All trainees have been assigned to a Rapid-Fire Talk Group and are expected to participate in these group discussions, even if they are not presenting a talk. The groupings and room assignments appear below. Both trainee and non-trainee poster numbers appear below. Non-trainees will not present a talk. All meeting participants are encouraged to participate in the group discussions.

**THURSDAY, MAY 12**

<table>
<thead>
<tr>
<th>Trainee Rapid-Fire Oral Poster Talks (Small Groups)</th>
<th>3:15 - 4:45 pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attended Poster Session and Reception (First half even; second half odd)</td>
<td>4:45 - 6:15 p.m.</td>
</tr>
</tbody>
</table>

*All posters to be installed in the Junior and Pavilion Foyers and Pavilion A&B*

**GROUP 1 ROOM: JUNIOR A&B**

<table>
<thead>
<tr>
<th>PAGE &amp; POSTER</th>
<th>PRESENTER</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 R Aitken, Amelia</td>
<td>The Cellular And Immune Responses To An Oncolytic Virus Targeting The RNAi Pathway</td>
<td></td>
</tr>
<tr>
<td>2 Blankstein, Anna</td>
<td>Siramesine And Lapatinib Induce Ferroptosis In Glioblastoma And Lung Adenocarcinoma Cells</td>
<td></td>
</tr>
<tr>
<td>3 R Burston, Helen</td>
<td>Identification Of An Essential Autocrine Signaling Loop Involving Relaxin And RXFP1 In High Grade Serous Ovarian Cancer</td>
<td></td>
</tr>
<tr>
<td>4 R Chan, Fong Chun</td>
<td>Clonal Dynamics Shape The Histological Transformation And Progression Of Follicular Lymphoma</td>
<td></td>
</tr>
<tr>
<td>5 R Dargahi, Daryanaz</td>
<td>Pan-Cancer Identification And Prioritization Of Cancer-Associated Alternatively Spliced And Differentially Expressed Genes: A Biomarker Discovery Application</td>
<td></td>
</tr>
<tr>
<td>6 R Firmino, Natalie</td>
<td>Hypoxia Is Induced In The Tumour-Draining Lymph Node</td>
<td></td>
</tr>
<tr>
<td>7 R Gangeh, Mehrdad</td>
<td>Cancer Therapy Assessment Using Multiview Learning And Quantitative Ultrasound Methods</td>
<td></td>
</tr>
<tr>
<td>8 R Gebremeskel, Simon Hao, Jun</td>
<td>Combining Natural Killer T Cell Immunotherapy With Chemotherapy Induced Immunogenic Cell Death To Target Breast Cancer Metastasis</td>
<td>Role Of GRB10 In The Development Of AR+ Castration-Resistant Prostate Cancer</td>
</tr>
<tr>
<td>9 R Harmatys, Kara</td>
<td>Progress Towards Prostate Cancer Targeted, Nanoparticle Enabled Photoacoustic Imaging</td>
<td></td>
</tr>
<tr>
<td>10 R LeBlanc, Veronique</td>
<td>Elucidating The Mechanisms By Which CIC Mutations Contribute To Malignancy</td>
<td></td>
</tr>
<tr>
<td>11 R Skowron, Patryk</td>
<td>Convergent Evolution Of Medulloblastoma Metastatic Tumors</td>
<td></td>
</tr>
<tr>
<td>12 R Skulimowski, Michael</td>
<td>Senescence-Associated Biomarkers Predict Clinical Outcome In High-Grade Serous Ovarian Cancer</td>
<td></td>
</tr>
<tr>
<td>13 R Vernier, Mathieu</td>
<td>The Estrogen Related Receptor Alpha Regulates The Methionine Cycle And DNA Methylation</td>
<td></td>
</tr>
</tbody>
</table>

*R=Rapid Fire Talk*
### All posters to be installed in Junior and Pavilion Foyers and Pavilion A&B

#### GROUP 2 ROOM: PARKSVILLE

<table>
<thead>
<tr>
<th>PAGE &amp; POSTER</th>
<th>PRESENTER</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Alkayyal, Almohanad</td>
<td>Utilizing An IL-12 Expressing MARABA MG1 Virus To Improve Autologous Tumour Infected Cell Vaccine</td>
</tr>
<tr>
<td>16 R</td>
<td>Arthur, Sarah</td>
<td>Single-Cell Sequencing And CTDNA Resolve Clonal Structure And Clonal Evolution Patterns An Diffuse Large B-Cell Lymphoma</td>
</tr>
<tr>
<td>17 R</td>
<td>Bydoun, Moamen</td>
<td>Functional Assessement Of The Plasminogen Receptor P11 As A Contributor To Cell Invasion And A Prognostic Marker In Pancreatic Cancer</td>
</tr>
<tr>
<td>18 R</td>
<td>Chun, Hye-Jung</td>
<td>Extra-Cranial Malignant Rhabdoid Tumours Exhibit Heterogeneous DNA Methylation and Gene Expression Profiles</td>
</tr>
<tr>
<td>19 R</td>
<td>Clairefond, Sylvie</td>
<td>Biomarker Validation By Immunofluorescence: A Novel Approach To Follow Prostate Cancer Progression</td>
</tr>
<tr>
<td>20 R</td>
<td>Hopkins, Julia</td>
<td>Mitochondrial Mutations in Prostate Cancer Crosstalk With Nuclear Mutations</td>
</tr>
<tr>
<td>21 R</td>
<td>Kridel, Robert</td>
<td>Defining the Mutational Landscape of Transformed and Treatment-Resistant Follicular Lymphoma</td>
</tr>
<tr>
<td>22</td>
<td>Luk, Iris Sze Ue</td>
<td>Targeting The Inhibitor Of Apoptosis Protein, BIRC6, As A Novel, Potential Strategy For Therapy Of Advanced Enzalutamide-Resistant Prostate Cancer</td>
</tr>
<tr>
<td>23 R</td>
<td>Maeda, Azusa</td>
<td>Investigating The Response Of Pancreatic Tumours To High-Dose Irradiation Using In Vivo Imaging</td>
</tr>
<tr>
<td>24 R</td>
<td>Sung, Vanessa</td>
<td>Met and FGFR1 Cooperate To Regulate Tumour-Initiating Cells In A Subset Of Triple Negative Breast Cancer</td>
</tr>
<tr>
<td>25 R</td>
<td>Timilshina, Narhari</td>
<td>A Contemporary Analysis Of Active Surveillance Uptake For Low Risk Localized Prostate Cancer (PC) In Canada</td>
</tr>
<tr>
<td>26 R</td>
<td>Wadsworth, Brennan</td>
<td>A Timecourse Strategy To Identify Transiently Hypoxic Cells In Solid Tumours</td>
</tr>
<tr>
<td>27 R</td>
<td>Zhang, Wen</td>
<td>Proteome Signatures And New Cancer Drivers</td>
</tr>
</tbody>
</table>

#### GROUP 3: ROOM PORT ALBERNI (4TH FLOOR)

<table>
<thead>
<tr>
<th>PAGE &amp; POSTER</th>
<th>PRESENTER</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 R</td>
<td>Allard, Bertrand</td>
<td>CD73 Expression Is An Independent Prognostic Biomarker In Prostate Cancer</td>
</tr>
<tr>
<td>29</td>
<td>Baxter, Katherine</td>
<td>Murine Models Of Pancreatic Cancer Can Be Effectively Treated With An Infected Cell Vaccine</td>
</tr>
<tr>
<td>30 R</td>
<td>Becker-Santos, Daiana</td>
<td>Developmental Transcription Factor NFIB Is A Target Of Oncofetal MiRNAs And Is Associated With Tumour Aggressiveness In Lung Adenocarcinoma</td>
</tr>
<tr>
<td>31 R</td>
<td>Boyne, Devon</td>
<td>The Association Between Adiposity And Repetitive Element DNA Methylation In Healthy Post-Menopausal Women</td>
</tr>
<tr>
<td>32 R</td>
<td>Bramhecha, Yogesh</td>
<td>The 16p13.3 Genomic Gain: A Biomarker For Disease Progression in Prostate Cancer</td>
</tr>
<tr>
<td>33 R</td>
<td>Cromwell, Ian</td>
<td>Cost-Effectiveness Analysis Of Genome-Guided Management Of Premalignant Oral Lesions</td>
</tr>
</tbody>
</table>

*R=Rapid Fire Talk*
<table>
<thead>
<tr>
<th>PAGE &amp; POSTER</th>
<th>PRESENTER</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>34 R</td>
<td>Filiaggi, Corey</td>
<td>Using The Zebrafish to Model High-Risk, NUP98-NSD1 Induced Pediatric Acute Myeloid Leukemia</td>
</tr>
<tr>
<td>35 R</td>
<td>Kondratyev, Maria</td>
<td>Novel Therapeutic Targets In Head And Neck Cancer</td>
</tr>
<tr>
<td>36 R</td>
<td>Kutovaya, Olga</td>
<td>The Role Of UBR5 Mutations In The Pathogenesis Of Mantle Cell Lymphoma</td>
</tr>
<tr>
<td>37 R</td>
<td>Li, Luolan</td>
<td>Regulatory Landscape Of Developing Human Brain</td>
</tr>
<tr>
<td>38 R</td>
<td>Lim, Liang</td>
<td>Clinical Study and Analysis of Ex Vivo Photoacoustic Imaging in Endoscopic Mucosal Resection Tissues in Barrett’s Esophagus</td>
</tr>
<tr>
<td>39</td>
<td>Qu, Sifeng</td>
<td>Inhibition of Prostate Cancer Invasion and Metastasis by the Combination of Docetaxel and Aneustat Via TargetinG EZH2</td>
</tr>
<tr>
<td>40 R</td>
<td>Xie, Stephanie</td>
<td>Dependence on Sphingolipid Metabolism in the Normal and Leukemic Human Hematopoietic Hierarchy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**GROUP 4 ROOM: BELUGA**

<table>
<thead>
<tr>
<th>PAGE &amp; POSTER</th>
<th>PRESENTER</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>41 R</td>
<td>Bulaeva, Elizabeth</td>
<td>Overexpression Of C-MYC Enhances The Growth Of Primitive Human Hematopoietic Cells And Induces A Human Leukemia De Novo In Transplanted Mice</td>
</tr>
<tr>
<td>42 R</td>
<td>Enfield, Katey</td>
<td>ELF3 Amplification Circumvents Dependency On Upstream Driver Mutations In Lung Adenocarcinoma</td>
</tr>
<tr>
<td>43 R</td>
<td>Fleury, Hubert</td>
<td>Synergistic Effect Of NER And PARP Inhibitor Combinations In Epithelial Ovarian Cancer</td>
</tr>
<tr>
<td>44</td>
<td>Hammond, Colin</td>
<td>Global Transcriptome Analysis of CD34+ Chronic-Phase CML Cells</td>
</tr>
<tr>
<td>45</td>
<td>Jones, Laura</td>
<td>Alternatively Phosphorylated STAT3 Supports Pro-Tumourigenic Metabolic Changes in Breast Cancer</td>
</tr>
<tr>
<td>46 R</td>
<td>Kaufmann, Kerstin</td>
<td>Extracting the Key Regulators of Leukemia Stem Cell Self-Renewal Using An Advanced Competitive In Vivo Screen</td>
</tr>
<tr>
<td>47 R</td>
<td>Krishnan, Ramya</td>
<td>First-In-Class Small Molecule Potentiators of Cancer Virotherapy</td>
</tr>
<tr>
<td>48 R</td>
<td>Lim, Emilia</td>
<td>Comprehensive Sequence Analysis Of Relapse And Refractory Pediatric Acute Myeloid Leukemia Identifies Transcripts Associated with Treatment Resistance</td>
</tr>
<tr>
<td>49 R</td>
<td>Liu, Kelly</td>
<td>Nodal Disease Remains A Poor Prognosis At Surgery Or During Follow-Ups – A COOLS’ Experience</td>
</tr>
<tr>
<td>50 R</td>
<td>Mollen, Erik</td>
<td>Targeting Peroxiredoxin4 In Pancreatic Cancer</td>
</tr>
<tr>
<td>51 R</td>
<td>Overchuk, Marta</td>
<td>High-Density Lipoprotein Mimicking Nanoparticles For Localized Prostate Cancer Imaging and Image-Guided Therapy</td>
</tr>
<tr>
<td>52</td>
<td>Son, Hwan Hee</td>
<td>Molecular And Histopathological Determinants Of Successful Oncolytic Virotherapy</td>
</tr>
<tr>
<td>53 R</td>
<td>Watt, Kathleen</td>
<td>Discovery Of Novel Prometastatic Targets Of MicroRNA-206 In Human Lung Adenocarcinoma</td>
</tr>
</tbody>
</table>

*R=Rapid Fire Talk*
## CONTENTS

All posters to be installed in Junior and Pavilion Foyers and Pavilion A&B

### GROUP 5 ROOM: FINBACK

<table>
<thead>
<tr>
<th>PAGE &amp; POSTER</th>
<th>PRESENTER</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>54 R</td>
<td>Boudhraa, Zied</td>
<td>Therapeutic Relevance of Ran GTPase in Epithelial Ovarian Cancer</td>
</tr>
<tr>
<td>55 R</td>
<td>Bushell, Kevin</td>
<td>Detection Of Hotspot Mutations In Plasma With A Highly Multiplexed Platform</td>
</tr>
<tr>
<td>56 R</td>
<td>Cardin, Sophie</td>
<td>Modeling Of Pediatric Acute Megakaryoblastic Leukemia (AMKL) Using Cord Blood Stem/Progenitor Cells</td>
</tr>
<tr>
<td>57 R</td>
<td>Lightbody, Elizabeth</td>
<td>PPARγ Loss Increases Metastasis of HER2+ Breast Tumours</td>
</tr>
<tr>
<td>58 R</td>
<td>Lo, Winnie</td>
<td>RNA Synthesis Of Homologous Recombination Repair Pathway Under Hypoxia</td>
</tr>
<tr>
<td>59 R</td>
<td>MacAldaz, Margarita</td>
<td>Analysis of Human Hematopoietic Cells Generated from Human Induced Pluripotent Stem Cells in Differentiating Teratomas</td>
</tr>
<tr>
<td>60 R</td>
<td>MacLeod, Graham</td>
<td>Genome-Wide CRISPR/CAS9 Screening Reveals Modulators Of Temozolomide Response in Glioblastoma</td>
</tr>
<tr>
<td>61 R</td>
<td>Martinez, Victor</td>
<td>Analysis of Piwi-Interacting RNA Transcriptomes Identify Cancer Type-Specific Expression Patterns and Signatures Predicting Lung Tumour Behaviour</td>
</tr>
<tr>
<td>62 R</td>
<td>Murugesan, Alli</td>
<td>Novel Therapeutic Approach To Target Multiple Myeloma: Inhibition Of 3' IGH Enhancer Using Small Molecules</td>
</tr>
<tr>
<td>63</td>
<td>Papadopoli, David</td>
<td>The Functional Link Between Mitochondrial One-Carbon Metabolism And Cellular Bioenergetics In Breast Cancer</td>
</tr>
<tr>
<td>64 R</td>
<td>Selman, Mohammed</td>
<td>Phosphatase Inhibitor Synergizes With Oncolytic Virotherapy</td>
</tr>
<tr>
<td>65 R</td>
<td>Topham, James</td>
<td>KMT2D Loss Of Function Is Associated With Increased Mutational Load And Downregulation Of Genes Involved In DNA Damage Response Pathways</td>
</tr>
<tr>
<td>66</td>
<td>Tran, William</td>
<td>Baseline Textural Features Of Diffuse Optical Spectroscopy Parameters To Predict Chemotherapy Response In Locally Advanced Breast Cancer-Preliminary Results</td>
</tr>
</tbody>
</table>

### GROUP 6 ROOM: ORCA

<table>
<thead>
<tr>
<th>PAGE &amp; POSTER</th>
<th>PRESENTER</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>67 R</td>
<td>Calvo Gonzalez, Lilians</td>
<td>Redundant Cyclin-Dependent Kinase Inhibitors Regulate Beneficial Therapy-Induced Senescence In High Grade Serous Ovarian Cancer</td>
</tr>
<tr>
<td>68</td>
<td>Chung, Philip E.D.</td>
<td>Targeted Inactivation Of Rb and p53 Via WAP-CRE Induces Pineoblastoma</td>
</tr>
<tr>
<td>69</td>
<td>Desreumaux-Communal, Laudine</td>
<td>Validation Of Therapeutic Targets In Ovarian Cancer</td>
</tr>
<tr>
<td>70</td>
<td>El Naggar, Amal</td>
<td>YB-1 Regulates Metabolic Adaptation And Cancer Progression Through Selective mRNA Translation</td>
</tr>
<tr>
<td>71 R</td>
<td>Halvorsen, Elizabeth</td>
<td>CCR5 Antagonists As Immune-Modulating Agents For The Treatment Of Breast Cancer Metastasis</td>
</tr>
<tr>
<td>72 R</td>
<td>Healy, Shannon</td>
<td>Recurrent TMEM30A Loss-Of-Function Mutation Improves Prognosis In Diffuse Large B Cell Lymphoma</td>
</tr>
</tbody>
</table>

*R=Rapid Fire Talk*
<table>
<thead>
<tr>
<th>PAGE &amp; POSTER</th>
<th>PRESENTER</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>73 R</td>
<td>Keller, Brian</td>
<td>Creating And Characterizing A Transposon-Mutagenized Library Of Vaccinia Virus Clones For The Treatment of Human Cancer</td>
</tr>
<tr>
<td>74 R</td>
<td>Kent, Oliver</td>
<td>Transcriptional Regulation of MIR-31 By Oncogenic KRAS Mediates Metastatic Phenotypes By Repressing RASA1</td>
</tr>
<tr>
<td>75 R</td>
<td>Mahamud, Osman</td>
<td>Hypoxia Induces Contextual ‘Loss-Of- Heterozygosity’ And Promotes PARPi Sensitivity</td>
</tr>
<tr>
<td>76 R</td>
<td>Pararajalingam, Prasath</td>
<td>Mantle Cell Lymphoma Sequencing Implicates Novel Genes In Malignancy</td>
</tr>
<tr>
<td>77 R</td>
<td>Robichaud, Nathaniel</td>
<td>Translational Control Of The Tumour Microenvironment</td>
</tr>
<tr>
<td>78 R</td>
<td>Schachter, Nathan</td>
<td>Identifying Genes That Cooperate With Mutant P53 And Activated STAT3 In Breast Cancer</td>
</tr>
<tr>
<td>79 R</td>
<td>Sultan, Mohammad</td>
<td>Identification Of Paclitaxel Response Mediators In Breast Cancer Using An In Vivo Genome-Wide Knockdown Screen</td>
</tr>
</tbody>
</table>

**NON-TRAINEES**

<table>
<thead>
<tr>
<th>PAGE &amp; POSTER</th>
<th>PRESENTER</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>Bergeron, Alain</td>
<td>Validation Of The Prognostic Value Of Ki-67 And P27 In Prostate Cancer: The Canadian Prostate Cancer Biomarker Network (CPCBN) Experience</td>
</tr>
<tr>
<td>81</td>
<td>Chaudary, Naz</td>
<td>Plerixafor Inhibits Myeloid Cell Recruitment And Improves The Radiocurability Of Cervical Cancer</td>
</tr>
<tr>
<td>82</td>
<td>Chevalier, Simone</td>
<td>Fer-Activated Androgen Receptor (pY223AR): A Predictive Biomarker Of Prostate Cancer Progression</td>
</tr>
<tr>
<td>83</td>
<td>Couetoux du Tertre, Mathilde</td>
<td>Multi-Omics-Based Approach To Identify Biomarkers Of Therapeutic Resistance In Colorectal Cancer Patients Through Analyses Of Sequential Metastatic Tissue And Liquid Biopsies; Q-CROC-01: NCT00984048</td>
</tr>
<tr>
<td>84</td>
<td>Haynes, Jennifer</td>
<td>Using DNA Barcoding To Elucidate Colorectal Cancer Cell Heterogeneity and Clonal Dynamics At Baseline And In Response To Chemotherapy</td>
</tr>
<tr>
<td>85</td>
<td>Liu, Jeff</td>
<td>A Comprehensive Map Of Critical Pathways And Networks In Cancer Stem Cells</td>
</tr>
<tr>
<td>86</td>
<td>Ouellet, Veronique</td>
<td>Quality Assessment Of The Canadian Prostate Cancer Biomarker Network (CPCBN) Platform</td>
</tr>
<tr>
<td>87</td>
<td>Stapleton, Shawn</td>
<td>Modulating Nanoparticle Drug Delivery Using Radiation And Heat</td>
</tr>
</tbody>
</table>

*R=Rapid Fire Talk*
1. THE CELLULAR AND IMMUNE RESPONSES TO AN ONCOLYTIC VIRUS TARGETING THE RNAi PATHWAY

Aitken Amelia¹,², Carolina Ilkow¹,², Donald Bastin¹, Vicki Jennings¹, John Bell¹,²

¹Ottawa Hospital Research Institute, Centre for Innovative Cancer Research ²University of Ottawa

Introduction: We have demonstrated that encoding an RNA interference (RNAi) suppression gene enhances the efficacy of oncolytic viruses in approximately 80% of cancer cell lines in a human cancer panel. This project will aim to characterize the cellular and immune responses to infection with an oncolytic virus (OV) targeting the RNAi pathway.

Methods: We have engineered an OV to express an inhibitor of RNAi (OV-I-RNAi). This virus was used to screen a panel of human cancer cell lines to assess efficacy of viral killing relative to control virus. Viral replication in a selection of these cell lines was assessed by qPCR of viral genomes and immunoblotting for viral proteins. Stimulation of an antiviral response and expression of immune related cytokines was studied by qPCR and ELISA. Transcriptome analysis by microarray was used to assess cellular changes unique to the OV expressing the RNAi inhibitor. Immune assays will be performed by flow cytometry of tumours established in sensitive murine cancer models.

Results: The expression of a RNAi inhibitor enhances the efficacy of OV in a large number of human cancer cell lines, representing a variety of tissue types. OV-I-RNAi shows enhanced replication in sensitive cell lines in vitro and in vivo. OV-I-RNAi leads to increased activation of the Type I interferon pathway, TNF alpha pathway and expression of immune related cytokines and chemokines, suggesting the potential for activation of a robust anti-tumour immune response.

Conclusions: We have engineered a novel OV expressing an inhibitor of the RNAi pathway and demonstrated its enhanced efficacy in a panel of cancer cell lines. We have begun to unravel the molecular and immune response to this virus and characterize the mechanisms behind the improved efficacy of this oncolytic virus.

Outcome/Impact: This project will begin to characterize the role of RNAi as an intrinsic antiviral mechanism in certain cancer cells and aim to demonstrate how its inhibition may be used to improve OV efficacy. This will also provide insight on the biology of viral defense mechanisms and contribute to our knowledge on the rational design of OV therapies.

Keywords: Oncolytic virus, RNAi pathway
2. SIRAMESINE AND LAPATINIB INDUCE FERROPTOSIS IN GLIOBLASTOMA AND LUNG ADENOCARCINOMA CELLS

Blankstein Anna R.¹,²,³, Shumei Ma¹,² and Spencer B. Gibson¹,²,³

¹Department of Biochemistry and Medical Genetics, University of Manitoba; ²Research Institute in Oncology and Hematology; ³CancerCare Manitoba

Introduction: In cancer cells, the two most common forms of cell death, apoptosis and autophagy, are often actively inhibited, contributing to drug resistance. Identifying and exploiting alternative cell death pathways are essential to overcoming resistance. Ferroptosis, a morphologically and biochemically distinct cell death mechanism is characterized by iron-dependent cellular accumulation of reactive oxygen species. The combination of siramesine, a lysosome disruptor, and lapatinib, a dual tyrosine kinase inhibitor, synergistically induced cell death in breast cancer cells. This effect was blocked by the ferroptosis inhibitor ferrostatin-1 (Fer-1) and the iron chelator deferoxamine (DFO). The objective of this study was to determine whether the siramesine/lapatinib combination induced ferroptotic death in additional cancer cell lines.

