely have to be sorted in order to enrich the glandular component; enough cells must be isolated to yield 10 ng of DNA.

5. Management of Early Disease

Opportunities for innovation in EAC management (*Paul Krzyanowski*)

Dr. Krzyanowski discussed work recently described in the literature around serum biomarkers of BE. Researchers have tested a series of biomarker risk scores for identifying patients with GERD who are more likely to have concomitant BE, using clinical criteria combined with the presence or absence of circulating plasma proteins. He also discussed work by a second group which suggests that serum glycoproteins are able to distinguish between healthy patients and those with BE and EAC. For the resulting TRI, he believes we should include a combination of genomics and other 'omics approaches that can be used to detect disease progression regardless of GERD status.

During Q&A, Dr. Philips described how Australian esophageal researchers share protocols and samples in order to maximize the size of research cohorts. He volunteered to share lessons learned.

Monitoring of esophageal adenocarcinoma via circulating DNA (*Tony Godfrey*)

Dr. Godfrey noted that circulating, cell-free tumour DNA (ctDNA) can be detected in the plasma of cancer patients, however, there's very little detail available regarding ctDNA in EAC. He described an approach in which digital PCR (dPCR) is used to amplify DNA isolated from plasma; the amplified DNA is then subjected to next generation sequencing (NGS). He then described a recent technique that uses PCR barcodes to reduce the error rate of dPCR to approximately 0.1% (from a raw rate of 1-3%). This allows extremely rare mutational events to be identified accurately. Applied to EAC patients, this technique has confirmed the presence of ctDNA carrying the expected EAC mutations.

During Q&A, Dr. Godfrey described ctDNA as a potentially useful biomarker for prognosis, or as a management tool to measure the response of the tumour to adjuvant or neoadjuvant therapy.

Biomarkers to optimize multimodal therapy of patients with gastroesophageal adenocarcinoma (GEAC) (*Elena Elimova*)

Dr. Elimova discussed the need for biomarkers that indicate how patients are likely to respond to treatment. In work she performed in her previous position at MD Anderson, she identified 185 patients who underwent chemoradiation and pre-

treatment biopsy, and tested the predictive value of a transcription factor, p63. Using Illumina sequencing and hierarchical clustering, she found that the patients had poorly differentiated tumours. Her data suggest that a pre-treatment p63-associated subtype of EAC highly correlates with pathological complete response (pathCR). Dr. Elimova then described a second study, currently open at MD Anderson, which is using combination therapy to overcome resistance. They have found that inhibition of hedgehog signalling by cyclopamine combined with docetaxel is synergistic and helps to overcome resistance to chemotherapy. She stressed the importance of finding novel agents/biomarkers which can treat patients with extreme resistance, while providing appropriate levels of treatment for other subtypes.

6. Management of Advanced Disease

Spatial Mapping of Mucosal Disease to Radiological Imaging using Endoscopic Tracking: Applications in Radiation Therapy (Robert A. Weersink)

Dr. Weersink shared his work on integrating endoscopic and radiological imaging (2D → 3D) in order to accurately define the radiation therapy treatment area. The work allows the endoscopists' view of the lesional area to be mapped accurately onto the 3D reconstruction provided by CT scanning.

Targeting TP53 in Esophageal cancer (Wayne A. Philips)

Dr. Philips discussed his work which uses p53 as a potential target for esophageal cancer. His group is performing pre-clinical work using a pharmaceutical that is believed to reactivate mutant p53 protein: PRIMA-1 (ARP-246). ARP-246 was used to treat esophageal cell lines with mutant p53; treatment stopped the growth of these cells (p53 null cells and wild type showed no effect). They are now using mouse xenografts and have found that treatment with ARP-246 blocks tumour growth. Tumour growth is inhibited for as long as the animals are treated with drug, however, once the drug is removed, the tumour re-starts growing. They are now developing a patient derived xenograft (PDX) system. They have found that the *in vivo* activity of APR-246 in the PDX model shows the expected response. The drug is well tolerated (little to no toxicity) and they are hoping to move to a clinical trial in esophageal cancer.