Methods: U87 (glioblastoma) and A549 (lung adenocarcinoma) cells were treated with siramesine and lapatinib, separately or in combination, for 24 hours, and cell death was measured by trypan blue staining and flow cytometry. To test whether cell death was occurring via ferroptosis, cells were pre-treated with either Fer-1 or DFO for one hour before incubated with the drug combination. Statistical significance was determined by an unpaired t-test comparing siramesine/lapatinib to the other conditions.

Results: In both cell lines, siramesine or lapatinib alone produced 10-15% cell death at 24 hours. The drug combination, siramesine/lapatinib, induced significantly more cell death than either drug alone [U87: 45.71±6.55% (p<0.0001)], [A549: 43.31±5.25% (p<0.0001)]. Pre-treatment with Fer-1 or DFO decreased the synergistic effect on cell death. Death of U87 cells in the presence of Fer-1 was 33.34±6.06% and in the presence of DFO 30.66±5.81% (p<0.005). Death of A549 cells in the presence of Fer-1 was 31.54±4.43% and in the presence of DFO 34.77±5.64% (p<0.01).

Conclusions: In U87 and A549 cells, siramesine/lapatinib synergistically induced cell death, which was inhibited by Fer-1 and DFO, suggesting that the cell death mechanism triggered by this drug combination is ferroptosis. The siramesine/lapatinib combination appears to be effective in more than one type of cancer.

Outcome/Impact: Inducing ferroptotic cell death is a potential approach to therapy for cancers, such as glioblastoma and lung adenocarcinoma, with poor response to available treatments.

Key Words: Cell death, ferroptosis, glioblastoma, adenocarcinoma
3. IDENTIFICATION OF AN ESSENTIAL AUTOCRINE SIGNALING LOOP INVOLVING RELAXIN AND RXFP1 IN HIGH GRADE SEROUS OVARIAN CANCER

Burston H.E. 1, Kent O.A. 1, Communal L. 2, Mes-Masson A.M. 2, and Rottapel R. 1,3

1Princess Margaret Cancer Centre, UHN, Toronto ON, 2CRCHUM, Montreal QC; 3Department of Immunology, 3Division of Rheumatology, St. Michael’s Hospital, Toronto ON

Introduction: Ovarian cancer is the most lethal gynecologic malignancy due to a poor understanding of disease mechanisms and lack of effective and specific treatments. Here, we have identified an essential role for the relaxin receptor (RXFP1) in high grade serous ovarian cancer (HGSOC) and established an autocrine loop involving RXFP1 and its ligand, the ovarian hormone relaxin (RLN2), in supporting cancer cell proliferation.

Methods: An shRNA genetic screen was used to identify RXFP1 essentiality. The phenotypic consequences of RXFP1/RLN2 siRNA knockdown were evaluated in cell based assays. Apoptosis was measured by PI annexin staining. RLN2 signaling pathways were studied by western blotting, quantitative PCR, and promoter luciferase assays. Immunofluorescence was used to detect RLN2 expression in microarray samples.

Results: We have identified RXFP1 as an essential GPCR in 20 of 30 HGSOC cell lines through genome-wide shRNA screening. Validation of the shRNA screen confirmed that knockdown of RXFP1 or its ligand RLN2 impaired proliferation and colony formation, and increased apoptosis in sensitive lines. Furthermore, RXFP1 knockdown was accompanied by loss of signaling through pro-survival AKT and MAPK pathways. Treatment of sensitive cell lines under serum-limiting conditions with recombinant RLN2 sustained survival, induced proliferation, promoted migration, and activated AKT and MAPK signaling. RLN2 expression is induced by its own signaling pathway, in addition to pro-inflammatory cytokines IL-6 and TNF-α via activation of the transcription factor NF-kB. Importantly, strong RLN2 expression was observed in multiple patient derived HGSOC microarray cases and in fallopian tube epithelium.

Conclusions: We have identified a subset of HGSOC cell lines dependent on RLN2 signaling for survival and proliferation. The strong expression of RLN2 in HGSOC samples and normal fallopian tube epithelium coupled with regulation of RLN2 by pro-inflammatory cytokines strongly implicates this pathway in the initiation and maintenance of ovarian tumours.

Outcome/Impact: The dependency of ovarian cancer cells on RLN2 signaling exposes novel therapeutically tractable targets for treatment of HGSOC. Antibody therapy or small molecules targeting RXFP1 or RLN2 offer promising new treatments for HGSOC.

Keywords: HGSOC, RLN2, RXFP1, Inflammation
4. CLONAL DYNAMICS SHAPE THE HISTOLOGICAL TRANSFORMATION AND PROGRESSION OF FOLLICULAR LYMPHOMA

Chan FC*1, Kridel R*1, Steidl C1, Marra M1,2, Gascoyne RD1, Shah S1,3

1Centre for Lymphoid Cancer, BCCA; 2Genome Sciences Center, BCCA; 3Department of Molecular Oncology, BCCA

Introduction: Follicular lymphoma (FL) is the most common form of indolent lymphoma. Despite extensive knowledge of the genomic alterations associated with FL, the clonal dynamics associated with transformation to an aggressive phenotype (2-3% of patients/year) and treatment resistance (20% of patients within 2 years of treatment) are poorly understood.

Methods: We assembled a cohort of 21 patients dichotomized into 2 categories: 15 transformed FL (TFL), and 6 progressed FL (PFL). For each patient, we obtained primary samples (T1; taken at diagnosis), samples at transformation/progression (T2), and matched normal samples and performed whole genome sequencing (60X) and validated somatic single nucleotide variants (sSNVs), associated with extinction of T1 clones and expansion of T2 clones, using targeted deep sequencing (10000X).

Results: 87% (n=13/15) of TFL patient tumours exhibited T2 dominant clones that were absent or extremely rare in T1 samples. Digital droplet PCR was used to confirm the existence of both scenarios suggesting that de novo sSNVs can arise between T1 and T2 or exist in minor subclones present at T1. By contrast, 67% (n=4/6) of PFL patients exhibited readily detectable clones at T1 that then expanded to become dominant at T2.

Conclusions: Our study provides the first comparison of the clonal dynamics of TFL and PFL patients. Our findings suggest that diagnostic samples of TFL patients may not be reliable predictors of transformation and that the properties of transformation are acquired after diagnosis. By contrast, diagnostic samples of PFL patients appear to be comprised of clones that already harbour treatment resistant properties and disease progression is attributed to the selection of these clones.

Outcome/Impact: The absence of T2 dominant clones at diagnosis suggests that TFL patients require rigorous monitoring for the detection of transformation. By contrast, readily detectable clones at diagnosis, in PFL patients, suggest that these diagnostic samples can be used to predict for treatment resistance.

Keywords: Lymphoma, clonal dynamics, disease progression.
5. PAN-CANCER IDENTIFICATION AND PRIORITIZATION OF CANCER-ASSOCIATED ALTERNATIVELY SPLICED AND DIFFERENTIALLY EXPRESSED GENES: A BIOMARKER DISCOVERY APPLICATION

Dargahi Daryanaz 1,2, Richard Swayze 3, Leanna Yee 3, Peter Bergqvist 3, Bradley Hedberg 3, Alireza Heravi-Moussavi 1, Edie Dullaghan 3, Ryan Dercho 3, Jianghong An 1, Christopher Bond 3, John Babcock 2,3, Steven Jones 1,2.

1 BC Cancer Agency, Genome Sciences Center, Canada; 2 Department of Molecular Biology and Biochemistry, Simon Fraser University, Canada; 3 Center for Drug Research and Development (CDRD), Canada

Introduction: Large-scale gene expression analysis, using techniques such as RNA-sequencing, provides a powerful tool to identify genes involved in human cancers. The goal of current study is to identify potential novel targets for cancer immunotherapy.

Method: We developed an analysis pipeline to identify novel cancer-associated alternatively spliced (AS) and/or abnormally expressed genes from human cancer RNA-sequencing datasets. Using our pipeline, we conducted a pan-cancer analysis in the RNA-sequencing data from more than 5,000 patients from 25 different cancer types generated by The Cancer Genome Atlas (TCGA). We identified differentially expressed and AS genes (present in at least 5% of samples in a tumour type) which seemed to be cancer-associated in comparison to a large compendium of normal transcriptomes gathered from Genotype-Tissue Expression (GTEx), illumina BodyMap, TCGA, and an in-house database. In order to further rank the candidate genes based on their antigenic potential, we performed an extensive literature search and systematic review to collect the characteristics of an ideal tumour antigen. We implemented an R package, Prize, based on the Analytic Hierarchy Process (AHP) method – a multiple-criteria decision making solution, and developed an AHP model to depict antigen properties for ranking and prioritizing the putative biomarkers.

Results and Conclusions: We identified 892 putative tumour-associated differentially expressed genes, as well as 267 recurrent AS events that occur in more than one tumour type. Our AHP model successfully ranks and recognizes the known tumour antigens in the top 10 of the ranked list.

Outcome/Impact: We are currently validating the top-ranked novel antigens in an orthogonal panel of tumour and normal tissues and cell lines using PCR.

Keywords: RNA-sequencing, Prioritization, Biomarker, Alternative splicing
6. HYPOXIA IS INDUCED IN THE TUMOUR-DRAINING LYMPH NODE

Firmino Natalie¹,², Nancy E. LePard¹, Wan L. Lam¹,², Kevin L. Bennewith¹,²

¹Department of Integrative Oncology, BC Cancer Research Centre, Vancouver; ²Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver

Introduction: Lymph node metastasis has historically been considered a passive process, with cancer cells draining through the lymphatic system and becoming trapped in downstream lymph nodes; however, recent evidence suggests that the development of metastases in the tumour-draining lymph nodes (TDLN) requires pre-metastatic changes in the lymph node microenvironment. Such changes include increased lymphangiogenesis and the dilation of the subcapsular sinus (SCS), a region of the lymph node that lymph-borne tumour cells first encounter. Since the SCS is devoid of blood vessels, we hypothesized that tissue near the SCS of TDLNs and therefore local immune cell populations are hypoxic, resulting in impaired anti-tumour immune responses and a permissive environment for metastatic colonization of the lymph node.

Methods: Murine mammary carcinoma cells (4T1 and 4TO7) were injected into the fat pad of immune-competent BALB/c mice. After the establishment of mammary tumours (3-4 weeks post-implant), the hypoxia-specific marker pimonidazole was administered to label all hypoxic cells in tumour-bearing and control mice. Tissue sections of TDLNs and healthy inguinal lymph nodes were stained with antibodies against pimonidazole to assess local hypoxia.

Results: Our results show pimonidazole staining within the cortex of TDLNs, rather than the SCS. Pimonidazole staining was not present in the inguinal lymph nodes of non-tumour bearing mice. These preliminary results indicate that these regions of hypoxia develop prior to the establishment of lymph node metastases.

Conclusions: Initial results suggest that TDLNs are hypoxic relative to control lymph nodes. Future studies will determine which immune cells are exposed to TDLN hypoxia and how this environment affects their phenotype.

Outcome/Impact: Our findings add to the repertoire of early tumour-induced changes in the lymph node microenvironment, and may have important implications for the development of anti-tumour immune responses.

Keywords: Tumour-draining lymph nodes, hypoxia, tumour immunology
7. CANCER THERAPY ASSESSMENT USING MULTIVIEW LEARNING AND QUANTITATIVE ULTRASOUND METHODS

Gangeh Mehrdad, Brandon Fung, Hadi Tadayyon, William Tran, Gregory Czarnota

Department of Medical Biophysics and Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto, Canada

Introduction: Therapeutic cancer response assessment is presently limited; results may not be available to the clinician for typically months. This can lead to ineffective cancer treatments continued needlessly as no faster feedback mechanisms have yet reached broad biomedical adoption. Quantitative ultrasound (QUS) methods provide a promising alternative framework that can non-invasively, inexpensively, and quickly assess tumour response to cancer treatments.

Methods: Fifty six patients with locally advanced breast cancer (LABC) who received neoadjuvant chemotherapy were imaged before and at 4 times during treatment, i.e., weeks 1, 4, 8 and pre-operatively. Data was acquired using a Sonix RP ultrasound machine. Mid-band fit, 0-MHz intercept, and spectral slope parametric maps were computed by employing QUS spectroscopy techniques. The patients were grouped into responders and non-responders based on their ultimate clinical and pathological response to treatment. Since there are multi-parametric maps for each scan, we propose to use supervised dictionary learning, an emerging machine learning algorithm in recent years, to compute one dictionary per view (parametric map), and corresponding sparse representations, which were eventually combined to generate the ultimate feature set. The feature sets computed for the “pre-” and “mid-treatment” scans were compared using a dissimilarity measure as an indication of treatment effectiveness. Eventually, the patients were classified as either responders or non-responders using a classifier.

Results: The classification of 56 LABC patients treated with neoadjuvant chemotherapy to responders or non-responders using the proposed method resulted in an accuracy of 88.8 and 89.6, sensitivity of 93.8% and 88.5%, and specificity of 83.8% and 90.8% after 1 and 4 weeks of treatment, respectively.

Conclusions: In this study, an advanced machine learning technique based on multiview learning was proposed to combine complementary information in multi-parametric maps computed using QUS methods. The effectiveness of developed method was assessed on 56 LABC patients treated with neoadjuvant chemotherapy. The proposed system achieved a promising accuracy and sensitivity early after the start of treatment.

Outcome/Impact: The results of this study would permit clinicians to receive feedback and switch to alternate treatments far earlier, in a step towards the goals of personalized medicine.

Keywords: LABC, response monitoring, quantitative ultrasound, multiview learning
8. COMBINING NATURAL KILLER T CELL IMMUNOTHERAPY WITH CHEMOTHERAPY INDUCED IMMUNOGENIC CELL DEATH TO TARGET BREAST CANCER METASTASIS

Gebremeskel Simon¹, ⁴, Lynnea Lobert¹, ⁴, Kaitlyn Tanner¹ and Brent Johnston¹, ², ³, ⁴

¹Department of Microbiology & Immunology, ²Pediatrics, ³ Pathology, Dalhousie University, ⁴Beatrice Hunter Cancer Research Institute, Halifax, Nova Scotia, Canada, B3H 4R2

Introduction: Breast cancer is the most common cancer in Canadian women and the second leading cause of cancer deaths. Given that most mammary tumours are surgically resectable and over 90% of breast cancer-associated deaths are due to metastasis, new therapeutic strategies targeting metastasis are required. Natural killer T (NKT) cells are a rare population of immune cells that have been shown to limit primary tumour growth and target distant metastatic disease in various animal models.

Method: We have shown that NKT cell activation improves survival in a model of postsurgical metastatic breast cancer. We are now expanding this work to determine whether NKT cell activation can be combined with chemotherapies to improve outcomes. In our model, 4T1 mammary carcinoma cells were injected into the mammary fatpad of syngeneic BALB/c mice. Tumours were resected at day 12, and mice were treated with cyclophosphamide or gemcitabine. On day 17, NKT cells were activated by transfer of dendritic cells loaded with the glycolipid antigen α-GalCer. We also examined whether Gemcitabine or mafosfamide (active component of cyclophosphamide) would induce immunogenic cell death of 4T1 cells in culture.

Results: Chemotherapeutics did not affect NKT cell activation as measured by serum IFNγ levels. Treatment with cyclophosphamide, gemcitabine, or α-GalCer-loaded dendritic cells alone reduced metastasis and prolonged survival. Combined treatments significantly enhanced survival. NKT cell activation decreased the frequency and immunosuppressive function of myeloid derived suppressor cells (MDSCs). Treatments resulted in enhanced tumour specific immunity as surviving mice exhibited slower tumour growth following secondary tumour challenge. Gemcitabine and mafosfamide also increase the immunogenicity of cancer cells in vitro by increasing the exposure/release of MHCI, MHCII, CD1d, Calreticulin, HMGB1 and ATP.

Conclusion: NKT cell activation therapy can successfully be combined with low doses of Gemcitabine or cyclophosphamide to enhance protection against tumour metastasis and recurrence.

Outcome/Impact: This work provides a clear rationale for combining chemotherapy with NKT cell immunotherapy to target metastatic disease in the clinical setting.

Keywords: Breast cancer, Immunotherapy, Natural Killer T-cell, Immunogenic cell death.
9. ROLE OF GRB10 IN THE DEVELOPMENT OF AR* CASTRATION-RESISTANT PROSTATE CANCER

Hao Jun¹,²,³, Dong Lin¹,², Hui Xue¹, Yuwei Wang¹, Xin Dong¹, Rebecca Wu¹, Peter W. Gout¹, Yuzhuo Wang¹,²

¹Experimental Therapeutics, BC Cancer Agency, Vancouver, BC, Canada; ²Vancouver Prostate Centre; ³Interdisciplinary Oncology Program, Faculty of Medicine, UBC.

Introduction: The standard treatment for advanced prostate cancer is androgen deprivation therapy (ADT). However, the cancers inevitably return as more aggressive, castration-resistant prostate cancer (CRPC). We have identified that the expression of GRB10, a multi-domain adaptor protein regulating more than one treatment resistance-related pathways, is elevated in prostate cancer after ADT.

Methods: High fidelity patient-derived xenograft (PDX) models were used to monitor ADT response of prostate cancer throughout the treatment. By comparing the gene expression data of 3 PDX models at multiple time points, we identified potential CRPC driving genes. Then, the genes were validated in clinical prostate cancer patient cohorts for its clinical relevance. siRNA mediated gene silencing was used to study the function and mechanism of the potential candidates in CRPC development.

Results: First, we compared the gene expression data of relapsed, dormant and parental prostate cancer samples in three PDX models. We found that GRB10 was a gene commonly and continuously elevated in these models throughout the ADT treatment. Importantly, we found that GRB10 was significantly upregulated in patients' CRPC samples compared to untreated primary prostate cancers. Patients with higher GRB10 expression also have a shorter progression free survival time. Functionally, we demonstrated GRB10 is upregulated by ADT in LNCaP and C4-2 cells. Moreover, combined with ADT, GRB10 silencing significantly inhibited prostate cancer cell growth. Finally, we found that the GRB10 silencing inhibited both AKT and MAPK pathways which play critical roles during the development of CRPC.

Conclusions: Based on the pilot study, we propose GRB10 is a potential CRPC driving gene and the expression of GRB10 is correlated with the aggressiveness in clinical prostate cancers. Moreover, the expression of GRB10 is critical to cell proliferation and survival of prostate cancer cells.

Outcome/Impact: We have identified GRB10, a potential CRPC driving gene, by using high fidelity prostate cancer PDX models; and analyzed the role of GRB10 in CRPC, which may finally lead to development of novel therapeutic strategies for CRPC patients.

Keywords: Prostate cancer, CRPC, GRB10, SRC-PDX models
10. PROGRESS TOWARDS PROSTATE CANCER TARGETED, NANOPARTICLE ENABLED PHOTOACOUSTIC IMAGING

Harmatys Kara\textsuperscript{1-3}, Marta Overchuk\textsuperscript{1-3}, Martin Pomper\textsuperscript{4}, Juan Chen\textsuperscript{1-3}, Gang Zheng\textsuperscript{1-3}

\textsuperscript{1}Princess Margaret Cancer Centre; \textsuperscript{2}University Health Network; \textsuperscript{3}University of Toronto; \textsuperscript{4}Johns Hopkins School of Medicine

Introduction: A major obstacle of photoacoustic imaging (PAI) of diseased tissue using exogenous contrast agents is low sensitivity and specific detection of tumours. A small-molecule conjugate containing a prostate specific membrane antigen (PSMA) target ligand and a photosensitizer, bacteriochlorophyll (BChl), was used for light-induced enhanced accumulation of PAI-capable nanoparticles (NPs) to solid tumours.

Methods: The \textit{in vivo} tumour-targeting capabilities of the small-molecule near-infrared (NIR) BChl—PSMA probe was tested in tumour-bearing Balb-c mice. Mice were injected with PSMA positive and the PSMA negative cells subcutaneously to each flank. After the tumors reached 5 mm in size, each mouse was injected with BChl—PSMA and subjected to whole-body fluorescence imaging to monitor probe targeting. A separate cohort of mice was injected with PSMA positive cells to both flanks. BChl—PSMA probe was injected 12 hours prior to light irradiation (right tumour; 750nm laser; 50 J/cm\textsuperscript{2}). One hour after treatment, fluorescent nanoparticles were injected and fluorescence imaging was performed to monitor NP accumulation.

Results: BChl—PSMA showed significant tumour targeting to the PSMA positive tumours. Additionally, mice with two positive tumours and subjected to laser treatment with subsequent injection of NP showed significant NP accumulation in comparison to the tumour subjected to no laser.

Conclusions: A small-molecule PSMA inhibitor was conjugated to a NIR photosensitizer, which is capable of targeting tumour neovasculature where PSMA is overexpressed. This allows for localized and rapid cell death in perivascular cells when activated by light. The results indicate selective targeting of the probe to PSMA positive tumours. Additional laser treatment of tumours indicated enhanced NP accumulation with fluorescence imaging. After proof-of-concept fluorescence imaging is achieved, enhanced PAI of tumours will be monitored.

Outcome/Impact: Due to the small size of these probes in comparison to antibodies, they can be used for better penetration into the tumour and enhanced accumulation of NPs. This enhancement will be impactful to patients for more sensitive photoacoustic signal from a PAI-capable nanoparticle system.

Keywords: Prostate specific membrane antigen (PSMA), targeted photosensitizer, enhanced delivery, fluorescence imaging, photoacoustic imaging
11. ELUCIDATING THE MECHANISMS BY WHICH CIC MUTATIONS CONTRIBUTE TO MALIGNANCY

LeBlanc Veronique G. 1,2, Marlo Firme 1,2, Susanna Y. Chan 2, Jungeun Song 2, Angelica Lee 2, Stephen Yip 3, Suganthi Chittaranjan 2, Marco A. Marra 2,4

1Genome Science & Technology graduate program, UBC; 2Canada’s Michael Smith Genome Sciences Centre, BC Cancer Agency; 3Department of Pathology & Laboratory Medicine, UBC; 4Department of Medical Genetics, UBC

Introduction: Somatic mutations in the Capicua (CIC) gene, which encodes a transcriptional repressor, have been identified in different cancer types, most prominently low-grade gliomas (LGGs) and stomach adenocarcinomas (STADs). Only three of its target genes have been established in human cells (ETV1/4/5), and the role of these somatic mutations in malignancy has yet to be established.

Methods: We developed CIC knockout cell lines and performed transcriptomic analyses in these and in control cell lines expressing wild type CIC. We also used RNA-seq data from The Cancer Genome Atlas (TCGA) for Type I LGGs and STADs to perform additional differential expression analyses between CIC-deficient and CIC-wild type or CIC-amplified samples.

Results: We identified a total of 582 differentially expressed (DE) genes between CIC knockout and CIC wild type cell lines. We also identified 1900 DE genes in Type I LGGs with CIC inactivating mutations compared to those expressing wild type CIC, and 207 DE genes in STADs with CIC deletions compared to those with CIC amplifications. Though gene-level overlap was limited between the three contexts, we found that CIC appears to regulate the expression of genes involved in cell-cell adhesion, metabolism, and nervous system development. In the CIC knockout cell lines and in LGGs, we also found that loss of CIC is associated with a MEK activation signature.

Conclusions: Our results suggest that CIC-deficient cells have dysregulated expression of genes involved in differentiation and adhesion mechanisms, which could help explain why CIC mutations have been associated with poorer outcome within Type I LGGs. Loss of CIC may also present a novel mechanism for the dysregulation of the RTK/MEK signalling pathway, which is frequently altered in glioma.

Outcome/Impact: CIC mutations occur at relatively high frequency (~40-70%) in specific subtypes of different cancer types, and are thus likely to contribute to malignancy. These results shed light on the pathological role of CIC mutations and may help inform novel targeted treatment options.

Keywords: Transcription factor, CIC, glioma, stomach adenocarcinoma
12. CONVERGENT EVOLUTION OF MEDULLOBLASTOMA METASTATIC TUMOURS

Skowron Patryk¹, ², Livia Garzia¹, Sorana Morrissy¹, and Michael Taylor¹, ²

¹The Hospital for Sick Children, ²University of Toronto

Introduction: Medulloblastoma initiates within the cerebellum and in 30% of cases disseminates throughout the brain and spinal cord. Little is known about the genes driving dissemination since matching primary and metastatic samples are rare. The medulloblastoma Sleeping Beauty (SB) mouse model uses random integration of transposons to initiate tumourigenesis. Insertions that confer a growth advantage are selected upon as the cancer progresses. Recent literature has demonstrated divergent evolution between the primary and metastatic sites and phenotypic convergent evolution between independent metastatic sites in multiple cancers. The extent of convergent evolution in medulloblastoma metastasis is unknown and its investigation may reveal important therapeutic targets.

Methods: Independent metastatic samples were from each mouse and the SB transposon/normal genomic DNA junctions were sequenced. From every sample, only the most abundant (i.e. clonal) insertions were kept for downstream analysis. Convergently selected genes were located by identifying unique insertion sites targeting the same gene in different metastatic compartments. For each mouse the probability of a random convergent event was modelled using the binomial distribution and compared to the observed rates to identify genes undergoing selective pressure.

Results: There were 15 significant genes undergoing convergent selective pressure across multiple mice. The most recurrent of these were CRebbp, Lgals3, Rabgap1l, Ak7, Ncoa3, Ptk2, Gabrb3, and Ophn1. CRebbp and Ncoa3 are chromatin remodellers part of the same complex, they play an essential role in growth control and embryonic development. While Ptk2, Gabrb3 and Ophn1 regulate cell-to-cell junction maintenance. Subsequent gene set enrichment analysis revealed a multitude of pathways essential for metastasis in medulloblastoma such as cell adhesion and Hedgehog signalling.

Conclusions: Convergent evolution plays a prominent role in medulloblastoma metastasis progression. Independent metastases have unique insertions in the same gene indicative of strong selective pressure.

Outcome/Impact: This project will shed important aspects of medulloblastoma tumour evolution. The genes functionally validated will be an important contribution to the understanding of medulloblastoma dissemination and can be used to discern therapeutic targets for the dismal metastatic disease.

Keywords: Metastasis, Medulloblastoma, Convergent evolution, Sleeping Beauty
13. SENESCENCE-ASSOCIATED BIOMARKERS PREDICT CLINICAL OUTCOME IN HIGH-GRADE SEROUS OVARIAN CANCER

Skulimowski M¹, Clément I¹, Martinez A¹, Provencher D¹,², Mes-Masson AM¹,², and Rodier F¹,²

¹CRHUM et Institut du cancer de Montréal; ²Université de Montréal

Introduction: Cellular senescence is a stable growth arrest induced by chemotherapy and characterized by a pro-inflammatory senescence-associated secretory phenotype largely mediated by NF-κB, which regulates the tumour microenvironment and alters tumour growth and resistance. Although we have previously shown that cellular senescence is a major response of ovarian cancer (OvCa) cells to treatment, the role of senescence in the context of pre-treatment tumours remains unclear.

Methods: Immunofluorescence staining for the senescence-associated (SA) biomarkers NF-κB, IL-6, IL-8 and phospho-Chk2 was performed on the TFRI COEUR validation tissue microarray consisting mostly of pre-chemotherapy high-grade serous (HGS) OvCa tissue (n=200). Using Visiopharm VIS, the expression of SA biomarkers was quantified in the epithelial (tumoral) and stromal compartments, and was normalized accurately between patients using a novel protein mask. Patients were stratified for Kaplan-Meier analysis into high and low groups for normalized expression of SA biomarkers using the median or the lowest quartile. Overall and disease-free survival were compared between groups. Univariate Cox regression was performed without stratification.

Results: Cox regression revealed correlations between increased survival and epithelial NF-κB (HR=0.168, p=0.002) levels, as well as stromal NF-κB (HR=0.220, p=0.005) and IL-8 (HR=0.147, p=0.002) levels. Additionally, increased disease-free survival correlated with epithelial NF-κB (HR=0.188, p=0.002) and stromal IL-8 (HR=0.165, p=0.002) expression. Further Kaplan-Meier analyses yielded correlations between increased disease-free survival and high levels of stromal NF-κB (p=0.001), IL-8 (p<0.001), IL-6 (p=0.033) and phospho-Chk2 (p=0.001). The use of a normalizing protein mask greatly increased concordance between duplicates and significance of discovered correlations.

Conclusions: We have developed an efficient protein mask to normalize TMA data that increases the significance of discovered correlations. Our quantitative data demonstrates that high expression of SA biomarkers in chemotherapy-naïve tissues predicts good clinical outcomes in HGS-OvCa.

Outcome/Impact: Our results suggest that senescence-competence in HGS-OvCa plays an important role in disease treatment-response and that senescence may be an interesting target for future therapeutic strategies.

Keywords: Senescence, ovarian cancer
14. THE ESTROGEN RELATED RECEPTOR ALPHA REGULATES THE METHIONINE CYCLE AND DNA METHYLATION

Vernier M¹, Audet-Walsh E¹, Tam I¹, Giguère V¹

¹McGill University, Montreal, Qc, Canada.

Introduction: Epigenetic mechanisms such as DNA methylation are implicated in the acquisition of a malignant phenotype and therefore epigenetic drugs are a promising strategy for anti-cancer therapy. DNA methylation is the process by which DNA methyltransferases (DNMT) add methyl groups to cytosines, using S-adenosine methionine (SAM) as a methyl donor. Thus, this process relies on DNMT expression and the availability of SAM, a component of the methionine cycle. In breast cancer, cell metabolism is tightly regulated by the nuclear receptor estrogen-related receptor alpha (ERRα) and thus, we hypothesized that ERRα could regulate SAM levels and DNA methylation.

Methods: We first analyzed chromatin immunoprecipitation experiments followed by DNA sequencing (ChIP-seq) of ERRα in breast cancer cells to identify ERRα target genes involved in the methionine cycle. We then inhibited ERRα by RNA interference or with the specific inhibitor C29 and measured gene expression and SAM levels. Finally, we treated breast cancer cells with inhibitors of ERRα and/or DNMT to evaluate the potential of a combined therapeutic approach.

Results: ChIP-seq data showed that ERRα binds the promoter of MAT2A and AHCY, two genes in the methionine cycle. ERRα Inhibition decreased the expression of these genes leading to a reduction in SAM levels. Moreover, ERRα binds the promoter of DNMT1 and again, inhibition of ERRα decreased its expression. Altogether, global DNA methylation was reduced when ERRα function was impaired. Unexpectedly, inhibition of DNMT1 itself decreased ERRα levels. Thus, combination treatment of breast cancer cells with pharmacological inhibitors of ERRA and DNMT1 efficiently impaired cell growth as observed with colony assay experiments.

Conclusions: We have uncovered a novel crosstalk between cell metabolism and epigenetics that modulate cancer cell biology and drug sensitivity. Importantly, we unravelled the existence of a feedback loop between DNMT1 and ERRα.

Outcome/Impact: We propose that targeting these two pathways in combination possesses great therapeutic potential for treating breast cancer and eventually for other cancers as well.

Keywords: epigenetics, metabolism, DNMT, breast cancer
15. UTILIZING AN IL-12 EXPRESSING MARABA MG1 VIRUS TO IMPROVE AUTOLOGOUS TUMOUR INFECTED CELL VACCINE

Alkayyal Almohanad1,3,6, Lee-Hwa Tai1, Jiqing Zhang1,2, Christiano deSouza1, Charles Lefebvre3, Michael Kennedy1, Greg Cron3, Andrew Makrigiannis3, Blair Macdonald8, David Stojdl3, John Bell1,3 and Rebecca Auer1,4.

1Cancer centre, OHRI; Departments of 2CMM; 3BMI; 4Surgery, 8Medical Imaging, uOttawa, Canada; 6Department of Laboratory Medicine, uTabuk, KSA

Introduction: Peritoneal carcinomatosis (PC) is the most common cause of death for abdominal cancers. Immunotherapies have demonstrated efficacy in selected solid malignancies but their potential in PC has only been explored to a limited extent. Here we report the utility of an intraperitoneal (IP) injection of the MG1-IL12-ICV in a murine model of PC.

Method: Interleukin-12 (IL-12) was cloned into an oncolytic MG1 virus. The infected cell vaccine (ICV) was created by infecting autologous tumour cells ex-vivo with MG1 or MG1-IL12 following irradiation. The measured immune response to ICV on NK cells included migration (transwell assay), activation (flow cytometry) and cytotoxicity (chromium release assay) in-vivo and in-vitro. PC was generated by inoculating mice IP with CT26 or B16F10 cells. The treatment was administered IP on day 3-10 following tumor seeding or when a measurable tumour was confirmed by MRI imaging.

Results: MG1-IL12-ICV promoted migration and activation of NK cells in the presence of DCs and was dependent on their secretion of the chemokine IP10 and this was not seen with MG1-ICV. In a murine model of PC, MG1-IL12-ICV treatment stimulated recruitment of IFNγ+ NK cells to the peritoneal cavity associated with a durable cures in >90% compared to 0% survival in MG1-ICV treated mice. Even in mice with bulky PC (tumours >8 mm), a complete radiological response was demonstrated in 100% of mice within 14 weeks. The effect was dependent on intact NK cell function as NK cell depletion abrogated any survival advantage. The effect of MG1-IL12-ICV on NK cell migration, activation were recapitulated in-vitro using human lymphocytes.

Conclusions: MG1-IL12 ICV treatment IP recruits activated NK cells to the peritoneal cavity resulting in a dramatic reduction in tumour burden and improved survival.

Impact: Our promising results in preclinical models of PC provide a proof of principle that an MG1-IL12-ICV platform has the potential to provide a personalized immunotherapy for patients who are diagnosed with terminal PC each year.

Keywords: Peritoneal carcinomatosis, Oncolytic virus, Interleukin-12, NK cells.
16. SINGLE-CELL SEQUENCING AND CTDNA RESOLVE CLONAL STRUCTURE AND CLONAL EVOLUTION PATTERNS IN DIFFUSE LARGE B-CELL LYMPHOMA

Arthur Sarah¹, Stephen Yu¹, Daniel Fornika¹, Miguel Alcaide¹, Joseph Connors², Randy Gascoyne², Nathalie Johnson³ and Ryan Morin¹*

¹Molecular Biology & Biochemistry, Simon Fraser University; ²Department of Lymphoid Cancer Research, BC Cancer Agency; ³Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University

Introduction: Non-Hodgkin lymphoma represents the sixth most common cancer among Canadians, with diffuse large B-cell lymphoma (DLBCL) being the most prevalent subtype. We are using single-cell sequencing methods to resolve sub-clonal populations and discover the clonal evolution patterns of DLBCL from diagnosis to relapse.

Methods: Exome and amplicon sequencing was performed on a DLBCL patient’s diagnostic tumour, relapsed tumour and plasma samples (containing circulating tumour DNA, ctDNA) from multiple time points. To verify and further resolve genetic features of individual sub-clonal populations, the Fluidigm AccessArray system, allowing the simultaneous amplification of 48 samples with 48 primer sets, was used to amplify single cells isolated from samples expected to represent the founding clone and each sub-clone prior to sequencing.

Results: Mutations and sub-clonal populations were identified in a DLBCL patient using exome and amplicon sequencing at diagnosis, first relapse and second. Mutations seen in single cells from the two relapse samples in this patient confirmed the genetic features of suspected sub-clones. An evolutionary history was derived from these results showing a major and minor clonal population at the initial relapse. In the subsequent relapse, the minor clone replaced the initial major clone and acquired additional mutations. We found new mutations at relapse in two genes, MS4A1 and NR3C1 that are each suspected to confer resistance to the chemotherapy drugs rituximab and prednisone respectively, two R-CHOP therapy drugs used to treat DLBCL.

Conclusions: Single-cell and exome sequencing of tumour and ctDNA was used to determine the clonal evolution pattern of a multiple relapsed DLBCL patient, including determining mutations in two genes at relapse likely leading to treatment resistance.

Outcome/Impact: Single-cell sequencing of DLBCL tumours allows validation and more precise determination of clonal evolution patterns determined through bulk tumour sequencing. This allows for better understanding of tumour heterogeneity and decisions to be made about course of treatment.

Keywords: DLBCL, single-cell sequencing, clonal evolution

Supported by the TFRI New Investigator Award (grant #1043) and the BC Cancer Foundation.
17. FUNCTIONAL ASSESSMENT OF THE PLASMINOGEN RECEPTOR P11 AS A CONTRIBUTOR TO CELL INVASION AND A PROGNOSTIC MARKER IN PANCREATIC CANCER

Bydoun Moamen J1, Henry D Liptay2, Andrea Uzans3, Weei Y Huang1, Alec C Kimmelman5, David M Waisman1,4

Departments of 1Pathology, 2Biology, 3Dalhousie Medical School, and 4Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia. 5Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts.

Introduction: Pancreatic cancer (PC) is an especially deadly cancer which has a five-year survival rate of 4%. This poor prognosis is coupled with late diagnosis and high metastatic propensity of PC cells ultimately leading to patient death. The initial trigger for metastasis occurs at the primary tumour site where a few cancer cells acquire proteolytic properties which degrade the heavily crosslinked extracellular matrix (ECM), penetrate the basement membrane and enter blood vessels. Proteolytic cleavage of ECM proteins is regulated by protease receptors.

Methods: Our group has shown that cancer cells utilize the protease receptor p11 to promote the activity of surface proteases allowing them to invade and metastasize. Here, we investigate the involvement of p11 in PC by 1) examining p11 protein levels in a tissue microarray of 89 resected PC patient samples 2) utilizing an inducible in vivo PC mouse model and 3) establishing if the protease-activating function of p11 regulates PC cell invasion in vitro.

Results: Immunohistochemical (IHC) staining of the tissue microarrays shows higher levels of p11 in tumor regions compared to normal ducts. P11 expression correlated with lesion invasiveness (pancreatitis: negative/weak, precancerous lesions: moderate, cancerous lesions: strong). In addition, IHC of tumors extracted from the KRASG12D-driven mouse model revealed similar p11 upregulation. P11 expression was also found to be driven by KRASG12D, a commonly-mutated driver oncogene in PC. In vitro depletion of p11 in PANC-1, BxPC3 and AsPC-1 PC cells reduced their proteolytic activity and invasiveness.

Conclusions: P11 plays an important role in PC cell invasion and is regulated by oncogenic KRAS.

Outcome/Impact: The study may place p11 as a functional prognostic marker in pancreatic cancer that can be used clinically.

Keywords: pancreatic cancer, p11, prognostic marker, invasion, KRAS
Extra-cranial Malignant Rhabdoid Tumours Exhibit Heterogeneous DNA Methylation and Gene Expression Profiles

Chun Hye-Jung E¹, Alireza Heravi-Moussavi¹, Annaick Carles⁵, Tina Wong¹, Eric Chuah¹, Jacquie E Schein¹, Daniela S Gerhard², Andrew J Mungall¹, Richard A Moore¹, Yussanne Ma¹, Steven JM Jones¹,³,⁶, Elizabeth J Perlman⁴, Martin Hirst¹,⁵, Marco A Marra¹,⁶

¹Canada’s Michael Smith Genome Sciences Centre, BCCA, ²Office of Cancer Genomics, NCI, US NIH, Bethesda, MD, USA; ³Department of Molecular Biology and Biochemistry, SFU; ⁴Department of Pathology and Laboratory Medicine, Lurie Children’s Hospital, Northwestern University’s Feinberg School of Medicine and Robert H. Lurie Cancer Center, Chicago, IL, USA; ⁵Department of Microbiology and Immunology; ⁶Department of Medical Genetics, UBC

Introduction: Malignant rhabdoid tumour (MRT) is one of the most lethal solid cancers, with an overall 4-year survival rate of 23% and no effective therapy established to date. MRTs predominantly affect infants, accounting for nearly 20% of cancers in kidneys and brain, in which MRT frequently arises. Nearly all MRTs exhibit loss of SMARCB1, a subunit of the SWI/SNF chromatin-remodeling complex that mobilizes nucleosomes and mediates transcription regulation and epigenetic reprogramming. We have previously shown that the effect of SMARCB1 loss on transcriptome and epigenome was heterogeneous, revealing sub-groups with distinct patterns of gene-expression and CpG island-associated gene promoter methylation. To further our understanding of epigenetic reprogramming in MRT, we sought to profile genome-wide DNA methylation patterns and identify genes with correlating gene expression changes.

Methods: We carried out integrative analyses of gene expression and genome-wide DNA methylation in 40 primary extra-cranial MRT cases using RNA-Seq and WGBS-Seq approaches.

Results: We identified methylation sub-groups that correlated with patients’ age, and exhibited differential methylation levels in genes that significantly enriched for homeobox-related terms. Our preliminary analysis also revealed variable methylation levels in imprinting control regions affecting H19 and IGF2, which are development-regulating genes that are differentially expressed between gene-expression sub-groups in MRTs.

Conclusions: MRTs exhibit heterogeneous genome-wide DNA methylation and expression patterns in genes that regulate development.

Outcome/Impact: Our study presents evidence of epigenetic reprogramming of developmentally regulating genes in MRTs, suggesting potential avenues for therapeutic intervention.

Keywords: Malignant rhabdoid tumours; SMARCB1; Gene expression; DNA methylation
19. BIOMARKER VALIDATION BY IMMUNOFLUORESCENCE: A NOVEL APPROACH TO FOLLOW PROSTATE CANCER PROGRESSION

Clairefond S¹, Ouellet V¹, Péant B¹, Barrès V¹, Fragoso G¹, Mes-Masson AM¹, Saad F¹

¹Centre de recherche du Centre hospitalier de l’Université de Montréal-ICM

Introduction: Prostate cancer (PCa) is the most frequently diagnosed form of cancer and the third leading cause of cancer-related mortality in Canada. As PCa patient management varies widely, there is a need to find putative biomarker for clinical use to identify patients with a poor prognosis. Our aim is to evaluate several biomarkers by immunofluorescence (IF) and correlate their expression with patient clinical data in order to identify the best prognostic biomarkers, either alone or in combination.

Methods: Biomarker antibodies (PUMA, NOXA, ErbB3, EGFR) were verified for specificity using western blots and optimisation tissue microarrays (TMA), which contain cell lines and xenografts obtained from human PCa cell lines injected in mice. Evaluation of biomarker expression was performed on radical prostatectomy specimen arrayed on TMAs (286 patients). Each biomarkers were multiplexed with CK8 and CK18 (epithelium) and DAPI (nucleus). Moreover, p63 a basal membrane markers included to visualize non-cancerous glands. The analysis of biomarker expression was semi-automated using the VisiomorphDP software.

Results: Analysis has been completed for PUMA. As expected from previous results, we found no significant association with PCa patient prognosis. The predictive PUMA/NOXA combination that has previously been shown to be prognostic remains under investigation. Three commercial NOXA antibodies were non-specific in western blot and a fourth is presently under investigation. Specificity of ErbB3 has been demonstrated and the expression of ErbB3 was increased in tumour glands. Preliminary statistical analyse showed a trend toward association with patient prognosis. The EGFR antibody has been validated and the staining by IF is ongoing.

Conclusions: Our multi-marker approach allows us to quantitatively compare the expression of individual markers within the tumour micro-environment as well as at the sub-cellular level, providing rich datasets for biomarker selection and validation.

Outcome/Impact: Validation of biomarkers on a large cohort of patients is necessary to translate their use in a clinical setting for PCa patient management.

Keywords: Prostate cancer, biomarkers, stratification
20. MITOCHONDRIAL MUTATIONS IN PROSTATE CANCER CROSSTALK WITH NUCLEAR MUTATIONS

Hopkins Julia F.¹, Veronica Y. Sabelnykova¹, John Watson¹, Rached Alkallas¹, Jennifer Aguiar¹, Lawrence E. Heisler¹, Junyan Zhang², Michael Fraser², Theodorus van der Kwast³, Robert G. Bristow²,⁴,⁵, Paul C. Boutros¹,⁴,⁶

¹ Informatics and Bio-computing Program, Ontario Institute for Cancer Research, Toronto
² Princess Margaret Cancer Centre, University Health Network, Toronto
³ Department of Pathology and Laboratory Medicine, Toronto General Hospital/University Health Network, Toronto
⁴ Department of Medical Biophysics, ⁵ Department of Radiation Oncology, ⁶ Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada

Introduction: Prostate cancer remains the most prevalent and second most lethal non-skin cancer in men. Whole genome studies have provided important insights into specific driver genes, however most of these studies have not assessed one key portion of the genome: the mitochondrial genome.

Method: To gain a complete understanding of the most commonly-diagnosed sub-groups of prostate cancer: low- and intermediate-risk localized disease, we surveyed the mitochondrial genomes from next-generation sequencing (NGS) data of over 300 tumour samples from prostate cancer patients. These samples were mainly from prostate cancer patients with clinical Gleason Scores of 3+3, 3+4 and 4+3. All had at least 5 years of follow-up data (median > 8 years), allowing identification of clinical associations with identified somatic mutations via Cox Proportional Hazards modeling and machine-learning.

Results: These mutations appear to be associated with age of patient. The mtDNA region with the majority of mutations was the regulatory control region, although certain proteins had high numbers of mutations. Specific identified candidate somatic mutations were validated via Sanger sequencing. Clinical associations between somatic were also integrated with existing copy-number alteration (CNA) biomarkers using machine learning methods to evaluate performance. mtDNA mutations were also compared to identified aberrations (CNA, PGA, SNVs) within the nuclear genome to determine correlations between the two genomes, in addition to other somatic mutations or altered-expression in nuclear-encoded mitochondrial proteins.

Conclusions: Taken together, these data demonstrate a key role for mitochondrial mutations in prostate cancer.

Keywords: mitochondria, mtDNA, prostate cancer, biomarkers
21. DEFINING THE MUTATIONAL LANDSCAPE OF TRANSFORMED AND TREATMENT-RESISTANT FOLLICULAR LYMPHOMA

Kridel Robert¹, Fong Chun Chan¹, Marco A. Marra², Randy D. Gascoyne¹, Sohrah P. Shah³

¹Centre for Lymphoid Cancer; ²Michael Smith Genome Sciences Centre; ³Department of Molecular Oncology, BC Cancer Agency, Vancouver, BC.

Introduction: Follicular lymphoma (FL) is an indolent, yet incurable disease. A subset of patients experience increased mortality rate driven by two distinct clinical endpoints: histological transformation and early progression after therapy. The genetic basis of these events is imperfectly understood and we aimed at filling this discovery gap in a large cohort.

Methods: We performed targeted capture-based sequencing of 94 genes in a cohort of 205 samples from 205 patients in order to decipher disrupted biology in the two clinical endpoints. The cohort was split up as follows: 1) TFL cohort: 116 patients experiencing transformation, including 80 samples from the FL timepoint (T1) and 83 samples from the transformed FL (TFL) timepoint (T2); 2) Clinical extremes cohort: 97 patients presenting either with early progression after therapy (n=30 T1 samples) or with late/never progression (n=67 T1 samples).

Results: We computed significantly mutated genes while accounting for differences in background somatic mutation rate. In the TFL cohort, 14 genes were significant in T2 only, including CCND3, RHOA and FOXO1. Mutations in these genes are inferred to occur late in the aetiology of TFL. B2M was the most differentially mutated gene and B2M mutations were associated with significantly reduced infiltration by CD8-positive T-cells. In the clinical extremes cohort, we found 12 genes were significantly altered in diagnostic specimens of early progressers, including B2M, BTG1 and XBP1. None of the early progression-associated genes were mutated at a frequency >20%. Thus, early progression appears to be related to relatively infrequent genetic alterations.

Conclusions: We found novel associations of gene mutations with transformation and with treatment resistance. Gene content of mutations associated with transformation and early progression differed.

Outcome/Impact: Our study reveals novel insights into the genetic basis of transformation and treatment resistance, thereby opening avenues for understanding mechanism of treatment resistance and improving currently available prognostic tools.