Targeting Neutrophil Extracellular Traps (NETs) to Prevent Cancer Progression (Lorenzo Ferri)

Dr. Ferri discussed his work on Neutrophil Extracellular Traps (NETs). He presented work suggesting that NETs can trap circulating EAC cells in the liver, thereby seeding metastases. In a mouse system, inhibition of NET inhibitors reduces the rate of liver mets. He hopes to apply this clinically to EAC patients in the perioperative period. During Q&A, he was asked if there were any side-effects from NET-inhibitor treatment, and he replied that no major complications have been reported in Japan, where NET-inhibitors have been used for some time.

Integrin-related targets in chemoresistant esophageal adenocarcinoma (Jules Lin)

Dr. Lin discussed the role of integrin signaling in chemoresistant EAC. His group has found that SPP1/OPN is upregulated in EAC, and that overexpression is associated with decreased survival. He presented work suggesting that high expression of distinct OPN isoforms is associated with different combinations of increase in cell migration, adhesion, and invasion. During Q&A, he suggested that integrin inhibitors might help in combination with other therapies, and that such inhibitors appear to be well tolerated.

7. Pre-clinical Models

Establishment of Animal Models of Barrett's Esophagus, Esophageal Dysplasia and Esophageal Adenocarcinoma in the Rat (*Gang Zheng*)

Dr. Zheng was unable to attend today's session, but his student, Wenlei Jiang presented in his absence. The lab is focused on medical imaging and nanomedicine. They have been working to create a surgery-based reflux esophagitis model in rats. To date, they have created 180 animal models via esophagogastroduodenal anastomosis (EGDA). Most of the animals have developed BE, but none have yet progressed to dysplasia or EAC. During Q&A, it was suggested that progression could be accelerated using P53 haplo-insufficient animals, by adding bile acid to the animals' drinking water, or by operating on younger animals.

Novel aberrations in Barrett's esophagus and esophageal adenocarcinoma identified through whole transcriptome sequencing (*Oliver Fisher*)

Dr. Fisher discussed his work using RNAseq to gain a better view of the transcriptome, with an aim to develop/identify new biomarkers with prognostic and predictive potential. He summarized his work on 51 tissues from 44 patients (normal, BE, BE + low grade dysplasia, EAC). They have found that expression differences separate the disease states fairly well, and that the differences validate in external data sets. Dr. Fisher also described work on long non-coding RNAs (lincRNAs), which are expressed at a much lower levels than coding RNAs, are tissue specific and tend to be upregulated in cancer compared to normal cells.

8. Next Steps/Wrap Up

During the summary/wrap up session, the participants discussed a series of key challenges in the management of esophageal cancer:

Most patients present when they become symptomatic, at which point prognosis is poor. Discussion around this point focused on the prospects for early detection using a one or more of the following technologies:

- Sponge cytology
- Circulating serum protein/nucleic acid markers
- Less invasive imaging, such as nasogastric endoscopy

Prior to resection, it is difficult to distinguish early tumours that are confined to the lamina propria (T1A) from those that invade the subucosa (T1B). The latter is more likely to involve lymphatics and to metastasize to regional lymph nodes. As a result, some patients are undertreated, whereas others are over treated. Discussion focused on the identification of biomarkers (radiological, molecular, serum-based) that can distinguish T1A from T1B tumours prior to resection, so that the most appropriate therapy can be applied.

Conventional chemotherapeutic agents are frequently ineffective for treatment of advanced disease. The group discussed potential targeted therapies, including the ARP-246 p53-targeting drug described by Dr. Philips, the hedgehog and integrin inhibitors described by Drs. Elimova and Lin, as well as the new generation of checkpoint inhibitors.

A dearth of good experimental models inhibits preclinical research into esophageal malignancy. The group discussed prospects for creating such models,

including approaches based on Dr. Zheng's rat EGDA procedure, the creation of patient-derived xenografts or organoids, or the production of cell lines.

Drs. Darling and Stein volunteered to create a collaborative document in which these focus areas were organized in terms of potential projects that could form the basis of a TRI. The document will be distributed to participants by mid-March.