Keywords: follicular lymphoma, transformation, progression
22. TARGETING THE INHIBITOR OF APOPTOSIS PROTEIN, BIRC6, AS A NOVEL, POTENTIAL STRATEGY FOR THERAPY OF ADVANCED ENZALUTAMIDE-RESISTANT PROSTATE CANCER

Luk Iris Sze Ue¹,², Raunak Shrestha¹, Hui Xue¹,², Yuwei Wang², Fang Zhang², Dong Lin¹,², Anne Haegert¹, Rebecca Wu², Xin Dong², Colin C. Collins¹, Martin Gleave¹, Peter W Gout² and Yuzhuo Wang¹,²

¹Vancouver Prostate Centre, Vancouver, BC; ²Department of Experimental Therapeutics, BC Cancer Agency, Vancouver, BC.

Introduction: Enzalutamide (ENZ) resistance has emerged as a major problem in the management of advanced castration-resistant prostate cancer (CRPC). The Inhibitor of Apoptosis Protein (IAP) family is well recognized for its role in promoting treatment resistance of cancers by inhibiting drug-induced apoptosis. Here we examined whether IAP family members play a role in ENZ resistance and could provide a target for therapy of ENZ-resistant CRPC.

Methods: The LTL-313BR transplantable patient-derived CRPC tissue xenograft line showing primary ENZ resistance, and xenografts based on CRPC cell lines showing treatment-induced (acquired) ENZ resistance were used. BIRC6-targeting was carried out using antisense oligonucleotide (ASO-6w2). Molecular pathways associated with growth-inhibitory effects were assessed via gene expression profiling and gene enrichment analysis.

Results: Of eight IAPs examined, BIRC6 was the only one showing elevated expression in both primary and acquired ENZ resistance models. Treatment with ASO-6w2 markedly suppressed growth of both resistance models, with the LTL-313BR model showing increased tumour apoptosis without major host toxicity. Pathway enrichment analysis indicated that GPCR and matrisome signalling were most significantly altered pathways. Furthermore, ASO-6w2 inhibited expression of pro-survival genes up-regulated in the LTL-313BR line.

Conclusions: BIRC6 was the top upregulated IAP member in ENZ-resistant CRPC and BIRC6-targeting inhibited the growth of both primary and acquired ENZ-resistant CRPC models.

Impact: BIRC6-targeting therapy may represent a novel treatment strategy for patients with ENZ-resistant CRPC.

Keywords: Enzalutamide-Resistance, Patient-derived Xenograft, BIRC6, Castration-resistant Prostate Cancer

This project is funded by TFRI. Terry Fox New Frontiers Program on Prostate Cancer Progression, Project 5: The Anti-apoptosis Protein, BIRC6: A Potential Therapeutic Target for Prostate Cancer (Y. Wang (PI) and M. Gleave).
23. INVESTIGATING THE RESPONSE OF PANCREATIC TUMOURS TO HIGH-DOSE IRRADIATION USING IN VIVO IMAGING

Maeda Azusa1,2, Ralph Dacosta1,2

1Princess Margaret Cancer Centre, University Health Network; 2Department of Medical Biophysics, University of Toronto. Toronto, ON.

Introduction: The number of clinical trials investigating the use of stereotactic body radiotherapy (SBRT) for pancreatic cancer is increasing; however, the biological mechanisms underlying the effect of SBRT remain unclear. A better understanding of the biological effects of high-dose irradiation on tumour, vasculature and microenvironment will inform SBRT regimen to achieve a better clinical outcome.

Methods: Subcutaneous BxPC3 pancreatic tumour xenograft was established in the dorsal skinfold window chamber (DSWC) and hind leg models. Tumours were irradiated with a single dose of 4-24 Gy, and the following parameters were assessed using in vivo imaging techniques: tumour size, microvascular function, vascular permeability, platelets/leukocytes adhesion and tumour hypoxia.

Results: Radiation induced significant but transient vascular dysfunction in a dose-dependent manner, while maintaining most of the vascular structure intact. Platelet and leukocyte adhesion to the vascular endothelium was observed within hours to days after irradiation, which could contribute to the reduced blood flow. Such acute reaction was followed by an increase in vascular permeability and tumour hypoxia occurring 1-2 weeks after irradiation. Similar results were obtained in the hind leg model, with tumour hypoxia increasing 2 weeks after irradiation with 24 Gy. Long-term assessment of tumour response demonstrated that the single dose of 24 Gy was insufficient to cause tumour control.

Conclusions: A single dose of irradiation caused functional alterations to the tumour vasculature, followed by revascularization and tumour recurrence. Irradiation-induced endothelial cell activation could contribute to the increase in vascular permeability and development of tumour hypoxia. The current data supports the concept that a high single dose of radiation does not simply cause vascular ablation, suggesting the need for further investigation to improve therapeutic outcome of SBRT.

Outcome/Impact: The results of this work could lead to a better understanding of the underlying mechanisms of radiation response in pancreatic tumours, leading to development of more effective pancreatic cancer treatment strategies.

Keywords: Radiation therapy, vasculature, hypoxia, pancreatic cancer
Introduction: Triple negative breast cancers (TNBC) lack effective targeted therapies and remain a challenge clinically. Elevated levels of the MET receptor are correlated with TNBC and poor outcome, but its roles in tumour initiation and progression are not well understood.

Methods: We have employed the Metmt;Trp53fl/+ transgenic mouse model of TNBC, human breast cancer cell lines, and breast patient-derived xenografts (PDXs) to investigate the role of MET signaling and its collaborating pathways in TNBC. To study tumour-initiation, we utilized a range of techniques including sphere-forming assays, flow cytometry, and in vivo transplant experiments.

Results: Metmt;Trp53fl/+ tumours genetically resemble human claudin-low tumours, a TNBC subtype enriched for mesenchymal and stem-like signatures. We have identified fibroblast growth factor receptor 1 (FGFR1) as a key signaling partner for Met in the regulation of Metmt;Trp53fl/+ TICs, where dual Met-FGFR1 inhibition abrogates sphere-formation in vitro and prolongs tumour-free survival in mice transplanted with Metmt;Trp53fl/+ cells. Consistent with our findings in mouse model, human claudin-low cell lines highly co-express MET and FGFR1, and dual inhibition of both receptors results in impaired sphere-formation and a depleted TIC compartment. Furthermore, a subset of TNBC PDXs co-express MET and FGFR1 and are similarly sensitive to MET and FGFR inhibitors in TIC assays. We are currently investigating the efficacy of combining chemotherapies with MET and FGFR inhibitors to eliminate both TICs and bulk tumour populations in candidate tumours.

Conclusions: Our findings support a role for Met and FGFR1 in TIC regulation in a subset of TNBC. The requirement for MET and FGFR1 signaling for TIC maintenance and efficient tumour outgrowth highlights the therapeutic potential of inhibiting both receptors in these tumours.

Outcome/Impact: TICs promote both cancer initiation and relapse. Validating a role for MET and FGFR1 signaling in TICs provides valuable insight into TNBC tumourigenesis, and proposes a patient stratification strategy and rationale for combination therapies targeting MET and FGFR1.

Keywords: breast cancer, tumour-initiating cells, MET, FGFR1
25. A CONTEMPORARY ANALYSIS OF ACTIVE SURVEILLANCE UPTAKE FOR LOW RISK LOCALIZED PROSTATE CANCER (PC) IN CANADA

Timilshina Narhari¹, Veronique Ouellet², Shabbir M.H. Alibhai¹, Anne-Marie Mes-Masson², Nathalie Delvoye², Darrel Drachenberg², Antonio Finelli¹, Marie-Paule Jammal², Pierre Karakiewicz², Hélène Lapointe², Jean-Baptiste Lattouf², Kenny Lynch², Jean-Benoît Paradis², Paula Sitarik², Alan So², Fred Saad²

¹UHN, ²Canadian Prostate Cancer Biomarker Network

Introduction: In active surveillance (AS), practitioners delay curative treatment in low-risk patients until there is evidence of disease progression, at which time active treatment is initiated. Although the uptake of AS appears to be increasing, the actual uptake in Canada remains largely unknown. The objective of the study is to determine the practice patterns, predictors of AS uptake and persistent use of AS for 12 months in low risk PC in Canada.

Methods: We evaluated the use of AS in men who underwent a prostate biopsy in 2010 in six centres in four provinces (BC, QC, MB and ON). At each centre, clinical and pathological information were collected prospectively for 12 months following the patients who underwent a first prostate biopsy in 2010. Outcomes initial uptake and persistent use of AS for 12 months were evaluated using logistic regression.

Results: Of 986 patients, 781 patients (mean age 64 years) were incident cases and eligible for AS. There were significant over three-quarters (77.3%) of patients chose AS at diagnosis and differences in uptake of AS by region (range 65.0-98.0%, p≤ 0.05). Key multivariate predictors of pursuing AS included age (p=0.044), region (p=0.021), number of cores (p=0.025), number of positive biopsy cores (p<0.001), and percent core involvement (p<0.001). 516 (85.4%) men remained on AS over 12 months. Maintenance with AS over 12 months differed by region, ranging from 64.1-93.9% (p=0.001). Predictors of maintenance with AS over 12 months included age, region, and number of positive cores.

Conclusions: AS is widely practiced across Canada, but important regional differences exist in patterns of AS use. Further analyses are required to understand the root causes of differences, and also to determine whether AS uptake is changing over time.

Outcome/Impact: The study result shows significant uptake of this management approach but important differences in patterns of AS use between provinces. In addition, our study provides important baseline data on Canadian rates of AS use in low-risk PC.

Keywords: Active surveillance, prostate cancer, population based study, treatment
A TIMECOURSE STRATEGY TO IDENTIFY TRANSIENTLY HYPOXIC CELLS IN SOLID TUMOURS

Wadsworth Brennan¹,², Elizabeth Halvorsen¹, Ada Young¹,², Natalie Firmino¹,², Kevin Bennewith¹,²

¹BC Cancer Research Centre Integrative Oncology Department; ²UBC Pathology and Laboratory Medicine Department. Vancouver, BC.

Introduction: Solid tumours often develop two distinct forms of inadequate oxygen supply (or ‘hypoxia’); chronic hypoxia due to low vascular density and transient hypoxia from cyclical perfusion loss and re-oxygenation. In vitro data has displayed substantial differences in metastatic potential of these populations, which we aimed to build upon by first improving our ability to identify and separate these hypoxic populations in tumour xenografts.

Methods: Tumour-bearing mice were injected with two hypoxia reporters, pimonidazole and EF5, two hours apart. After tumour harvest, reporters were detected via both fluorescent immunohistochemistry (IHC) and flow cytometry. Cells labeled by only one of the two reporters indicate a change in oxygenation. We validated reporter response to tumour perfusion using nicotinamide and hydralazine, two vasoactive agents known to increase and decrease tumour perfusion respectively. As chronically hypoxic cells exist even in the presence of perfusion, we hypothesized that nicotinamide-induced perfusion would specifically re-oxygenate transiently hypoxic cells and tested this via distance mapping of IHC images.

Results: Our reporter system could detect singly labeled cells at a significant rate (30% of hypoxic area in IHC N=4 p<0.01). The hypoxia reporters demonstrated a significant response to both perfusion gain (25% drop in EF5 signal N=4 p<0.05) and perfusion loss (3-fold increase in EF5 signal N=4 p<0.01). When hypoxic regions in IHC images were mapped based on distance from blood vessels (CD31 staining), we observed that nicotinamide only re-oxygenated tumour regions more proximal to vessels (50% drop in hypoxic staining).

Conclusions: Our observations suggest that hydralazine can be used to induce a tumour-wide bout of transient hypoxia and that nicotinamide can specifically re-oxygenate the transiently hypoxic tumour cell population.

Outcome/Impact: Future work will use hydralazine as a tool to amplify the transiently hypoxic tumour cell population in vivo for confirmation of expression and migration phenotypes. Our reporter system will be combined with nicotinamide to separate the hypoxic populations for assessment of pro-metastatic gene expression and biomarker discovery.

Keywords: Hypoxia, Tumour Microenvironment, Tumour Perfusion, Detection Methods
27. PROTEOME SIGNATURES AND NEW CANCER DRIVERS

Zhang Wen1,2, Shingo Sakashita3, Melania Pintilie1,3, Bethany Pitcher3, Jiefei Tong2, Paul Taylor2, Nhu-An Pham3, Ming Li3, Tao Wang3, Jing Xu3, Wendy So3, Leanne Wybenga-Groot2, Ming S. Tsao1,3, and Michael F. Moran1,2,3

1University of Toronto; 2Hospital for Sick Children, Program in Cell Biology; 3Princess Margaret Cancer Centre, Toronto, ON, Canada

Introduction: The ability to establish a primary tumour-derived xenograft is a poor prognosis indicator in early stage non-small cell lung carcinoma (NSCLC) and other cancers, suggesting engraftment selects for aggressive aspects of the cancer phenotype linked to disease progression. We hypothesized engrafting NSCLC have distinctive proteome features which may reveal novel cancer drivers. We contend proteome signature discovery is a vital component of an “integrated omics” platform, and broadly applicable to various types of cancer.

Methods: 53 NSCLC primary tumours were analyzed by quantitative proteomics. An optimized method was developed involving a stable isotope labeled protein mixture from representative NSCLC cell lines added to each sample as an internal standard. Proteome signatures of engraftment were assessed for significant links to clinical features. Validation involved analysis of signatures in an independent patient cohort, and a determination of the consequences of signature protein modulation on in vitro and in vivo cancer phenotypes.

Results: Proteomes of engrafting NSCLC tumours were different from non-engrafting tumours. Most significantly altered was a signature comprising certain metabolism proteins. High level expression of one particular metabolism protein in non-engrafting tumours was validated in an independent NSCLC cohort for association with better survival. Ectopic expression of the protein inhibited NSCLC cell proliferation and xenograft tumour growth.

Conclusion: Aggressive NSCLC have a distinctive proteome signature suggestive of an altered metabolic state linked to overall survival. An enzyme, not previously implicated by genome or transcriptome analyses, is implicated as a negative cancer driver in NSCLC.

Outcome/Impact: Our results illustrate the power of proteomics to uncover cryptic cancer drivers, and portend innovative anti-metabolism therapeutic modalities.

Keywords: non-small cell lung cancer, cancer metabolism, proteomics/proteome profiling
28. CD73 EXPRESSION IS AN INDEPENDENT PROGNOSTIC BIOMARKER IN PROSTATE CANCER

Allard Bertrand¹,², Bruno G. Leclerc¹,², Roxanne Charleboix¹,², Guillaume Chouinard¹, Sandra Pommey¹, Fred Saad¹, and John Stagg¹,²

¹Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Institut du Cancer de Montréal, Montréal, Québec, Canada.
²Faculté de Pharmacie, Université de Montréal, Pavillon Jean-Coutu, Montréal, Québec, Canada.

Introduction: CD73 is an adenosine-generating ecto-enzyme that suppresses antitumour immunity in mouse models of cancer, including prostate cancer. Although high levels of CD73 are associated with poor prognosis in various types of human cancers, the clinical impact of CD73 in prostate cancer remains unclear.

Methods: We evaluated the prognostic value of CD73 protein expression and CD8+ T cell density in 285 cases of prostate cancer on tissue microarray (TMA). Normal adjacent and tumour tissues were evaluated in duplicates.

Results: Univariate and multivariate analyses revealed that high levels of CD73 in normal adjacent prostate epithelium were significantly associated with shorter biochemical recurrence (BCR)-free survival. Notably, CD73 expression in normal epithelium conferred a negative prognostic value to prostate-infiltrating CD8+ T cells. Surprisingly, high levels of CD73 in the tumour stroma were associated with longer BCR-free survival in univariate analysis. In vitro studies revealed that adenosine signaling inhibited NF-κB activity in human prostate cancer cells via A2B adenosine receptors. Consistent with these results, CD73 expression in the prostate tumour stroma negatively correlated with p65 expression in the nuclei of prostate tumour cells.

Conclusions: Our study revealed that CD73 is an independent prognostic biomarker in prostate cancer. Our data support a model in which CD73 expression in the prostate epithelium suppresses immunosurveillance by CD8+ T cells, whereas CD73 expression in the tumour stroma reduces NF-κB signaling in tumour cells via A2B adenosine receptor signaling. CD73 expression, including in normal adjacent prostate epithelium, can thus effectively discriminate between aggressive and indolent forms of prostate cancer.

Outcome/Impact: Our work identified CD73 as a new biomarker capable of stratifying prostate cancer patients and predicting outcome. Our data also confirm the negative prognostic value of CD8+ T cell infiltration in prostate cancer.

Keywords: CD73, prostate cancer, NF-κB, CD8+ T cells, biomarker.
29. MURINE MODELS OF PANCREATIC CANCER CAN BE EFFECTIVELY TREATED WITH AN INFECTED CELL VACCINE

Baxter Katherine¹,², Almohanad Alkayyal¹,², Lee Hwa-Tai¹, Kyle Stephenson³, Carolina Ilkow¹, Michael Kennedy¹, John C Bell¹,², Brian D. Lichty², Rebecca C Auer¹,⁴

¹Center for Innovative Cancer Research, OHRI, Ottawa, ON; ²Department of Biochemistry, Microbiology, and Immunology, uOttawa,; ³Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON; ⁴Department of Surgery, Division of General Surgery, The Ottawa Hospital

Introduction: Pancreatic cancer has a dismal prognosis with <5% survival. A recent clinical trial with an immunomodulatory allogeneic cancer vaccine showed prolongation of progression free survival in pancreatic cancer patients, suggesting this approach may hold promise. In this project we sought to explore whether a replicating, autologous infected cell vaccine (ICV) could stimulate an immune response against tumour cells to prevent metastases and improve survival in an animal model of pancreatic cancer.

Methods: An ICV was prepared using irradiated Pan02 cells and infected with Maraba MG1 virus expressing IL-12 at a multiplicity of infection (MOI) of 10. The effectiveness of the vaccine was explored using both prophylactic and therapeutic treatment regimens of a subcutaneous and a peritoneal murine model of pancreatic cancer. The therapeutic effect was measured by assessing tumour outgrowth and immune responses were evaluated using a flow cytometry based assay of tumour-specific T cell (CTL) cytotoxicity.

Results: The ICV inhibited tumour growth in the subcutaneous model, including a complete response in 28% of mice after three doses. In a prophylactic vaccination model of peritoneal disease, mice exhibited partial protection from tumour challenge with reduced tumour burden 20 days after Pan02 challenge. An in vitro CTL killing assay demonstrated effective targeting of the endogenous tumour associated antigen, p15E.

Conclusions: The MG1-IL12 ICV demonstrates efficacy in a murine model of pancreatic cancer associated with an adaptive tumour-specific CTL response against the endogenous tumour associated antigen p15E. Future directions will be to combine the ICV with immunotherapies to further increase survival times.

Outcome/Impact: Pancreatic cancer is deadly but ICVs are a promising treatment strategy that would increase survival for the 4400 Canadians diagnosed with pancreatic cancer each year.

Keywords: pancreatic cancer, oncolytic vaccine, Maraba MG1, Interleulin-12
30. DEVELOPMENTAL TRANSCRIPTION FACTOR NFIB IS A TARGET OF ONCOFETAL MiRNAs AND IS ASSOCIATED WITH TUMOUR AGGRESSIVENESS IN LUNG ADENOCARCINOMA

Becker-Santos Daiana D.¹, Kelsie Thu¹, John English³, Larissa Pikor¹, Victor D. Martinez¹, Calum MacAulay¹, William Lockwood¹, Wendy Robinson³, Igor Jurisica², Stephen Lam¹, Wan Lam¹

¹BC Cancer Research Centre, Vancouver BC; ²Princess Margaret Cancer Center, Toronto ON; ³University of British Columbia, Vancouver BC

Introduction: Genes involved in fetal lung development are thought to play crucial roles in the malignant transformation of adult lung cells. Consequently, the study of lung tumour biology in the context of lung development has the potential to reveal key regulatory pathways reactivated in lung cancer.

Methods: 131 pairs of non-small cell lung cancer (NSCLC) tumour and non-malignant lung tissues and 15 human fetal lung tissue samples were profiled by miRNA-sequencing. To investigate protein-coding genes controlled by the oncofetal miRNAs identified, miRDIP algorithm was applied followed by luciferase-reporter assays. Associations between patient survival and mRNA expression of selected oncofetal miRNA-gene targets were evaluated in ~1,400 NSCLC cases. Immunohistochemical analysis of oncofetal miRNA targets was performed on a lung adenocarcinoma (LUAD) tissue microarray.

Results: We describe for the first time a comprehensive characterization of miRNA expression in human fetal lung tissue, and identified numerous miRNAs that recapitulate their fetal expression patterns in NSCLC. Nuclear Factor I/B (NFIB), a transcription factor essential for lung development, was identified as being frequently targeted by these oncofetal miRNAs. Concordantly, analysis of NFIB expression in multiple NSCLC cohorts revealed its frequent underexpression in tumours (>60%). Remarkably, low expression of NFIB was significantly associated with higher grade, biologically more aggressive subtypes of LUAD, and ultimately, poorer survival in LUAD patients.

Conclusions: This work has revealed a prominent mechanism for the downregulation of NFIB, a developmental transcription factor essential for lung differentiation, which we found to be associated with aggressive phenotypes of LUAD and consequently, poor patient survival.

Outcome/Impact: Restoration of NFIB expression in LUAD may induce lung cell differentiation, and therefore has the potential to reduce tumour aggressiveness.

Keywords: miRNAs, miRNA-sequencing, lung adenocarcinoma (LUAD), lineage transcription factors
Introduction: Although DNA methylation is recognized as having an important role in the etiology of cancer and other diseases, the extent to which it is determined by lifestyle factors such as adiposity remains unclear. We examined the association between various measures of adiposity with DNA methylation within Long Interspersed Nuclear Element-1 (LINE-1) and Alu repeat regions amongst a group of healthy post-menopausal women.

Methods: We conducted a cross-sectional study using baseline information from 289 post-menopausal women who enrolled in the Alberta Physical Activity and Breast Cancer Prevention Trial (2003-6). Baseline measures of adiposity were objectively assessed using a DXA scan, CT scan, and balance beam scale. Weights at different ages were self-reported in a baseline health questionnaire. DNA methylation was analysed from white blood cells using a bisulfite-PCR pyrosequencing assay. Linear mixed effects model was used to assess the associations of interest while controlling for potential confounders. All effect sizes are reported as the percent methylation increase per standard deviation increase in the exposure of interest.

Results: LINE-1 methylation was significantly positively associated with intra-abdominal fat area (β: 0.21, p = 0.03), body fat percent (β: 0.20, p = 0.05), fat mass (β: 0.26, p = 0.01), waist circumference (β: 0.21, p = 0.03), hip circumference (β: 0.33, p = 0.001), BMI (β: 0.21, p = 0.03), baseline weight (β: 0.30, p = 0.002), weight at age 20 (β: 0.22, p = 0.02), and weight gain (β: 0.20, p = 0.03). No significant associations were found between any of the adiposity measures and Alu methylation.

Conclusion: Increased levels of adiposity are associated with higher levels of LINE-1 methylation in healthy post-menopausal women.

Impact: These findings contribute to our growing understanding of the determinants of DNA methylation and suggest that the epigenome remains susceptible to influence later in life.

Keywords: epigenetics, DNA methylation, adiposity, cancer
INTRODUCTION: Prostate cancer (PCa) metastases are characterized by high frequency of DNA copy number alterations including an uncharacterized gain at 16p13.3 region. With its clinical relevance unexplored, we hypothesized that the 16p13.3 gain might be associated with PCa progression and predict clinical outcome, if detected early in primary tumours.

METHODS: Fluorescent in situ hybridization (FISH) was used to detect 16p13.3 gain on tissue microarrays (TMAs) representing primary radical prostatectomy (pRP) cohort with clinical follow-up (n=304) and Castrate resistant PCa (CRPC) from a series of transurethral resection of the prostate (TURP) for advanced disease (n=37).

RESULTS: The 16p13.3 genomic gain was detected in 42% (113/267) of the pRP specimens assessed by FISH wherein the gain status was significantly associated with clinico-pathological features of aggressive PCa including high Gleason score (GS), advance tumour stage and high post-operative risk scores defined by the Cancer of the Prostate Risk Assessment (CAPRA-S) nomogram (p<0.05, respectively). Importantly, the 16p13.3 gain status significantly predicted early biochemical recurrence in these patients (log rank, P=0.003, HR=2.04), independent of the standard prognostic indicators (GS, pre-operative prostate specific antigen (PSA), and tumour stage). Furthermore, the association between 16p13.3 gain and shorter recurrence free survival was still observed in a subgroup of patients with GS≤7. The combination of 16p13.3 gain with the CAPRA-S or the standard prognostic indicators improved the overall prognostication as compared to these markers alone (log rank, P<0.001, respectively). In CRPC, the 16p13.3 gain was detected in 70% (26/37) of cases, wherein, the level and frequency of gain were significantly higher than that in pRPs (P=≤0.01).

CONCLUSIONS: For the first time, our study demonstrates the prognostic significance of 16p13.3 genomic gain in primary PCa patients and suggests its potential as a marker of PCa progression.

OUTCOME/IMPACT: Identification and characterization of such novel biomarkers might aid in appropriate patient risk-stratification and allow for efficient clinical management of PCa.

KEYWORDS: Prostate cancer, prognosis, 16p13.3
33. COST-EFFECTIVENESS ANALYSIS OF GENOME-GUIDED MANAGEMENT OF PREMALIGNANT ORAL LESIONS

Cromwell Ian¹,², Dean A Regier¹,²,³, Stuart J Peacock¹,²,⁴, Catherine F Poh²,⁵

¹Canadian Centre for Applied Research in Cancer Control, BC Cancer Agency; ²Department of Cancer Control Research, BC Cancer Agency; ³School of Population and Public Health, UBC; ⁴Faculty of Health Sciences, SFU; ⁵Department of Oral Oncology, BC Cancer Agency

Introduction: A recent prospective study showed that a specific molecular panel of biomarkers, using loss of heterozygosity (LOH) is the most significant predictor of progression of an Oral Premalignant Lesion (OPL) to an invasive cancer. Patients presenting with an OPL can be stratified into high-, intermediate-, or low-risk group that corresponds to the likelihood of developing cancer. Adjusting clinical management to fit this risk would result in a change in patterns of health system resource utilization. We examined the potential cost-effectiveness of using a genomic assay to inform such a risk-stratification approach.

Methods: We constructed a cost-effectiveness Markov model that simulates a cohort of patients with newly-diagnosed OPLs. Frequency of follow-up is based on LOH risk score: high-risk patients are given immediate OPL resection, intermediate-risk patients are re-assessed every two years, and low-risk patients are re-assessed every five years. The costs and outcomes experienced by this cohort were compared to those of a cohort receiving current standard practice (re-assessment every six months with three-year comparative re-biopsy, regardless of risk). Model parameters values were determined from retrospective BCCA data, the published literature, expert opinion, and data from the COOLS Trial, a TFRI-funded phase III randomized surgery trial for early tumour-stage oral cancer.

Results: The risk-stratified cohort experienced an average on 0.64 additional quality-adjusted life years (QALYs) and cost $8123 less to manage than the standard care cohort, and retained a 95% probability of being cost-saving even if LOH risk identification costs $1275 per assessment, well above the baseline estimate of $500. Sensitivity analysis suggests that these findings are robust to both expected and extreme variation in all parameter values.

Conclusion: Our model suggests that LOH risk-stratification is cost-saving and produces better patient outcomes than standard practice.

Impact: Development of an LOH risk-identification assay could dramatically change clinical practice for patients with OPLs.
34. USING THE ZEBRAFISH TO MODEL HIGH-RISK, NUP98-NSD1 INDUCED PEDIATRIC ACUTE MYELOID LEUKEMIA

Filiaggi Corey¹, Adam P. Deveau², Sergey Prykhozhij², Jason N. Berman¹,²

¹Dalhousie University; ²IWK Health Centre; Halifax, NS.

Introduction: Acute myeloid leukemia (AML) is a common pediatric leukemia caused by the accumulation of genetic and epigenetic changes leading to expansion and differentiation arrest of myeloid cells in the bone marrow. Overall AML survival rates are poor, which is even more apparent when patients are stratified into high-risk groups based on cytogenetics, like pediatric patients with the NUP98-NSD1 (NND1) translocation; we hypothesize that a transgenic zebrafish model expressing NND1 will recapitulate critical disease aspects of this high-risk pediatric AML.

Methods: Plasmid constructs containing NND1 were generated with Gateway® cloning, and incorporated into the zebrafish using Tol2-mediated transgenesis. Whole-mount in situ hybridization (WISH) experiments were performed at 24, 28, and 36 hours post fertilization (hpf) with erythroid (gata1), myeloid (lcp1, mpx), and hematopoietic stem cell (HSC) (runx1, c-myb) markers to assess the effects of NND1 expression on hematopoiesis in the zebrafish.

Results: Plasmid-injected zebrafish expressing NND1 under a ubiquitous (ubi) or pan-leukocyte (cd45) promoter display green fluorescence (indicating NND1 expression) in circulating myeloid cells, as well as in sites of zebrafish embryonic hematopoiesis. WISH experiments demonstrate increased expression of myeloid markers with decreased erythroid gene expression, and no differences in HSC markers in fish expressing NND1 compared to uninjected control fish.

Conclusions: Our zebrafish model of NND1 demonstrates a disruption in normal hematopoiesis which is similar to the pre-leukemic state observed in other zebrafish models of high-risk AML containing a NUP98 gene fusion.¹

Outcome/Impact: Preclinical NND1 animal models are lacking. Zebrafish expressing the NND1 fusion can be used to interrogate underlying leukemogenic mechanisms, and as a platform for discovering small molecules that restore normal hematopoiesis. These studies have the potential to elucidate the pathogenesis of this high-risk form of pediatric AML and identify promising targets and therapies to pursue to improve outcome.

Keywords: Leukemia, Hematopoiesis, NUP98-NSD1, Zebrafish

NOVEL THERAPEUTIC TARGETS IN HEAD AND NECK CANCER

Kondratyev Maria¹, Aleksandra Pesic¹, Stephano Marastoni¹, Reider Grenman², Marianne Koritzinsky¹ and Bradly G. Wouters¹

¹Princess Margaret Cancer Centre, Department of Radiation Oncology, University Health Network, Toronto, Ontario; ²Dept. of Otorhinolaryngology - Head and Neck Surgery, Turku University and Turku University Hospital, Turku Finland

Introduction: Head and neck carcinoma is the sixth most common malignancy in the world. Despite advances in diagnosis and treatment, the survival rates remain unacceptably low due to high rates of local and regional recurrences. The biology of underlying patients with poor prognosis remains unclear.

Methods: We used functional genomic technologies to identify new potential therapeutic targets for metastatic disease in HNSCC. These targets were identified by conducting whole genome shRNA screens in matched sets of cell lines derived from primary tumours and their respective metastatic sites, with the goal of identifying genes that become essential for cell survival only following metastasis. To test if knockdown of selected targets will inhibit metastasis in a therapeutic setting, we established orthotropic model of HNSCC that metastasize to regional lymph nodes in the mouse.

Results: Several components of the Notch signaling pathway (Notch3, Jag2) were identified and validated as essential for survival of cells derived from metastatic sites. In one patient, whole exome sequencing identified a novel mutation in one of the EGF domains of Notch3 that was acquired only in the metastatic line. Mutations in EGF domains have been reported to influence interaction with specific ligands, dictating which ligand can activate Notch signalling. Our preliminary data indicate that metastatic, but not primary tumour cells, undergo apoptosis upon knockdown of Notch3 and that a distinct set of target genes is induced upon interaction between Notch3 and the Jag2 ligand (expressed at considerably higher levels in metastatic cells). Furthermore, our results to date indicate that suppression of Notch3 improves survival in mice bearing orthotopic tumours derived from the metastatic HNSCC lines.

Conclusions/Impact: Our data demonstrates that metastatic cells from head and neck tumours acquire dependency on Notch3 signaling. Novel treatments targeting components of this pathway may prove effective in targeting metastatic cells alone or in combination with conventional therapies.

Keywords: head and neck cancer, Notch signalling, novel therapies.
36. THE ROLE OF UBR5 MUTATIONS IN THE PATHOGENESIS OF MANTLE CELL LYMPHOMA

Kutovaya Olga A.¹,², Stacy Hung¹, Christopher Hughes³, Gregg B. Morin³,⁴, Randy Gascoyne¹,², Christian Steidl¹,².

¹Centre for Lymphoid Cancer, Department of Experimental Therapeutics, BC Cancer Agency; ²Department of Pathology and Laboratory Medicine, UBC; ³Michael Smith Genome Sciences Centre, BC Cancer Agency; ⁴Department of Medical Genetics, UBC.

Introduction: Mantle Cell Lymphoma (MCL) accounts for 7% of non-Hodgkin lymphomas and represents a challenging disease with patient outcomes inferior to most other lymphomas. Using targeted capture sequencing of MCL biopsies, we reported frequent mutations in UBR5, a gene encoding an E3 ubiquitin-protein ligase that has not previously been implicated in lymphomagenesis. All mutations were clustered within 100bp in or around Exon58 and truncate the reading frame. These findings implicate UBR5 mutations as critical pathogenic events and might be therapeutically targetable. The aim of this study is to determine the specific role of UBR5 mutations in the pathogenesis of MCL.

Methods: As seen in MCL patients, mutations in Exon58 of UBR5 were generated in three MCL cell lines (Granta-519, Jeko-1, and Mino) using the CRISPR-Cas9 genome engineering tool. First, immunoprecipitation-mass spectrometry-based (IP-MS) was employed to identify UBR5 interacting partners in UBR5 mutant and wildtype (WT) samples. Next, global proteomes of these samples were analyzed by TMT-based mass spectrometry.

Results: The IP-MS analysis of WT vs UBR5 mutants revealed a number of histone proteins (H1, H4, and H2AFX) as candidate UBR5 interacting proteins. The global proteome analysis was performed to identify differentially expressed genes common among the MCL cell lines with the same fold change and direction of change. Gene ontology analysis indicated that DNA damage response, chromosome organization, cell cycle response, and mRNA processing pathways were affected.

Conclusions: The proteome results are consistent with UBR5 functioning as a key regulator of cell signalling. Our results strongly suggest UBR5 is a novel regulator of histone modifications. These findings provide an experimentally valid platform for further investigation and functional validation.

Outcome/Impact: UBR5 mutations and associated pathways might emerge as novel drug targets for future therapies in personalized medicine initiatives. Our future goal is to conduct pre-clinical investigations on MCL biopsies as we believe this work has the potential to provide novel and predictive biomarkers in the modern era of MCL treatment.

Keywords: mantle cell lymphoma, UBR5 mutations, proteome, histones.
37. REGULATORY LANDSCAPE OF DEVELOPING HUMAN BRAIN

Li Luolan¹, Misha Bilenky², Annaick Carles¹, Michael Stevens⁴, Alireza Heravi-Moussavi², Chibo Hong³, Cecile Maire⁵, Angela Tam², Baljit Kamoh², Stephanie Cho², Dorothy Cheung², Irene Li², Tina Wong², Raman Nagarajan³, Andrew J Mungall², Richard Moore², Steven JM Jones², Marco A Marra², Ting Wang⁴, Keith L. Ligon⁵, Joseph Costello³, Martin Hirst¹,²

¹Department of Microbiology and Immunology, Centre for High-Throughput Biology, UBC, Vancouver; ²BC Cancer Agency Canada’s Michael Smith Genome Science Center, Vancouver; ³Departments of Neurological Surgery, Pathology and Microbiology and Immunology, University California San Francisco, San Francisco, CA, USA; ⁴Department of Genetics, Washington University, St. Louis, MO, USA; ⁵Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute, Boston, MA, USA.

Introduction: A comprehensive regulatory network of primary human brain cell types provides a foundation from which to interpret pathogenic deregulation.

Methods: Here we characterized epigenomes and transcriptomes of brain tissue and primary neural progenitor cell (NPC) types derived from four human fetuses at three developmental stages including a pair of monozygotic twins. Epigenetic regulatory states were identified for each stage and cell type, and compared between monozygotic twins, between NPC cells, and across different gestational weeks (GW).

Results: Strikingly, we found epigenetic signatures between monozygotic (MZ) twins derived from analogous brain regions were highly asymmetric in midgestation. Comparison between cortex and ganglionic eminence derived NPCs identified key regulators of fetal brain development to be epigenetically modified and differentially expressed. Comparison of NPCs between GW13 – GW17 revealed an increase in active regulatory states later in development, with an increase in hypomethylated regions, active enhancer states and upregulated genes at GW17. Moreover, the peak of transcriptional activation in different NPC cell types occurred at different developmental stages.

Conclusions: Individual specific epigenetic signatures between MZ twins arise as early as midgestation, and NPCs undergo epigenetic and transcriptional activation during early fetal brain development.

Outcome/Impact: Taken together, our analysis provides a comprehensive gene regulatory network of NPCs in early fetal brain development.

Keywords: epigenomics; human fetuses; brain development; monozygotic twins.
38. CLINICAL STUDY AND ANALYSIS OF EX VIVO PHOTOACOUSTIC IMAGING IN ENDOSCOPIC MUCOSAL RESECTION TISSUES IN BARRETT’S ESOPHAGUS

Liang Lim¹, Catherine J. Streutker², Norman E. Marcon²,³, Maria Cirocco², Alexandra Lao², Vladimir V. Iakovlev², Ralph Dacosta¹,³, Brian C. Wilson¹,³

¹Princess Margaret Cancer Centre, University Health Network, Toronto, ²St. Michael’s Hospital, Toronto, ³University of Toronto, Toronto

Introduction: Accurate endoscopic detection of dysplasia in patients with Barrett’s esophagus (BE) remains a major unmet clinical need. Current diagnosis uses multiple biopsies under endoscopic image guidance, where up to 99% of the tissue remains unsampled, leading to significant risk of missing dysplasia. We have conducted an ex vivo clinical trial using photoacoustic imaging (PAI) in BE patients undergoing endoscopic mucosal resection (EMR) with known high-grade dysplasia, for the purpose of characterizing the esophageal microvascular pattern.

Methods: Thirteen (13) tissue samples from 8 patients were analyzed, spanning a range of pathological classifications, including columnar type mucosa (5), dysplasia (5) and intramucosal adenocarcinoma (3). EMR tissues were mounted immediately after resection with the luminal side up on a clear agar slab and covered with ultrasound gel. The PAI transducer (40 MHz center frequency) was placed along the short axis of the tissue. Acoustic image slices (ultrasound and photoacoustic at 680, 750, 824, 850 and 970 nm) were simultaneously acquired covering the full length of the sample, each image slice having a field of view of 14 mm (width) by 15 mm (depth). The tissues were then sliced along the acoustic image slice at approximately 2 mm spacing and fixed in formalin for histopathology with H&E staining.

Results: From the acoustic images, we created 3D reconstruction of the full ex vivo tissue volume and generated images of the relative hemoglobin concentrations (oxy-, deoxy- and total) and oxygen saturation. We co-registered the acoustic images and the corresponding histological images. The photoacoustic signal distribution within the tissue appears to coincide with the distribution of blood, the main optical absorber in EMR tissue.

Conclusions: Analysis of total hemoglobin and the intrinsic acoustic signals are currently being assessed against histologically diagnosed H&E images for each histopathological classification.

Outcome/Impact: These initial PAI + ultrasound images have demonstrated the technical feasibility of this approach and point to the potential of PAI to reveal the microvascular pattern within EMR specimens.

Keywords: Photoacoustic imaging, esophageal cancer, Barrett’s Esophagus
Introduction: There is compelling evidence that polycomb genes play an essential role in malignancy progression, such as, EZH2, the catalytic subunit of polycomb repressive complex 2 (PRC2), which has been demonstrated to be important in prostate cancer invasion and metastasis. This study was to examine if EZH2 is responsible for the inhibition of invasion and metastasis by the combination of docetaxel and Aneustat.

Methods: Human metastatic, C4-2 prostate cancer cells and NOD-SCID mice bearing metastatic, patient-derived prostate cancer xenografts, LTL-313H were treated with docetaxel and Aneustat, alone and in combination. Wound-healing assay with C4-2 cells was performed. Lung metastasis and local invasion of xenograft tissue into mouse kidney were analyzed. Xenografts were profiled using gene expression microarrays. EZH2 expression after treatment was determined with microarray data and validated. The expression changes of EZH2-regulated genes were determined with the microarray data. Molecular functions were performed on differentially expressed genes using Ingenuity Pathway Analysis (IPA) software. The correlation of gene pattern changes after treatment with comparison of prostate cancer metastatic and primary events was determined using Oncomine.

Results: The combination of docetaxel and Aneustat synergistically inhibited C4-2 cell replication at a dose-dependent manner. Moreover, the combination significantly inhibited C4-2 migration and LTL-313H xenograft invasion and metastasis. The inhibition of xenograft invasion/metastasis was supported by a set of invasion/metastasis genes identified via microarray analysis. Among these genes, EZH2 was the most significantly inhibited by the combination. Furthermore, the change of gene expression with the combination treatment indicated the combination could reduce prostate cancer metastatic events using Oncomine.

Conclusions: This study demonstrated that the combination of docetaxel and Aneustat can significantly inhibit prostate cancer invasion and metastasis via affecting a set of metastasis-associated genes, including EZH2.

Outcome/Impact: Our study indicates that the clinical outcome of prostate cancer patients could be efficiently improved by the combination.

Keywords: prostate cancer, docetaxel, Aneustat, invasion and metastasis
40. DEPENDENCE ON SPHINGOLIPID METABOLISM IN THE NORMAL AND LEUKEMIC HUMAN HEMATOPOIETIC HIERARCHY

Xie Stephanie Z., Elisa Laurenti, Sasan Zandi, Naoya Takayama, Kerstin Kaufmann, Erwin Schoof, John Dick

Princess Margaret Cancer Centre, UHN

Introduction: Metabolic alterations are a hallmark of cancer but the lipid requirements of hematopoietic stem cells (HSC) in general, and leukemic stem cells (LSC) specifically are poorly understood. Sphingosine-1-phosphate (S1P) regulates HSC egress and lymphocyte trafficking. However, the intrinsic role of sphingolipid metabolism in the hematopoietic hierarchy or whether sphingolipid metabolism in LSC differs from HSC is unknown.

Method: By analyzing a comprehensive transcriptional roadmap of human hematopoiesis, and comparing this to a LSC signature developed from 84 human acute myeloid leukemia (AML) samples, we defined a lipid stem signature including de novo synthesis sphingolipid genes, whose expression is higher in HSC than progenitors. Unbiased clustering of RNA-seq data of 46 sphingolipid genes was sufficient to segregate mature erythroid, lymphoid, and myeloid cells suggesting a role in cell fate. To determine functionality of sphingolipid biology in normal hematopoiesis and human AML, xenograft assays, in vitro culture assays, lipidomics and proteomics were performed.

Results: Our lipid stem signature is enriched in LSC gene expression profiles by GSEA analysis, while S1P genes are enriched in non-LSCs suggesting that LSC and non-LSC have a differential dependence on sphingolipid metabolism as in normal hematopoiesis. Myeloid cells have the highest transcriptional expression of S1P receptors implicating the importance of S1P signaling in myeloid fate. Moreover, S1PR3 protein expression appears to be myeloid-specific. S1PR3 is highly overexpressed in AML relative to normal blood cells, suggesting S1P biology is dysregulated in AML. To assess a functional role in AML biology, we altered sphingolipid biology with inhibitors, including myriocin, which blocks de novo sphingolipid synthesis, and FTY720, a S1P mimetic, in mice bearing primary AML xenografts. Remarkably, myriocin and FTY720 both decreased leukemia burden in mice engrafted with primary AML, but only FTY720 treatment disrupted LSC function.

Conclusions: The normal human hematopoietic hierarchy displays a differential dependence on sphingolipid metabolism that is also distinct between HSC and LSC. Moreover, S1P receptor upregulation in AML over normal blood cells opens up a therapeutic window.

Outcome/Impact: Thus, targeting of bioactive sphingolipids is a viable novel therapeutic strategy in AML to eradicate LSC while sparing HSC.

Keywords: hematopoietic stem cells, leukemic stem cells, sphingolipid metabolism, acute myeloid leukemia.
Introduction: Increased expression of the transcription factor c-MYC has been reported in the leukemic cells of patients with chronic and acute myeloid leukemia (CML and AML), with unknown significance. This study was designed to examine the effect of c-MYC overexpression in primitive normal human hematopoietic cells and its potential role in the acquisition of leukemic properties.

Methods: We used a lentiviral gene delivery strategy to overexpress c-MYC in normal cord blood (CB) CD34+ cells and then assessed the effect on the number, phenotype and properties of cells subsequently produced in vitro and in vivo (in transplanted immunodeficient mice).

Results: When test-(MYC) and control-(empty vector) transduced CD34+ CB cells were cultured for 12 days in single-cell growth factor-supplemented liquid cultures, the number of clones obtained was similar (269/384 and 306/384 respectively), but the colonies produced from the MYC-transduced cells were, on average, 7 times larger. In more prolonged co-cultures containing mouse stromal feeders engineered to express human FLT3-ligand, Steel Factor, IL-3, and G-CSF, the MYC-transduced cells sustained a markedly increased output of cells compared to control-transduced cells (for up to 12 weeks). When MYC-transduced CD34+38- CB cells were transplanted into sublethally irradiated immunodeficient (NRG-3GS) mice immediately post-transduction, a fatal CD33+CD123+CD14-CD15- human leukemia was rapidly (within 7 weeks) and consistently produced from the MYC-transduced cells. The mice showed occasional blasts in the blood, suppression of host blood cell production, and splenomegaly caused by the presence of the leukemic cells.

Conclusions: Our results suggest that supra-normal levels of MYC in normal CB CD34+ cells activate pathways that significantly, rapidly and sustainably increase cell output from this source in vitro and are sufficient to induce an AML-like disease in transplanted mice.

Impact: An improved understanding of MYC-driven leukemogenesis in human cells may lead to better treatment options for AML/CML and the many other cancers that display MYC abnormalities.

Keywords: c-Myc, cord blood, CML, AML
42. ELF3 AMPLIFICATION CIRCUMVENTS DEPENDENCY ON UPSTREAM DRIVER MUTATIONS IN LUNG ADENOCARCINOMA

Enfield Katey1, David A Rowbotham1, Christine Anderson1, Erin Marshall1, Kevin W Ng1, Brenda de Carvalho Minatel1, Raj Chari2, Megan Fuller1, Daiana D Becker-Santos1, Calum MacAulay1, Stephen Lam1, Will Lockwood1, Aly Karsan1, Wan L Lam1

1BC Cancer Research Centre, Vancouver BC; 2Harvard Medical School, Boston, MA

Introduction: The transcription factor Elf3 was recently identified as a direct target of the tumour suppressor Smad4 in a mouse model of lung tumourigenesis, and high ELF3 expression was associated with poor outcome. We sought to further investigate this relationship in human lung adenocarcinoma (LUAD) to determine whether ELF3 could represent a novel therapeutic target in SMAD4-deficient LUAD.

Methods: Multi-omic data was analyzed from the BCCRC (n=83), The Cancer Genome Atlas (TGCA) (n=420), and four murine models of LUAD tumourigenesis (EGFR_L858R, EGFR_\Delta exon19, KRAS_G12D, MYC). Stable ELF3 knock-down (shELF3) and overexpression was achieved in SMAD4 wild type LUAD cell lines and immortalized bronchial epithelial cells (HBECs) by lentiviral vector delivery. Cell proliferation, cell death, and anchorage independent growth was assessed in vitro. Survival analyses were performed using the log-rank test.

Results: ELF3 was overexpressed in 62%-72% of LUAD. While only one third of these cases harbored SMAD4 mutation or copy number (CN) loss, the majority harboured CN gain, focal amplification, and/or promoter hypomethylation at the ELF3 locus with mutation observed in 1.5% of cases. High ELF3 expression occurred irrespective of driver mutation in human and murine LUAD. In vitro, shELF3 LUAD cells harboring representative driver mutations were less viable, less proliferative, and had a reduced ability to form colonies in soft agar. Similarly, proliferation rates of HBECs overexpressing ELF3 increased. ELF3 expression was significantly associated with overall survival independent of SMAD4 expression.