Esophageal Cancer Translational Research Initiative Planning Workshop

Wednesday, February 17, 2016

8:30 a.m. – 4:00 p.m.

Light breakfast will be served at 8:30 a.m.

Location: OICR | West Tower Boardroom 5-20/21

| TIME | AGENDA ITEM | PRESENTER |
|--------------------------------|--|---------------------------------------|
| 8:30 a.m. | Arrivals and light breakfast | |
| 9:00 a.m. - 9:10 a.m. | Opening remarks and welcome | <i>Gail Darling Lincoln Stein</i> |
| 9:10 a.m. - 9:30 a.m. | Background <ul style="list-style-type: none"> OICR Strategic Plan 2016-2021: overview Translational Research Initiatives | <i>Nicole Onetto</i> |
| 9:30 a.m. - 9:45 a.m. | Esophageal Cancer: Challenges in Detection and Management | <i>Gail Darling</i> |
| 9:45 a.m. - 11:00 a.m. | Early Diagnosis and Management of Early Disease <ul style="list-style-type: none"> Norman Marcon (SMH): “Barrett’s: can we do better” (15 min + 5) Lincoln Stein: Prospects for a sponge-cytology based genetic signature to detect patients at high risk of esophageal adenocarcinoma (10 min + 5) Tony Godfrey (BU): Characterization of copy number changes in esophageal cytology samples via FISH and FACS (10 min + 5) John Bartlett MLPA and Oncomine – high throughput CNV analysis from FFPE tissues. (10 min + 5) | <i>Lincoln Stein</i> |
| 11:00 a.m. - 11:15 a.m. | Break | |
| 11:15 a.m. - 12:15 p.m. | Management of Early Disease <ul style="list-style-type: none"> Paul Krzyanowski (OICR): Opportunities for innovation in EAC management (10 min + 5) Tony Godfrey (BU) Monitoring of esophageal adenoca via circulating DNA (10 min + 5) Elena Elimova (UHN) Biomarkers to optimize multimodal therapy of patients with GEAC (10 min + 5) | <i>Gail Darling</i> |
| 12:15 p.m. - 12:45 p.m. | Lunch | |

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| <p>12:45 p.m. - 1:45 p.m.</p> | <p>Management of Advanced Disease</p> <ul style="list-style-type: none"> • Robert A. Weersink (PMH) Spatial Mapping of Mucosal Disease to Radiological Imaging using Endoscopic Tracking: Applications in Radiation Therapy (15 min) • Wayne A. Phillips (Australia) Targeting TP53 in Oesophageal cancer (15 min) • Lorenzo Ferri (McGill) Targeting Neutrophil Extracellular Traps (NETs) to Prevent Cancer Progression (15 min) • Jules Lin (University Michigan) Integrin-related targets in chemoresistant esophageal adenocarcinoma (10 min + 5) | <p><i>Gail Darling</i></p> |
| <p>1:45 p.m. - 2:15 p.m.</p> | <p>Pre-clinical Models</p> <ul style="list-style-type: none"> • Gang Zheng (PMH) Establishment of Animal Model of Barrett's Esophagus, Esophageal Dysplasia and Esophageal Adenocarcinoma in the Rat (10 min + 5) • Oliver Fisher (Australia): Novel aberrations in Barrett's esophagus and esophageal adenocarcinoma identified through whole transcriptome sequencing (10 min + 5) | <p><i>Lincoln Stein</i></p> |
| <p>2:15 p.m. - 2:30 p.m.</p> | <p>Break</p> | |
| <p>2:30 p.m. - 4:00 p.m.</p> | <p>Discussion</p> <ul style="list-style-type: none"> • What are the greatest needs for esophageal cancer management? • Where can research have the greatest impact? <ul style="list-style-type: none"> ○ ...in understanding the biology of the disease ○ ...in early diagnosis ○ ...in management of early disease ○ ...in management of late disease • What unique resources does this group bring to the problems? • What projects can we propose that meet the broad translational goals of the OICR TRI mechanism? | <p><i>Gail Darling Lincoln Stein</i></p> |
| <p>4:00 p.m.</p> | <p>Adjourn</p> | |