Conclusions: Direct DNA-level alteration at the ELF3 locus is frequently observed in LUAD and bypasses the requirement for SMAD4-deficiency. ELF3 knock-down decreases growth and viability of SMAD4 wild type LUAD cells, indicating the high ELF3 expression observed in patient samples and murine models with diverse genetic backgrounds is biologically relevant to LUAD.

Outcome/Impact: Taken together, the high frequency of alteration, the phenotypic impact of ELF3 deregulation in vitro, and the associations with poor outcome suggest therapeutic inhibition of ELF3 could benefit a large proportion of LUAD patients and warrants further investigation.

Keywords: ELF3, SMAD4, lung cancer, genomics
43. SYNERGISTIC EFFECT OF NER AND PARP INHIBITOR COMBINATIONS IN EPITHELIAL OVARIAN CANCER

Fleury H.1, Carmona E.1, Karam H.1, Provencher D.1, Mes-Masson AM.1

1Centre de recherche du Centre hospitalier de l’Université de Montréal/ICM

Introduction: Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer largely due its late detection and high recurrence rate. In order to improve EOC survival and decrease chemoresistance, PAPR inhibitors (such as Olaparib) are now in clinical use. Although response rates are encouraging, predicting response remains a challenge. Our recent results revealed that PARPi sensitivity is associated with the combined deficiencies in homologous recombination (HR) and nucleotide excision repair (NER) or mismatch repair pathways. Biomarkers for these pathways were further validated. Based on these studies, we propose to investigate the effect of a combination treatment using an NER inhibitor (NSC130813) and Olaparib in EOC cell lines with low Olaparib sensitivity.

Methods: Olaparib and NSC130813 sensitivities were assessed by clonogenic assay and proliferation. A synergy index was calculated by the COMPUSYN software. DNA repair pathways were evaluated by Rad51 foci for HR and 6-4 photoproducts for NER. Apoptosis was measured by FACS and Western Blot.

Results: NSC130813 IC50 was calculated for three cell lines that respond poorly to Olaparib (TOV112D, OV90 and OV1369(2)) as well as for normal epithelial cells (ARPE-19). Its major effect on the NER pathways was evaluated through the present of 6-4PP although there was also a moderate effect of this inhibitor on RAD51 foci formation, suggesting it also impact HR. Our clonogenic assay and proliferation results show a synergistic effect of the combination of NSC130813 and Olaparib. An increasing number of apoptotic cells were observe in the combination treatment compared to single treatment of NSC130813 or Olaparib.

Conclusion: These promising results suggest that a combination therapy targeting simultaneously multiples DNA repair pathways might be effective to treat EOC patients that show no or limited response to Olaparib alone.

Impact: Our efforts towards understanding the mode of action of PARPi and identifying new predictive biomarkers uncovered the potential of combination treatments targeting multiple DNA repair pathway as a means to improve patients survival.

Keywords: Olaparib, NSC130813, high-grade serous epithelial ovarian cancer, DNA repair pathways, NER, MMR
44. GLOBAL TRANSCRIPTOME ANALYSIS OF CD34+ CHRONIC-PHASE CML CELLS

Hammond Colin A. 1,2, Davide Pellacani1, David J.H.F. Knapp1,2, Phillip A. Beer1, Martin Hirst3, Connie J. Eaves1,2,4.

1Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver. 2Department of Medicine, UBC. 3Centre for High-Throughput Biology, UBC. 4Department of Medical Genetics, UBC.

Introduction: The impact of the hallmark BCR-ABL1 fusion oncprotein of chronic myeloid leukemia (CML) on gene expression is believed to contribute to the malignant phenotype and competitive advantage of CML progenitor cells over their co-existing normal hematopoietic counterparts. While previous comparative investigations have mainly relied on microarrays to capture a subset of total mRNAs, we performed global gene expression profiling in primary samples of CML CD34+ and normal CD34+ hematopoietic cells using RNA-Seq.

Methods: Strand-specific RNA-Seq libraries were created from poly-adenylated RNA extracted from highly purified (>90%) CD34+ blood cells isolated from a single adult patient with chronic phase CML (all Ph+/BCR-ABL1+) and from a large pool of normal cord blood samples. Paired-end sequencing reads were aligned to a transcriptome reference consisting of a genomic sequence (GRCh37-lite July 2010) supplemented by read-length-specific exon–exon junction sequences. Differentially expressed genes were identified using the DESeq tool and custom R scripts (FDR ≤ 0.05).

Results: We found more than 200 genes to be significantly differentially expressed between the CD34+ cells isolated from normal cord blood and CML cells. Of these, only 79 genes were more highly expressed in the CD34+ CML cells. Interestingly, these included numerous genes involved in the regulation of apoptosis such as GAS2 and FAS. Conversely, several of the genes that were more highly expressed in cord blood CD34+ cells were involved in IGF-1 signaling and self-renewal control.

Conclusion: A number of the identified differentially expressed genes are consistent with previously observed age-related transcriptomic differences between primitive hematopoietic cells in fetal and adult mice and with several genes previously found overexpressed in CD34+ CML cells.

Outcome/Impact: These results set the stage for deeper analyses of the intrinsic mechanisms underpinning CML-associated changes in CD34+ stem and progenitor cells and a distinction between those associated with age versus leukemia.

Keywords: Chronic myeloid leukemia, gene expression, RNA-Seq
45. ALTERNATIVELY PHOSPHORYLATED STAT3 SUPPORTS PRO-TUMOURIGENIC METABOLIC CHANGES IN BREAST CANCER

Jones Laura M.1,2, Jill J. Ranger1,2, Simon-Pierre Gravel1,2, Angela Ahn2,3, Dongmei Zou1,2, Valérie Chénard1,2, Josie Ursisin-Seigel2,3, Julie St-Pierre1,2, and William J. Muller1,2

1McGill University, Goodman Cancer Centre1, Department of Biochemistry2, Lady Davis Institute3, Montreal, QC

Introduction: The transcription factor Stat3 is found to be constitutively activated in 35-60% of human breast cancers and its downstream targets are involved in many tumour promoting processes. In addition to its transcription factor role, recent studies have placed alternatively phosphorylated Stat3 in the mitochondria and shown the importance of this function in Ras-dependant cellular transformation, suggesting further complexity to Stat3’s role in cancer progression.

Methods: We utilized both a transgenic mouse model of activated ErbB2 mammary tumourigenesis (MMTV-NIC) with conditional Stat3 deletion and tumour-derived primary cell lines from this model. Additionally we have engineered polyoma virus middle T antigen (PyVmT) driven primary cell lines with Stat3 ablated using the CRISPR-Cas system. These tumour tissues and cell lines were subjected to various biochemical and metabolic profiling techniques.

Results: Preliminary results demonstrate that Stat3 ablation in both ErbB2 and PyVmT cancer cell lines causes a general suppression of both glycolysis and oxidative phosphorylation as mechanisms of cellular metabolism. This correlates with abnormal mitochondrial structure and a decrease in free amino acid levels. The importance of this altered metabolism and its effects on metastasis is under investigation with a particular focus on fatty acid oxidation as an alternative fuel source. We have also shown a decrease in ribosomal protein expression and a possible impairment of mTOR and the translational program.

Conclusions: This preliminary data suggests an underappreciated role for Stat3 in modulating metabolic phenotypes to enhance mammary tumour progression and metastasis. Data suggests that Stat3 ablation results in an overall depression of cellular metabolic fitness leading to a decreased ability to metastasize.

Outcome/Impact: Altered metabolism has been linked to cancer progression and site-specific metastasis. Further study of the metabolic role of Stat3 will improve our understanding of its multi-faceted role in cancer progression and metastasis and its suitability as a druggable target for metastatic breast cancer.

Keywords: breast cancer, Stat3, oncometabolism, metastasis
EXTRACTING THE KEY REGULATORS OF LEUKEMIA STEM CELL SELF-RENEWAL USING AN ADVANCED COMPETITIVE IN VIVO SCREEN

Kaufmann Kerstin¹, Stanley Ng², Peter van Galen³, Erno Wienholds¹, Stephanie Xie¹, Amanda Mitchell¹, Igor Jurisica³, Jean Wang¹, John Dick¹

¹PMCC, UHN, ²Institute of Biomaterials and Biomedical Engineering, UofT, Toronto, Canada, ³MGH, Boston, MA, USA

Introduction: Leukemia stem cells (LSC) can survive chemotherapy and initiate relapse in acute myeloid leukemia (AML). For development of novel therapies it is crucial to understand the mechanisms underlying LSC survival and self-renewal, properties shared with normal hematopoietic stem cells (HSC). To identify the key regulators of LSC/HSC self-renewal we assessed the potential of 64 genes to enhance self-renewal in a competitive in vivo screen.

Methods: Candidate genes were selected based either on high expression in functionally validated LSC vs. non-LSC fractions or derived from protein interaction analysis of LSC genes. In two gain-of-function screens we transduced human cord blood HSC and progenitor enriched fractions with 64 lentiviral vectors and assembled 16 pools, that were transplanted into NSG (for 20w) or NSG-SGM3 (for 4.5w) mice, respectively. We implemented a small-scale unbiased barcoding approach to facilitate in depth analysis of an individual vector’s contribution within a competitive cell pool by digital droplet PCR (ddPCR). A scoring algorithm considering individual gene abundance, competition and robustness across pools and both screens was developed to identify the most powerful regulators of self-renewal.

Results: The transplanted pools (mean input %BFP⁺: 13% and 28%) resulted in up to 69% BFP⁺ human CD45⁺ bone marrow cells and individual gene abundance reached up to 97% of BFP⁺ cells. We observed multiple diverse patterns of competition and robustness across cell subsets, individual mice, pools and both screens. Based on our scoring algorithm the 9 highest scoring genes were selected for individual evaluation in vivo. Of note, in the progenitor screen HOXA4, a known positive regulator of murine repopulating cells, received the highest score and expanded CD34+ cells in vivo in a first validation assay highlighting the power of our screening approach to find stemness regulators.

Conclusions: We successfully developed and applied an advanced competitive in vivo gain-of-function screen and thereby extracted potential regulators of self-renewal.

Outcome/Impact: Detailed functional studies on the candidate genes will uncover new therapeutic targets, setting the stage for eradication of LSC in AML.

Keywords: leukemia stem cell, AML, self-renewal, xenograft
47. FIRST-IN-CLASS SMALL MOLECULE POTENTIATORS OF CANCER VIROTHERAPY

Krishnan Ramya,1,2,5 Mark H. Dornan,3,5 Andrew M. Macklin,4,5 Mohammed Selman,1,2 Nader El Sayes,1,2 Hwan Hee Son,1,2 Colin Davis,1,2 Andrew Chen,1 Kerkeslin Keillor,3 Penny Le,3 Christina Moi,3 Paula Ou,1,2 Christophe Pardin,3 Carlos R. Canez,4 Fabrice Le Boeuf,1 John C. Bell,1,2 Jeffrey C. Smith,4 Christopher N. Boddy3, Jean-Simon Diallo,1,2

1Centre for Innovative Cancer Research, OHRI; 2Department of Biochemistry, Microbiology and Immunology, 3Departments of Chemistry and Biomolecular Sciences, University of Ottawa; 4Department of Chemistry and Institute of Biochemistry, Carleton University, Ottawa; 5Equal contribution

Introduction: The aim of this project is to enhance the tumour-killing ability of oncolytic vesicular stomatitis virus (VSVΔ51) with the compound Viral Sensitizer 1 (VSe1) and to understand its mechanism of action.

Methods: To study the structure-activity-relationship of VSe1, enhancement of VSVΔ51 by VSe1 analogs was assessed by a novel high-throughput assay. Plasma stability and electrophilicity were assessed by mass spectrometry and reactivity with glutathione, respectively. Selectivity of analogs for cancerous tissue was assessed by ex vivo treatment of tissues. In vivo tolerability of analogs was assessed in a dose escalation study, and in vivo efficacy was assessed in murine tumour models.

Results: In vitro assays and a rational design approach allowed identification of VSe1`s modifiable functional groups. Lead compounds increased VSVΔ51 growth up to 2000-fold in vitro and demonstrated remarkable selectivity for tumours over normal tissue ex vivo. Some analogs possess improved potency and stability with reduced electrophilicity. Analogs were better tolerated than VSe1 in vivo and enhanced tumour-specific VSVΔ51 replication.

Conclusions: We have developed a novel class of small molecules for enhancing tumour-specific VSVΔ51 replication. Structure-activity-relationship studies identified compounds with favourable pharmacological properties that enhance VSVΔ51 propagation selectively in resistant cancers, completing proof-of-concept studies for a pharmacoviral combination approach to enhancing oncolytic virus therapy.

Outcome/Impact: Pre-clinical and clinical studies have shown that oncolytic virus (OV) therapy is well tolerated in humans and can infect a broad range of cancers. Still, resistance in a subset of tumours highlights areas for improvement. Combining OV and drug therapy is a promising strategy to selectively enhance OV-mediated tumour cell death. VSe1 analogs sensitize cancer cells to OV infection and thus have high potential for use in combination with OV strategies for the treatment of cancer.

Keywords: oncolytic virus, viral sensitizer, combination therapy
Introduction: Induction chemotherapy results in complete remission in 80% of children with acute myeloid leukemia (AML). However, many patients either fail to achieve a remission, or relapse after an initial response and subsequently die of their disease.

Methods: To identify prognostic markers and therapeutic targets, we provided a sequence-based characterization of the pediatric AML transcriptome. We performed miRNA-seq on 637 primary, 22 refractory and 37 relapse samples, and mRNA-seq on 177 primary, 12 refractory and 47 relapse samples.

Results: Cox proportional hazards analyses identified miRNAs (including members of the miR-106a-363 & miR-500a/b clusters) that were associated with inferior overall survival (OS) and event free survival (EFS) (Hazards Ratio: 1.36-2.14; q-value<0.05). In addition, miR-106a-363 & miR-500a/b were abundantly expressed in relapse and refractory samples and in primary samples of refractory patients. Integrative miRNA:mRNA analyses further indicated that several candidate targets of miR-106a-5p are involved in oxidative phosphorylation, a process that is suppressed in treatment-resistant leukemic cells.

Conclusions: Through a detailed analysis of the transcriptome, we identified miRNAs whose expression levels were significantly associated with clinical outcome. In addition, we showed that abundant expression of miR-106a-5p might contribute to treatment resistance by modulating genes involved in energy metabolism.

Outcome/Impact: Overall, our transcriptome profiles provide clinically meaningful data for risk and response identification and define novel pathways that may be amenable to therapeutic targeting.

Keywords: miRNA, pediatric acute myeloid leukemia, treatment resistance
49. NODAL DISEASE REMAINS A POOR PROGNOSIS AT SURGERY OR DURING FOLLOW-UPS – A COOLS’ EXPERIENCE

Liu Kelly YP1,2 J. Scott Durham,2 Donald W. Anderson,2 Joseph Dort,3 Hadi Seikaly,4 Paul Kerr,5 Kevin Higgins,6 Jonn Yoo,7 Robert Hart8, Catherine Poh1,2

1BCCRC, 2UBC; 3University of Calgary; 4University of Alberta; 5University of Manitoba; 6Sunnybrook Health Sciences Centre; 7London Health Sciences Centre; 8Dalhousie University

Introduction: For oral cancer patients, cervical lymph node metastasis (N+) reduces survival by half. Prophylactic neck treatment (PNT) has been advocated for clinically node negative (cN0) necks. However, balancing between harm and benefit, there is a lack of objective approach to indicate PNT. In the TFRI-funded pan-Canadian COOLS trial, we prospectively recruited early-tumour stage OSCC with highly annotated information allowing us to investigate controversies of PNT.

Patients and Methods: A total of 352 patients were recruited between January 2010 and March 2015, and received curative local excision (LE) as the primary treatment. We analyzed cumulative incidence (CI) of OSCC-specific-death or RF; logistic regression on risk of developing RF; and performance analysis on tumour depth of invasion (DOI) in the event of RF.

Results: There were 313 (89%) cN0 patients at the time of surgery and 84 received PNT (51, ND; 33, ND+XRT). Among these, 65% had LE-only while 28% had concurrent PNT. Comparing to LE-only, there was significantly higher proportion of PNTs with moderately-to-poorly differentiated tumour ($p=0.01$) and greater DOI (5±5 vs. 9±7mm, $p<0.001$), with 77% of them ≥4mm. PNT group had higher incidence of N+ (53% vs. 20%, $p<0.001$) and worse DSS rate (65% vs. 54%, $p=0.001$). However, when comparing DSS to LE-only patients who developed RF and received subsequent salvage neck treatment, PNT failed to show better survival (53.8% vs. 40.4% $p=0.87$). For the 20% pN+ in the LE-only group, the median time to RF was only 7.8 months after surgery. Comparing to those who remained N0 throughout follow-up period, poorly tumour differentiation and DOI ≥4mm were associated with RF ($p=0.01$ and $p=0.02$, respectively). However, they had poor performance of discriminating N+ from N0 status. Interestingly, there is overlapping with transient difference in CI of death and the development of RF.

Conclusion: Nodal disease remains a prominent poor prognostic factor on OSCC survival.

Impact: Highly annotated, prospectively collected information are key sources to investigate questions around impact on survival outcome.
50. TARGETING PEROXIREDOXIN4 IN PANCREATIC CANCER

Mollen Erik¹,², Ravi N. Vellanki², Azin Sayed², Marianne Koritzinsky²,³

¹Maastricht University, The Netherlands, ²Princess Margaret Cancer Centre, Toronto, Canada, ³Department of Radiation Oncology and Institute of Medical Sciences, University of Toronto, Toronto, Canada.

Introduction: Pancreatic cancer is a fatal malignancy responding poorly to current therapies, only showing a 5% 5-year survival rate. There is an urgent need for novel therapeutic targets in pancreatic cancer. Whole genome screening, using a shRNA library, was utilized to identify essential genes in pancreatic cancer cell lines (Marcotte et al., Cancer Discovery 2012). By mining these data, we found peroxiredoxin4 (PRDX4) to be essential in 26% of the cell lines. PRDX4 is localized in the endoplasmic reticulum (ER) where it metabolizes H₂O₂. Here we sought to validate PRDX4 as an essential protein in pancreatic cancer, and unveil the underlying mechanism.

Methods: PRDX4 was depleted using siRNA/shRNA approach in 2 PRDX4 depletion-sensitive Pancreatic Ductal Adenocarcinoma cell lines from the screen (PANC-1 and SKPC-3) and one resistant cell line (CAPAN2). Cell lines were analyzed for changes in mRNA and protein expression. Analysis of proliferation rates was performed using the Incucyte-ZOOM (ESSEN Bioscience) automated microscopy system. Apoptosis was measured by fluorescence using an activated-caspase-3/7 recognition motif (DEVD) coupled to a DNA-intercalating dye. Senescence was assessed by β-galactose activity.

Results: We achieved 60% depletion of PRDX4 in all cell lines. Despite the transient and partial knockdown, PRDX4 depletion resulted in substantially reduced proliferation rates in PANC-1 and SKPC-3 cells. In contrast, the proliferation of CAPAN-2 cells was not affected by PRDX4 knockdown. These results hence replicated the data from the screen. Moreover, PRDX4 depletion resulted in increased levels of caspase and β-galactose activity, suggesting induction of apoptosis and senescence. Additionally, p53 expression was increased upon PRDX4 knockdown. Pathway analysis of genes whose transcripts were enriched in PRDX4 depletion sensitive cells suggested that these cell lines had increased expression of proteins involved in redox homeostasis.

Conclusions: Loss of PRDX4 results in apoptosis, senescence and growth arrest. We propose that PRDX4 is a critical factor for proliferation and survival in a subset of pancreatic cancer cells by protecting the cellular redox state. We are currently addressing this hypothesis and investigating the role of PRDX4 for growth of orthotopic pancreatic xenografts.

Keywords: Pancreatic cancer. Peroxiredoxin4. ROS. ER.
51. HIGH-DENSITY LIPOPROTEIN MIMICKING NANOPARTICLES FOR LOCALIZED PROSTATE CANCER IMAGING AND IMAGE-GUIDED THERAPY

Overchuk Marta¹ ², Juan Chen² ³, Gang Zheng² ³

¹Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada; ²Princess Margaret Cancer Centre and Techna Institute, University Health Network, ³Department of Medical Biophysics, University of Toronto

Introduction: One of the major challenges of the localized prostate cancer management is finding the balance between high treatment efficacy and preservation of the organ function. Focal phototherapies in combination with image guidance have a great potential to address a ubiquitous issue of organ overtreatment. Following advancements of light delivery technologies, new specific multimodal agents are highly required. We designed and evaluated in vivo novel high density lipoprotein-mimicking nanoparticles Bchl-HPPS(Pyro), for the targeted therapeutic agent delivery and visualization of the tumour margins.

Methods: Bchl-HPPS(Pyro) were synthesized by the previously described method (5 mol% Bchl-lipid, 0.3 μmol Pyro-oleate) [1]. Cellular uptake specificity was confirmed by incubating Bchl-HPPS(Pyro) with mSRBI (SRB1 “+”) and ldlA7 (SRB1 “−”) cells and evaluating nanoparticle binding by fluorescence microscopy. In vivo tumor accumulation of Bchl-HPPS(Pyro) was monitored by CRI Maestro in vivo imaging system in the PC3-Luc orthotopic prostate cancer mice.

Results: Bchl-HPPS(Pyro) were selectively internalized by SRB1 overexpressing cells in vitro. In vivo and ex vivo studies demonstrated that the nanoparticles preferentially accumulate in the prostate tumour, sparing the neighboring organs (healthy prostate, testes, seminal vesicles and rectal wall), which is very promising for the localized prostate cancer treatment. Current nanoparticle design allows us to benefit from the bacteriochlorophyll strong near infra-red fluorescence for image guidance, and high payload core delivery of the pyropheophorbide for high phototherapy efficacy.

Conclusions: The designed nanoparticles can be used as a highly selective agent for the delivery of NIR fluorescent photosensitizers, which can be applied for cancer imaging and image-guided focal phototherapy.

Outcome/Impact: We created a new multimodal agent, which has a high potential for cancer theranostics and can be further developed in the preclinical and clinical settings.

Keywords: lipoprotein mimetics, photodynamic therapy, image-guided PDT, prostate cancer.

52. MOLECULAR AND HISTOPATHOLOGICAL DETERMINANTS OF SUCCESSFUL ONCOLYTIC VIROTHERAPY

Son Hwan Hee1,2, Andrew Chen2, Rozanne Arulanandam2, Jean-Simon Diallo1,2

1Department of Biochemistry, University of Ottawa; 2Centre for Innovative Cancer Research, Ottawa Hospital Research Institute

Introduction: Pre-clinical and clinical data indicate that oncolytic viruses (OV) such as the attenuated Maraba-rhabdovirus MG-1 can lead to durable anti-cancer responses with minimal adverse effects; however, heterogeneous therapeutic response is evident. Therefore, it would be advantageous to gain insight regarding the factors important for successful OV therapy, such as to eventually use this information to select patients most likely to benefit from these promising therapies.

Methods: A panel of mouse tumour models was screened to characterize OV infection ex vivo and in vivo. Tumour cells were implanted in immunocompetent mice, and MG-1 was injected intratumourally or intravenously. 24 hours post infection (hpi), tumours were excised and cored. For ex vivo infection, tumours were cored before the administration of MG-1. 24 hpi (ex vivo) or following tumour tissue processing (in vivo), virus was quantified. Also, the cores were stained for immune cells and blood vessels. In vivo anti-tumour efficacy was assessed by measuring tumour size and survival following intratumoural or intravenous administration.

Results: Ex vivo infectivity of tumours did not correlate with in vivo infection irrespectively of the route of virus delivery. However, the number of CD31+ blood vessels positively correlated with in vivo infectivity; MG-1 was delivered more efficiently when tumours were better vascularized. In vivo efficacy data revealed little correlation with tumour infection and a potential role of innate immune response upon OV injection for tumour control in some models.

Conclusions: Ex vivo infectivity of MG-1 did not predict in vivo infection, but the histological assessment of tumour microenvironment identified high tumour vascularization as a potential biomarker for infection. However, the lack of correlation between tumour infection and therapeutic efficacy study highlights the complexity of therapeutic response to OV therapy.

Outcome/Impact: Clinical oncologists cannot foresee what will happen to enlisted patients based on systemic examinations performed before OV clinical trial enrollments; hence, they are currently incapable of predicting therapeutic response. If we are able to identify factors associated to therapeutic efficacy and resistance, this research could help oncologists to identify responsive patients suitable for clinical trials, which will facilitate the effective use of OV in the clinic.

Keywords: Oncolytic virotherapy, tumour vasculature
53. DISCOVERY OF NOVEL PROMETASTATIC TARGETS OF MICRORNA-206 IN HUMAN LUNG ADENOCARCINOMA

Watt Kathleen¹, Peter Truesdell¹, Elena Voorand¹, Neil Renwick², Andrew W.B. Craig¹

¹Department of Biomedical and Molecular Sciences; ²Department of Pathology and Molecular Medicine, Queen’s University, Kingston ON, Canada

Introduction: Lung cancer is the most common and deadly cancer, with a five-year survival rate of only 15%. When the disease becomes metastatic, treatment options are few and five-year survival drops to <5%. Most cases are non-small cell lung cancers (NSCLC), mainly comprised of squamous cell carcinomas, and adenocarcinomas. MicroRNA-206 (miR-206) is found to be downregulated in many cancers, including NSCLC. Low expression of miR-206 is correlated with poor prognosis and increased risk of metastasis in a variety of cancer contexts. Several targets of miR-206 including EGFR, MET, and KRAS are drivers of lung adenocarcinoma that are mutated or dysregulated in ~50% of cases. However, the role of miR-206 in lung adenocarcinoma and its effect on metastasis is still unclear.

Methods: Human lung adenocarcinoma cell lines (H1299, A549, HCC827, H1975) were engineered to express miR-206 constitutively, and in a doxycycline-inducible manner using lentiviral vectors. The effect of miR-206 on metastatic potential and tumour progression was examined both in vitro and in vivo. Additionally, cell lines and derived tumour xenografts were subjected to small RNA-sequencing and nanoString profiling to identify targets of miR-206 involved in lung adenocarcinoma progression and metastasis.

Results: Expression of miR-206 impaired cell migration and invasion in vitro. In subcutaneous tumour xenografts in mice, miR-206 expression impaired tumour growth and metastasis to the lungs. This reduction in lung metastases was also observed in tail vein lung seeding experiments. Profiling of the cell lines and tumour xenografts resulted in identification of novel genes directly or indirectly regulated by miR-206. Among these was Thrombospondin-1 (THBS1), which we have further validated as a miR-206-responsive gene by qRT-PCR and ELISA.

Conclusions: Enforced expression of miR-206 reduces metastatic potential and inhibits tumour progression by regulating a network of genes that drive these processes in lung adenocarcinoma. Among these, we have further validated THBS1 as a novel miR-206-responsive gene that may play an important role in metastasis.

Outcome/Impact: These findings encourage the use of microRNA-based therapies, and help to identify new therapeutic targets that are desperately needed for metastatic lung cancers.

Keywords: Lung adenocarcinoma, microRNA, metastasis, nanoString
54. THERAPEUTIC RELEVANCE OF RAN GTPASE IN EPITHELIAL OVARIAN CANCER

Boudhraa Z1, Tchakarska G1, Schmitt E1, Zaoui K1, Carmona E1, Mes-Masson AM1

1Centre de recherche du Centre hospitalier de l’Université de Montréal-ICM

Introduction: In our previous work, we have identified Ran GTPase as a potent biomarker of epithelial ovarian cancer (EOC), being highly expressed in malignant tumours. We have also demonstrated, using shRNA, that Ran plays a pivotal role in EOC proliferation. This suggests that, besides its prognostic value, Ran is a promising therapeutic target. It has been reported that, in contrast to normal cells, Ran depletion induces cancer cell death by abrogating cellular progression through mitosis. It has also been shown that aneuploidy affects the Ran gradient during mitosis. Since EOCs are characterized by widespread aneuploidy, we hypothesize that Ran sensitivity is driven by aneuploidy and that this can be explored therapeutically.

Methods: Ran depletion was performed by siRNA. Cell viability was assessed by clonogenic assay and FACS. Aneuploidy was induced by nocodazole treatment and verified by metaphase-spread experiments. To identify novel chemical compounds targeting Ran, the GDP-binding pocket was used for a virtual screening of the entire NCI chemical database. The top-ranking compounds were then tested in vitro.

Results: We found that healthy diploid epithelial cells and near diploid cancer cells (TOV21G) were more resistant to Ran depletion than aneuploid cells. Furthermore, by inducing aneuploidy, TOV21G cells became more sensitive to Ran depletion. Our in silico screening identified 45 compounds as potential inhibitors of Ran. Further in vitro characterization revealed that M26 and v188 compounds inhibited specifically the colony formation of aneuploid EOC cells.

Conclusion: Our results suggest that aneuploid EOC cells are more sensitive to Ran depletion than diploid cells. Furthermore, M26 and v188 compounds are potential inhibitors of Ran and are starting scaffolds for the development of new-targeted therapy of EOC.

Outcome/Impact: The identification of Ran as a potent biomarker of epithelial ovarian cancer allowed us to study its therapeutic significance and to identify for the first time promising inhibitors of this GTPase.

Keywords: Ovarian cancer, Ran GTPase, Aneuploidy, Inhibitors
55. DETECTION OF HOTSPOT MUTATIONS IN PLASMA WITH A HIGHLY MULTIPLEXED PLATFORM

Bushell Kevin¹, Sarah Arthur¹, Hagen Kennecke², Aly Karsan², Hui-Li Wong², David Shaeffer²,³, Daniel Renouf²,³, Ryan Morin¹.

¹Molecular Biology & Biochemistry, Simon Fraser University, Burnaby BC; ²BC Cancer Agency, Vancouver, BC; ³Vancouver General Hospital, BC.

Introduction: Circulating tumour DNA (ctDNA) holds promise as a non-invasive biomarker for solid cancers but current methods to detect ctDNA are limited in sensitivity or multiplexing capacity. We demonstrate the detection of ctDNA in patients with a broad range cancers malignancies using a sensitive and highly-multiplexed technology known as OnTarget™.

Methods: We received tumour tissue and pre-operative plasma samples for 18 cases of localized pancreatic ductal adenocarcinoma (PDAC). Post-operative plasma was available for nine. We received tumour tissue and plasma from 14 granulosa cell tumours (GCT), 81 colorectal (CR), and 57 lung cancer cases. We performed targeted sequencing on the CR and lung tumours and used OnTarget (Boreal Genomics) to detect mutations in GCT and PDAC tissues and all plasma samples. This platform pre-enriches samples for mutations prior to detection through next-generation sequencing. A 96-plex panel covering hotspots in FOXL2, BRAF, KRAS, EGFR, among other genes commonly mutated in solid tumours.

Results: Using OnTarget, we detected >1 mutations 16 of 18 PDAC tumours and ctDNA in the pre-operative plasma from 7 of these 16 (44%) patients. We detected post-operative ctDNA in four cases which was found to be significantly associated with earlier recurrence (p=0.022). We detected ctDNA in 4/14 (29%) GCT plasma samples using OnTarget; these results were in agreement with those obtained through digital PCR on the same samples. We detected >1 OnTarget-compatible mutations in 70 CR and 34 lung tumours and concordant mutations in the plasma of 43 (61%) and 18 (53%) of these cases, respectively. We also observed clinically-relevant discordant mutations in the plasma of five CR cases (affecting KRAS or BRAF) and five lung cases (affecting EGFR or KRAS).

Conclusions: OnTarget can detect ctDNA across numerous cancer types and enable the detection of clinically-relevant discordant mutations in the plasma.

Outcome/Impact: OnTarget combines sensitivity with high multiplexing capacity and may have utility across a range of cancer types.

Keywords: ctDNA, Biomarkers

Supported by the TFRI (grant #1021) and the BC Cancer Foundation.
56. MODELING OF PEDIATRIC ACUTE MEGAKARYOBlastic LEUKEMIA (AMKL) USING CORD BLOOD STEM/PROGENITOR CELLS

Cardin Sophie1, Louise Laramée1, Tara MacRae2, Jalila Chagraoui2, Guy Sauvageau2,3, Keith Humphries4, Josée Hébert2,3, Brian Wilhelm2, Sonia Cellot1

1Université de Montréal, CHU Sainte-Justine; 2Université de Montréal, IRIC; 3 Hôpital Maisonneuve-Rosemont, Montréal; 4BC Cancer Agency, Vancouver

Introduction: AMKL is a subset of pediatric acute myeloid leukemia associated with poor outcome (cure rates <50%) and molecular heterogeneity. A fusion between the genes encoding for the nucleoporin NUP98 and the histone demethylase KDM5A (NUP98-KDM5A fusion) is a recurrent mutation in 10-15% of AMKL. Modelling of AMKL in the physiologically relevant context of human cells is warranted to identify leukemia specific targets and biomarkers. We developed in vitro and in vivo models of AMKL through overexpression of NUP98-KDM5A in cord blood hematopoietic stem/progenitor cells (CB-HSPC).

Methods: Using lentiviral transduction and optimized culture conditions, the NUP98-KDM5A fusion was overexpressed in CD34+ cells isolated from cord blood units. The GFP reporter gene of the lentiviral construct enabled tracking of the transduced CB-HSPC (GFP+ cells). Fractions of independently seeded cell cultures were transplanted into immunodeficient mice or expanded in vitro. Cell cultures were analyzed at regular intervals for GFP content, cytology, immunophenotype and clonogenic activity. Expression profiling of CB-HSPC, NUP98-KDM5A overexpressing cells and pediatric AMKL samples was performed using RNAseq.

Results: In vitro, NUP98-KDM5A-transduced CB-HSPC displayed marked enrichment for GFP+ cells (~5% to >90%) in 2 independent cultures after 90 days. The emergence of this GFP+ cell population was associated with maturation arrest, increased progenitor frequency (30% vs 1%) and immunophenotype (CD34+, CD71+, CD96+) suggestive of leukemic blasts, vs control cells (100% mast cells). Expression profiling of the 2 cell lines unravelled de-repression of the 5'HOXB gene cluster, as well as other transcription factors and epigenetic regulators similar to NUP98-KDM5A AMKL patient samples. In vivo, NUP98-KDM5A-transduced HSPC (day8 of culture) gave rise to a myeloproliferative disorder that mirrors pediatric AMKL phenotype. Epigenetic/genomic studies and functional confirmatory assays are ongoing.

Conclusions: These results demonstrate that overexpression of NUP98-KDM5A in CB-HSCP can lead to leukemia development in vivo and to establishment of cell lines in vitro.

Outcome/Impact: These models will be exploited to identify leukemia specific targets, biomarkers (vs CB-HSPC and patient AMKL samples), biochemical studies and chemical/genetic screens.

Keywords: NUP98-KDM5A, acute megakaryoblastic leukemia, cord blood
57. PPARγ LOSS INCREASES METASTASIS OF HER2+ BREAST TUMOURS

Lightbody Elizabeth D.¹, O’Connell Kathleen M.², Rubino Rachel E.², Apostoli Anthony J.², SenGupta Sandip K.¹, and Nicol Christopher JB.¹-³

¹Departments of Pathology and Molecular Medicine; ²Cancer Biology and Genetics, Cancer Research Institute; and ³Biomedical and Molecular Sciences, Queen’s University, Kingston, ON.

Introduction: Human epidermal growth factor receptor 2 (HER2+) breast tumours have a poor patient prognosis compared to HER2- tumours. Peroxisome proliferator-activated receptor γ (PPARγ) is a transcription factor that regulates the expression of genes involved in sugar and fat metabolism. PPARγ has shown anti-cancer effects, however, the role of PPARγ during HER2+ breast tumourigenesis is unclear.

Methods: We interbred a spontaneous HER2+ breast tumour mouse model, MMTV-Neu-IRES-Cre (NIC) with our PPARγ-floxed mice (PPARγ-MG KO) to create a unique model (PPARγ-NIC KO). The in vivo model has PPARγ deletion in the same HER2+ transformed mammary epithelial cells that drive breast tumourigenesis. Western Blot analysis and immunofluorescence evaluated PPARγ and HER2 expression and localization in tumours. A mouse tumourigenic cell line from a PPARγ-NIC KO lung metastatic tumour (PPARγ-NIC KO metastatic) was used to define in vitro signaling interactions.

Results: PPARγ-NIC KO have high mammary tumour incidences and multiplicity, and enhanced lung metastases compared to the parental NIC strain. PPARγ protein levels decrease as the mammary gland progresses from normal to tumourigenic tissue, and is also inversely matched to increased HER2 phosphorylation at tyrosine 877. PPARγ-NIC KO primary and metastatic tumours had significantly higher IF HER2 H-Scores compared to PPARγ-WT and chemical-induced mammary tumours. PPARγ-NIC KO metastatic cells showed migration, invasion and tumourspheres were significantly enhanced after epidermal growth factor (EGF, 20ng/ml) treatment; co-treatment with a PPARγ activating drug (rosiglitazone, 10µM) decreased EGF-mediated cell migration, invasion, and tumoursphere formation potential.

Conclusions: This is the first evidence that loss of PPARγ enhances the metastatic spread of HER2+ mammary tumours, and suggests PPARγ ligands may benefit HER2+ breast cancer patients.

Outcome/Impact: This project will confirm PPARγ and HER2 signaling interactions that impact the metastatic potential of HER2+ breast tumours. It also unveils novel PPARγ upstream/downstream targets to be used as predictive biomarkers for HER2+ patients susceptible to metastasis.

Keywords: breast cancer, HER2, PPARγ, rosiglitazone
58. RNA SYNTHESIS OF HOMOLOGOUS RECOMBINATION REPAIR PATHWAY UNDER HYPOXIA

Lo Winnie W.¹, Gaetano Zafarana¹, Robert G. Bristow¹,²

¹Radiation Medicine Program, PMCC, UHN; ²Departments of Radiation Oncology and Medical Biophysics, University of Toronto

Introduction: The expression of homologous recombination (HR) repair pathway genes have been shown to be downregulated in hypoxia; however, the change in RNA synthesis of HR pathway has never been investigated. HR occurs in S and G2 phases of the cell cycle when sister chromatids are readily available. We use metabolic labeling of newly-synthesized RNA to investigate the change in RNA synthesis of HR pathway in cells under hypoxia.

Methods: 4-thiouridine (4sU), a uridine analogue, was added to the media of DU145 cells that have been cultured in 21% and 0.2% O₂ for 72 hours. Cells were sorted to G1, S and G2 phases based on DNA content. Total RNA was extracted and 4sU-tagged RNA was biotinylated and purified from pre-existing RNA using streptavidin coated magnetic beads. Real-time RT-PCR was used to compare the expression of key HR regulators, RAD51, BRCA1 and BRCA2 in total and newly-synthesized RNA.

Results: We found that the expression of RAD51, BRCA1, and BRCA2 is the highest in S phase, and the expression is decreased in each cell cycle phase of hypoxic cells. The overall percentage of total 4sU uptake is lower in hypoxic cells. Higher incorporation of 4sU was found in S and G2 phases of normoxic cells, which was also decreased in hypoxic cells. Highest expression of HR genes was found in the newly-synthesized RNA isolated from the S phase of normoxic cells.

Conclusions: Increased RNA synthesis was found in S and G2 phases, suggesting that cells may require more active RNA synthesis for DNA replication and mitosis. High expression of HR genes found in S phase corresponds with the function of the HR pathway, and can be explain by increased RNA synthesis of RAD51, BRCA1, and BRCA2 in S phase. Decreased RNA synthesis of HR genes in each cell cycle phase may also explain the decreased HR expression in hypoxic cells.

Outcome/Impact: Genetic instability can arise from decreased HR repair, and hypoxia can maintain or drive genetic instability by decreasing HR repair. This study provides understanding into the mechanisms behind how hypoxia alters the expression of HR pathway.

Keywords: prostate cancer, RNA synthesis, homologous recombination, hypoxia
59. ANALYSIS OF HUMAN HEMATOPOIETIC CELLS GENERATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS IN DIFFERENTIATING TERATOMAS

MacAldaz Margarita¹, Paul Miller¹, Melanie Kardel², Connie Eaves¹

¹Terry Fox Laboratory, BC Cancer Agency and University of British Columbia, Vancouver BC
²STEMCELL Technologies, Inc., Vancouver, BC

Introduction: Induced pluripotent stem cells (iPSCs) offer new opportunities for creating new and genetically corrected human tissues as well as for modeling and testing new treatment approaches for human cancers, including leukemia. Being that methods to generate transplantable normal or leukemic human hematopoietic stem cells in vitro have not yet been developed, in this project, we are exploring the possibility of obtaining such cells in human iPSCs-derived teratomas produced in vivo in immunodeficient mice.

Method: NOD-Rag1-null IL2Rgc-null W41/W41 producing human IL3, GM-CSF and SCF (NRG-W41±3GS) mice were injected with human iPSCs (10³-10⁶ cells/injection) ± fibroblasts engineered to produce human FLT3-L, SCF, IL3 and IL6. The teratomas obtained a few weeks later were enzymatically dissociated into single viable cell suspensions that were then analyzed for the presence of human CD34+ and/or CD45+ cells. In vitro assays for various progenitor cell types were also performed.

Results: Teratomas containing human CD45+ cells were generated in every experiment and in highest yields (up to 7x10⁶ CD45+ cells from a teratoma containing 4x10⁸ cells) in mice containing a source of human IL3, G-CSF, SCF. CD34+ cells were detected in all teratomas with >0.4% CD45+ human cells. Colonies of myeloid, erythroid and mixtures of these formed in standard growth factor-supplemented methylcellulose cultures, and hematopoietic cells able to produce granulopoietic progenitors for 6 weeks in vitro were also detected.

Conclusion: These experiments lay the foundation for future studies of the in vivo activities of the human hematopoietic cells produced in this system.

Impact: The information obtained could revolutionize the clinical use of human blood stem cell transplants and enable new investigations of how their properties are controlled and transformed.

Keywords: hematopoiesis, induced pluripotent stem cells, teratoma,
60. GENOME-WIDE CRISPR/CAS9 SCREENING REVEALS MODULATORS OF TEMOZOLOMIDE RESPONSE IN GLIOBLASTOMA

MacLeod Graham¹, Traver Hart², Jason Moffat²,³, Peter B. Dirks³,⁵, Stephane Angers¹,⁴

¹Department of Pharmaceutical Sciences and Leslie Dan Faculty of Pharmacy; ²Donnelly Centre; ³Department of Molecular Genetics; ⁴Department of Biochemistry, University of Toronto, Toronto, ON. ⁵Program in Developmental and Stem Cell Biology, Division of Neurosurgery, Hospital for Sick Children, Toronto, ON

Introduction: Glioblastoma (GBM) is a prevalent and highly lethal form of primary brain tumours. Presently, median survival time for GBM patients is only 15 months and thus improved treatment methods are in great need. Current treatment for GBM includes surgery, radiotherapy and the chemotherapeutic agent temozolomide (TMZ). However, TMZ offers only a modest improvement in survival time. With the aim of identifying new therapeutic targets for GBM we performed genome-wide CRISPR/Cas9 screening to identify genes that modulate response to TMZ in GBM.

Methods: A library of single-guide RNAs (gRNAs) targeting over 89,000 exons in 17,232 human genes was used to screen a patient-derived human GBM stem cell (GSC) line. GSC treated with DMSO were grown in parallel with GSC treated with either a lethal, or sub-lethal dose of TMZ. Next-generation sequencing was used to identify gRNAs that were increased/decreased in abundance in TMZ treated pools of cells.

Results: Positive selection screening revealed that loss of key components of the mismatch repair pathway—MLH1, MSH2, MSH6 and PMS2 confers resistance to lethal doses of TMZ. Negative selection screening using a sub-lethal dose of TMZ identified 116 genes whose loss sensitizes GSC to TMZ treatment. Functional annotation revealed enrichment for genes involved in multiple DNA repair pathways, most prominently Fanconi Anemia and double-strand break repair via homologous recombination. Further studies validated that deletion of either member of the MCM8/9 helicase complex sensitizes GBM cells to TMZ making this complex a promising therapeutic target. In addition, we characterized another TMZ sensitizing gene from our screen, the previously uncharacterized ZC3H7A which was revealed to be a cytoplasmic, stress-granule associated protein.

Conclusions: Genome-wide CRISPR/Cas9 screening identified genes that modulate response to the chemotherapeutic agent TMZ in GBM including MCM8, MCM9 and ZC3H7A.

Outcome/Impact: Genes whose loss provides increased sensitivity to TMZ represent promising therapeutic targets for increasing efficacy of chemotherapy and decreasing lethality in GBM

Keywords: Glioblastoma, CRISPR/Cas9, DNA Repair, Genomics
61. ANALYSIS OF PIWI-INTERACTING RNA TRANSCRIPTOMES IDENTIFY CANCER TYPE-SPECIFIC EXPRESSION PATTERNS AND SIGNATURES PREDICTING LUNG TUMOUR BEHAVIOR

Martinez Victor ¹, Brenda Minatel¹,², Erin Marshall¹, Kevin Ng¹, Katey Enfield¹, Natalie Firmino¹, Kevin Bennewith¹, Stephen Lam¹ and Wan Lam¹

¹British Columbia Cancer Research Centre

Background: PIWI-interacting RNAs (piRNAs) are small (24–32bp) RNAs with a key role in epigenetic regulation of gene expression and maintenance of genomic stability in germ cells. Recent evidence suggests they are also expressed and functionally active in somatic tissues and cancer. Aberrant expression of individual piRNAs have been associated with clinical features in some cancer types; however, their involvement in lung cancer remains to be deciphered. Towards this end, we analyzed piRNA transcriptomes from human tumours and adjacent non-malignant tissue. We investigated associations with clinical parameters in lung cancer for the purpose of identifying novel prognostics targets.

Methods: We developed a custom small-RNA analysis pipeline to deduce expression from ~32K human piRNAs. We generated 6,378 piRNA transcriptomes (non-malignant/tumour tissue) from 11 organs. In lungs, we analyzed 1,082 tumours and 209 non-malignant samples from two different cohorts (TCGA/BCCA). We evaluated clinical parameters of aggressiveness (nodal/distant metastasis) and outcome (overall/disease-free survival). Multiple-piRNA survival signatures were identified using Cox Proportional Hazard model.

Results: Our results reveal piRNA features related to tumour biology, particularly in lung. We found that: 1) A small set of known piRNAs are somatically-expressed, in an organ-specific manner, 2) piRNA expression patterns in tumours are markedly different from non-malignant tissues in a cancer-type specific mode and are associated with clinical features, 3) in lung, adenocarcinoma (AdC) and squamous cell carcinoma (SqCC) subtypes exhibit unique piRNA expression patterns, and 4) a specific aggressiveness-related piRNA expression signature was identified in SqCC.

Conclusions: We provide evidence of somatic, tissue-specific human piRNA expression. Aberrant expression patterns contribute to lung cancer subtype-specific biology. We discovered piRNA-based signatures that identify aggressive SqCCs and have prognostic value for this subtype.

Impact: Our findings suggest that piRNAs can be further explored to better understand the unique biology of lung tumours, and also as candidates for prognostic clinical markers.

Keywords: piRNAs, lung cancer, survival
**62. NOVEL THERAPEUTIC APPROACH TO TARGET MULTIPLE MYELOMA: INHIBITION OF 3' IGH ENHANCER USING SMALL MOLECULES**

**Murugesan Alli**¹,², René Boudreau², Bithika Ray¹, Anthony Reiman¹,²

¹University of New Brunswick, Saint John, NB; ²Dalhousie Medicine New Brunswick

**Introduction:** Multiple myeloma (MM) is an incurable, deadly bone marrow plasma cell cancer. Myeloma remains difficult to treat due to large number of aberrant biological processes that can lead to and sustain the disease. The most common one is the translocation of immunoglobulin heavy chain (IgH) locus. These translocations result in a genomic state where oncogenes located on juxtaposed chromosomes become overexpressed under the control of strong IgH enhancer. Treatment strategies individually targeting oncogenes are currently established or in development. Since no single oncogene is involved in more than 20% of MM cases, and multiple oncogenes can also be involved, we believe this strategy to be of limited scope. Hence, we investigated inhibition of Oct2, the key transcription factor regulating IgH enhancer activity that has been previously established by us a poor prognostic factor linked to reduced survival in MM patients, as a novel treatment for myeloma.

**Methods:** Human myeloma cell lines (HMCLs) KMS11, JJN3 and KMM1 harbouring IgH translocations t(4;14), t(6;14) and t(14;16), respectively were exposed to mono/dual therapy for 24hr. Since there are no direct Oct2 inhibitors, inhibitors to upstream regulators of Oct2 namely Butein an NF-kB inhibitor, PKC412 an all conventional-PKC isoform inhibitor, PKC isoform specific inhibitors Rottlerin and Enzastaurine were used. Cell viability was determined by PrestoBlue assays; Combination indexes (CI) determined by Chou-Talalay method; Oct2 and oncogene protein expression checked by immunoblotting method.

**Results:** All four inhibitors decreased cell viability in HMCLs. Combination studies revealed synergistic, additive, in certain conditions antagonist effects for dual therapy. CI equal to or greater than moderately synergistic was present in all three HMCLs at a minimum of one Butein concentration combined with Rottlerin/PKC412. Furthermore, protein expression analyses in KMS11 cells confirmed downregulation of both Oct2 and oncogenes upon inhibition.

**Conclusion:** Treatment of HMCLs with NF-kB and PKC inhibitors individually resulted in decreased cell viability, Oct2 and oncogene expression. Combination therapy led to a further decrease. This study demonstrates IgH enhancer inhibition through indirect Oct2 downregulation using NF-kB and PKC inhibitors.

**Outcome:** Our findings suggest Oct2 as a novel therapeutic target for myeloma.

**Keywords:** Myeloma, translocations, IgH enhancer, Oct2
63. THE FUNCTIONAL LINK BETWEEN MITOCHONDRIAL ONE-CARBON METABOLISM AND CELLULAR BIOENERGETICS IN BREAST CANCER

Papadopoli David¹, Julie St-Pierre¹

¹Department of Biochemistry, Goodman Cancer Research Centre, McGill University

Introduction: Cancer cells display an altered metabolism in order to fuel tumour growth. Indeed, many tumour types display an increased dependence on glycolysis contrary to untransformed cells that are more dependent on oxidative phosphorylation for ATP production. Mining of numerous publically available datasets revealed that the most significantly modulated metabolic gene across a number of cancer subtypes is MTHFD2, a one-carbon metabolism gene that is central for the production of purines in fast dividing cells. The dependence of cancer cells on one-carbon metabolism is exploited in oncology through the usage of anti-folate drugs like methotrexate. The fact that both embryonic and transformed cells, which are largely glycolytic, express high levels of MTHFD2, while oxidative untransformed cells have low expression of this gene suggests that the expression and activity of MTHFD2 is linked to the metabolic reprogramming of cancer cells, from an oxidative to a glycolytic phenotype.

Methods: Quantitative RT-PCR was performed to determine the expression of one-carbon metabolism genes in MCF7 and NT2196 breast cancer cell lines and their respective non-transformed controls, MCF10A and NMuMG, respectively. Respirometry and NOVA BioProfile Flex Analyser were used to assess the metabolic profile of cells. Bioinformatics analyses were conducted using the cBioPortal for Cancer Genomics.

Results: Both MCF7 and NT2196 breast cancer cell lines displayed increased glycolysis along with elevated expression of the one-carbon metabolism genes MTHFD1L, MTHFD2, and MTHFD2L. Both cancer cell lines also displayed reduced respiration relative to their non-transformed controls. These data indicate that the expression of one-carbon metabolism genes correlates positively with glycolytic activity and negatively with mitochondrial respiration. To directly assess whether there is a functional relationship between one-carbon metabolism and mitochondrial respiration, we treated NT2196 cells with methotrexate, which increased respiration in both NT2196 cells and controls. Interestingly, the growth of the glycolytic NT2196 cancer cells was less sensitive to methotrexate than controls, likely due to their elevated one-carbon metabolism.

Conclusions/Outcome: These results demonstrate that one-carbon metabolism correlates positively with the glycolytic phenotype, but inversely with oxidative phenotype. Furthermore, glycolytic cancer cells may be less sensitive to methotrexate, linking the response to methotrexate to the bioenergetics profile of cancer cells.

Keywords: One-carbon metabolism, MTHFD2, glycolysis, mitochondria, methotrexate
64. PHOSPHATASE INHIBITOR SYNERGIZES WITH ONCOLYTIC VIROTHERAPY

Selman Mohammed¹,², Chris Rousso¹, Andrew Chen¹, Jean-Simon Diallo¹,²

¹ Centre for Innovative Cancer Research, Ottawa Hospital Research Institute; ² Department of Biochemistry, University of Ottawa, Ontario, Canada

Introduction: Oncolytic viruses (OV) are an emerging class of anticancer bio-therapeutics, based on their selective replication and lysis of tumour cells, without causing damage to normal cells. Numerous studies have shown that viral oncolysis, spread and overall efficacy is improved using pharmacological compounds that manipulate the cellular innate anti-viral immune response. A number of viruses have evolved various strategies to antagonize the antiviral activity. For instance, some viral proteins target phosphatases to evade innate immune recognition. Hence, we tested a chemical panel of phosphatase inhibitors (PI) to identify novel compounds that could enhance OV activity.

Methods: A phosphatase inhibitor panel was screened for their ability to enhance of OV VSVΔ51 in 786-0 cells, a human renal carcinoma cell line which is highly resistant to infection with VSVΔ51. Promising compounds were tested for cancer specific enhancement of VSVΔ51 in ex vivo in tumours and normal organ samples. The mechanism of action was examined by microarray analysis. In vivo anti-tumour efficacy of the compounds alone and in combination with VSVΔ51 was tested in resistant mouse tumour models was also tested.

Results: We identified PI compounds that could enhance OV infection in vitro and ex vivo, in resistant tumor cell lines. Furthermore, one phosphatase inhibitor tested increased anti-tumour efficacy in combination with OV in several syngenic tumour models, leading to durable responses in models otherwise refractory to OV and drug alone. Microarray analyses suggest this potentiation may occur through enhanced immune-stimulation in addition to improved oncolysis.

Conclusions: We show that PI compounds can maximize anticancer immunity, and the ability to enhance the growth and spread of oncolytic rhabdoviruses, in addition to OV mediated viral lysis in cancer cells leading to increase efficacy of OV treatment in in vivo models.

Outcome/Impact: Overall, the present project will lead to a better understanding of important factors of viral infection and development of improved oncolytic therapy strategies.

Keywords: Phosphatase inhibitor, oncolytic virus, vsvd51, virotherapy
65. KMT2D LOSS OF FUNCTION IS ASSOCIATED WITH INCREASED MUTATIONAL LOAD AND DOWNREGULATION OF GENES INVOLVED IN DNA DAMAGE RESPONSE PATHWAYS

Topham James1*, Alessia Gagliardi1, Ryan D. Huff1, Diane L. Trinh1, Andrew J. Mungall1, Jacqueline Schein1 and Marco A. Marra1.

1Canada’s Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver BC

Introduction: Lysine (K)-Specific Methyltransferase 2D (KMT2D) is one of the most frequent targets of all types of somatic mutation in human cancer. However, despite its relatively high rate of mutation, the molecular consequences of KMT2D mutations in tumor cells are not fully understood.

Method: We investigated the impact of KMT2D mutations on the genomic and transcriptomic landscapes of tumor samples through analysis of whole exome sequencing data (8,366 samples, 32 cancer types) and RNA-sequencing data (3,633 samples, 11 cancer types) from The Cancer Genome Atlas project. Furthermore, we leveraged isogenic KMT2D-knockout HEK293A cell lines to compliment transcriptome results from TCGA tumour samples.

Results: We revealed robust association between KMT2D mutation status and overall mutational load (defined as the total number of mutations in a sample) in five cancer types. Furthermore, we identified consistent downregulation of genes involved in DNA damage response pathways in KMT2D-mutant primary tumour samples and KMT2D-knockout cell lines.

Conclusions: The significant association between KMT2D mutation status and mutational load may indicate a novel mechanism by which KMT2D loss-of-function could impact oncogenesis. Furthermore, downregulation of genes involved in DNA damage response pathways in both KMT2D-knockout HEK293 cell lines and KMT2D-mutant primary tumour samples may indicate a means by which KMT2D loss-of-function could increase mutational load in diverse tissue contexts.

Outcome/Impact: This study represents the first large-scale bioinformatics approach to understanding genomic and transcriptomic effects of KMT2D mutations in cancer. Furthermore, we propose a novel mechanism by which KMT2D mutations facilitate oncogenesis.

Keywords: KMT2D, bioinformatics, epigenetics, DNA damage response
Introduction: Locally advanced breast cancer is a treatment challenge. Typically, patients undergo neoadjuvant chemotherapy (NAC) to down-stage the disease prior to surgery. However, approximately 50% of patients will demonstrate recurrence within 5 years; necessitating improved imaging techniques to evaluate tumour response during treatment. There is evidence to suggest that diffuse optical spectroscopy (DOS) parameters can provide surrogate markers to pathological response at early stages of treatment. Additionally, textural features of those parameters have indicated statistically significant differences between responders and non-responders. In this study, we investigate baseline DOS textural features as predictors to NAC response.

Methods: This study was approved by the institution’s ethics committee and written informed consent was obtained. Patients (n=37) were imaged with a commercially developed diffuse optical tomography imaging device (ART technologies, Montreal, Canada) prior to the start of NAC. Texture-based features were analyzed using a grey-level co-occurrence matrix (GLCM) of the volumetric DOS images, within a region of interest (ROI) around the tumour. Pathological assessment of patient mastectomies determined clinical and pathological response to treatment after the completion of NAC. Discriminant analysis was conducted to classify patients into response groups using a linear discriminant function of the texture-based parameters.

Results: Responders and non-responders demonstrated significant differences (p<0.01) in texture-based DOS parameters (contrast, correlation, energy and homogeneity) at baseline. Baseline deoxy-haemoglobin [contrast] demonstrated a sensitivity and specificity of 85.2% and 80.0%, respectively (p<0.01). Similarly, other parameters such as the total optical index [homogeneity] demonstrated a sensitivity and specificity of 100% and 70%, respectively.

Conclusions: Study results suggest that DOS texture-based features can classify and predict clinical and pathological response to NAC in locally advanced breast cancer patients, using baseline measurements.

Outcome: Texture-based features of DOS images can be used as a clinical tool to help guide therapy for locally advanced breast cancer patients treated with neoadjuvant chemotherapy.

Keywords: Diffuse optical spectroscopy, breast cancer, texture analysis, chemotherapy
**Introduction:** A better understanding of high grade serous ovarian cancer (HGS-OvCa) cell fates decisions in response to chemotherapy is one of the key to improve treatment efficacy. Among many potential cell fate decisions including death or mitotic catastrophe, our study demonstrate that chemotherapy mostly trigger therapy-induced cellular senescence (TIS) in HGS-OvCa primary cells.

**Methods:** Key senescence hallmarks including altered morphology, senescence associated -galactosidase, DNA damage markers, cell cycle arrest, cyclin dependent kinase inhibitors (CDKi) expression, and the secretion of pro-inflammatory and pro-angiogenic factors were evaluated and detected in chemotherapy (carboplatin/paclitaxel/X-Ray) treated primary HGS-OvCa cells. We also performed stable gene expression depletion using short hairpin RNA (shRNA) to determine whether p16\(^{INK4a}\) is sufficient to induce senescence in HGS-OvCa. Using tissue microarrays (TMAs) built from HGS-OvCa tissue samples, we test whether p16\(^{INK4a}\) expression in tumours impact clinical outcome.

**Results:** We find 90% of HGS-OvCa primary cultures undergo culture-stress mediated growth arrest: they stop proliferating, become enlarged and flattened, and acquire most senescence hallmarks including p16\(^{INK4a}\) expression, a CDKi that prevent cell cycle progression. p16\(^{INK4a}\) depletion could delay, but not bypass senescence, suggesting that other CDKi may redundantly backup the senescence growth arrest. Indeed, chemotherapy-treated HGS-OvCa cells up-regulated another CDKi, p27\(^{Kip1}\), which become a *de facto* target for senescence regulation in HGS-OvCa. Importantly, in patient-derived TMAs, high p16\(^{INK4a}\) expression correlated with a delayed progression of the disease and a better survival.

**Conclusion:** Our data reveal that primary HGS-OvCa cells retain different mechanism to undergo TIS, including p16\(^{INK4a}\) and p27\(^{Kip1}\), and that this senescence response could be beneficial for the patient.

**Outcome/Impact:** Our results reveal an important role of TIS in HGS-OvCa treatment and suggest that senescence of tumour cells may be beneficial for patient’s survival. We now reckon that pharmaceutical manipulation of TIS could improve the outcome of HGS-OvCa treatment.

**Keywords:** ovarian cancer, p16\(^{INK4a}\), p27\(^{Kip1}\), therapy-induced senescence.
TARGETED INACTIVATION OF Rb AND p53 VIA WAP-CRE INDUCES PINEOBLASTOMA

Chung Philip E.D.1,2, Deena Gendoo3, Ronak Ghanbari1,2, Adrian Dubuc4, Marc Remke1,4, David Shih1,4, Jennifer Tsui1, Zhe Jiang2, Livia Garzia4, Sidney Croul1,5, Benjamin Haibe-Kains3, Michael D. Taylor1,3, Eldad Zacksenhaus1,2

1Dept. of laboratory medicine and pathobiology, University of Toronto, Toronto; 2Division of advanced diagnostic, TGH, Toronto; 3 Princess Margaret Bioinformatics and Computational Genomics Laboratory, PMH, Toronto; 4 Arthur and Sonia Labatt Brain Tumour Research Center, The Hospital for Sick Children, Toronto; 5 Dept. of Neuropathology, TGH

Introduction: Pineoblastoma (PB) is the most aggressive WHO IV tumour of the pineal gland. There is a need to develop and study PB mouse model to understand its biology and identify therapeutic targets.

Method: We used the cre-loxP system to inactivate Rb and p53 via a Whey Acidic Protein (WAP)-Cre transgene. H&E and IHC were performed to study histology/marker expression. To define cell/tissue of origin, Rb/p53-deleted transgenic mice were crossed with mT/mG reporter mice. Microarray analysis was done on Rb/p53-deleted PBs to study gene expression. Drug screening was done in silico to identify therapeutic targets.

Results: Rb/p53-deleted mice developed brain tumours with a median latency of 133 days (100% penetrance; 149 out of 149). The tumours originated in the pineal gland. H&E and IHCs staining showed features commonly seen in pineoblastoma (PB). These PB disseminated to the spinal cord and/or to the brain (22%; 7 out of 32). Interestingly, the Rb/p53-deleted PBs closely resembled Group 3 medulloblastoma (MB) and expressed similar photoreceptor genes. Of several therapeutic targets tested, gemcitabine had the greatest inhibitory effect in vitro (IC50=30nM). Using our mouse model, we demonstrated strong inhibition of PB tumour progression using gemcitabine monotherapy. In silico screen suggested tricyclic antidepressant drugs as potential therapeutic drugs, and we confirmed strong cytotoxic effect on PB cells with the antidepressant nortriptyline in vitro (IC50=15µM). In vivo analysis is underway.

Conclusions: We have generated a novel mouse model that spontaneously forms PB. These PBs readily metastasize, share similarity with group 3 MB and are highly sensitive to gemcitabine in vivo and nortriptyline in vitro.

Outcome/Impact: The Rb/p53-deleted mouse model may allow us to identify new therapy for PB.

Keywords: Pineoblastoma, Rb, p53
69. VALIDATION OF THERAPEUTIC TARGETS IN OVARIAN CANCER

Communal Laudine¹, Mauricio Medrano²,³,⁴, Fabrice Sircoulomb³,⁴, Isabelle Clément¹, Diane Provencher¹,⁵, Robert Rottapel²,³,⁴, Anne-Marie Mes-Masson¹,⁵,⁶

¹Institut du cancer de Montréal / Centre de recherche du Centre hospitalier de l’Université de Montréal, Montreal, QC, Canada; ²Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada; ³Ontario Institute for Cancer Research, Toronto, ON, Canada; ⁴Princess Margaret Cancer Center and University Health Network, Toronto, ON, Canada; ⁵Departement of Obstetrics and Gynaecology, Université de Montréal, Montreal, QC, Canada; ⁶Department of Medicine, Université de Montréal, Montreal, QC, Canada.

Introduction: The Selective Therapy Program (STP), launched by the TFRI/Ontario Institute for Cancer Research (OICR), has identified new promising therapeutic targets using an integrative genomic, proteomic and functional approach (1). Here we characterized and validated the relevance of selected candidates as therapeutic targets and biomarkers in High Grade Serous Epithelial Ovarian Cancer (HGS-EOC).

Methods: A systematic candidate characterization included expression evaluation by Western blot in EOC cell lines. Candidate expression was then evaluated by immunofluorescence (IF) on a tissue-microarray (TMA) consisting of 101 cases of HGS-EOC. Multi-labeling IF conditions were defined to allow the discrimination of epithelial and stromal cells as well as nuclei and cytoplasmic compartments. Candidate expression was accurately quantified with a powerful image analysis procedure (Visiomorph™) and were correlated with patient clinical parameters (SPSS) in order to determine their relevance and to prioritize them for further studies.

Results: Promising results were obtained for two candidates. High expression of CD151 protein was correlated with poor patient prognosis in our HGS-EOC TMA and in the pan-canadian TFRI COEUR cohort comprised of 833 HGS tumours. CD151 depletion impaired survival, proliferation and tumour growth in a subset of HGSOC cell lines in vitro and in vivo. APBB3 protein levels were also associated with poor prognosis and further analyses are ongoing to confirm its potential as biomarker and/or therapeutic target.

Conclusions: Some of the candidates seem promising as prognostic markers and therapeutic targets.

Outcome/Impact: Candidates associated with patient clinical data will be prioritized to be developed as new therapeutics or biomarkers to improve ovarian cancer management.

Keywords: Ovarian cancer, therapeutic target, biomarker, multi-mapping immunofluorescence

70. YB-1 REGULATES METABOLIC ADAPTATION AND CANCER PROGRESSION THROUGH SELECTIVE mRNA TRANSLATION

EL-Naggar Amal M\textsuperscript{1}, and Poul H Sorensen\textsuperscript{1*}

\textsuperscript{1}Dept of Molecular Oncology, BC Cancer Research Center, Vancouver, Canada
\textsuperscript{*}Corresponding author

Introduction: Cells respond to stress by blocking global protein synthesis to preserve energy, while maintaining translation of stress adaptive mRNAs such as \textit{HIF1A}. However, the basis for such selective mRNA translation remains largely unknown. One conserved mechanism for inhibiting translation under stress is to sequester mRNAs in ribonucleoprotein (RNP) complexes known as stress granules (SGs). SGs are composed of RNA binding proteins, stalled translation initiation complexes and silenced mRNAs, which are temporarily stored in SGs until the stress is abated. Emerging evidence implicates SGs in cancer biology, whereby SGs confer survival under stress and chemotherapeutic resistance to tumour cells. Recently, we found that the RNA binding protein, YB-1, facilitates sarcoma metastasis through two mechanisms. First, it directly binds to the \textit{HIF1A} 5'-UTR to enhance \textit{HIF1α} mRNA translation under hypoxia and drive metastatic capacity \textit{in vivo}. Second, under diverse stress forms, YB-1 mediates formation of SGs through 5'-UTR binding and translational activation of the G3BP1 SG nucleator. Unexpectedly, we found that YB-mediated SG formation is also critical for \textit{in vivo} metastasis of childhood sarcoma cells.

Methods: To study the potential role of \textit{HIF1α} in SG formation, we used U2OS, and MNNG (osteosarcoma), CHLA10 (Ewing sarcoma), PC3 (prostate carcinoma) and RCC4 and RCC4-VHL (renal cell carcinoma) tumour cell lines, and performed transient and stable YB-1 knockdown (kd), \textit{HIF1α} kd, and G3BP1 kd in each cell line. Then, cells were exposed to hypoxia and arsenite stress and subjected to different assays.

Results: We found that \textit{HIF1α} is essential for YB-1 mediated SG formation in tumour cells under stress, with \textit{HIF1α} lying upstream of G3BP1 in this process. Moreover, inactivation of the YB-1-\textit{HIF1α}-G3BP1-SG signaling axis inhibits AMPK energy signaling and reduces mitochondrial functions, thus blocking adaptation to metabolic stress.

Conclusions: The YB-1-\textit{HIF1α}-G3BP1-SG signaling axis represents a key link between cancer progression and metabolic adaptation.

Outcome/Impact: Targeting The YB-1-\textit{HIF1α}-G3BP1-SG signaling axis may represent a novel therapy in the treatment of cancer.
71. CCR5 ANTAGONISTS AS IMMUNE-MODULATING AGENTS FOR THE TREATMENT OF BREAST CANCER METASTASIS

Halvorsen E.C.¹,², Hamilton M.J.¹, Lee H.N.¹, LePard N.E.¹, Young A.¹,³, Wadsworth B.J.¹,³, Firmino N.¹,³, Lam W.L.¹,²,³, and Bennewith K.L¹,²,³*.

¹Integrative Oncology Department, British Columbia Cancer Agency, Vancouver, BC, Canada
²Interdisciplinary Oncology Program, University of British Columbia, Vancouver, BC, Canada
³Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Introduction: Regulatory T cells (Tregs) are an immunosuppressive population associated with the growth and metastasis of mammary tumours¹. Under homeostatic conditions, Tregs mediate peripheral tolerance and prevent autoimmunity; however, elevated levels of Tregs are associated with poor prognosis in several tumour types¹. A strategy to decrease tumour growth may be to target Tregs to disrupt Treg-mediated suppression of cytotoxic T cell activity, although therapies that systemically ablate Tregs may cause autoimmunity¹. Research to inhibit the homing of Tregs to primary tumours and peripheral tissues is warranted to minimize the off-target effects of systemic Treg depletion.

Results: We have identified elevated levels of Tregs expressing CCR5 in the lungs of mice bearing metastatic mammary tumours relative to naive control mice, and observed that CCR5⁺ Tregs co-express CTLA-4 and CD103, indicating an immunosuppressive phenotype. Production of the CCR5 ligand, CCL8, was dramatically increased in the lungs of mice bearing metastatic tumours, and we identified macrophages as the primary source of CCL8 in the lungs. We found that Tregs migrate towards CCL8 ex vivo, which can be inhibited by the FDA-approved CCR5 antagonist Maraviroc (MVC). Furthermore, treatment of tumour-bearing mice with MVC reduced pulmonary Treg accumulation and decreased lung metastases.

Conclusions: Our data support further testing of CCR5 antagonists to prevent the development of pro-metastatic pulmonary microenvironments that contain CCR5⁺ Tregs and to decrease metastatic tumour growth in the lungs.

Impact: The inhibition of CCR5 represents a viable therapeutic strategy to prevent Treg homing to sites of tumour growth without systemically depleting them, thereby avoiding the off-target effects of global Treg depletion such as autoimmunity. We anticipate that this work will advance the generation of targeted, immune-based therapeutics for the treatment of metastatic breast cancer.

Keywords: Regulatory T cells, CCR5, pulmonary metastases, breast cancer, Maraviroc.

1) Halvorsen et al., Cancer Met Rev. 2014; 33:1025-41
72. RECURRENT TMEM30A LOSS-OF-FUNCTION MUTATION IMPROVES PROGNOSIS IN DIFFUSE LARGE B CELL LYMPHOMA

Healy Shannon¹, Daisuke Ennishi¹, Randy Gascoyne¹, Christian Steidl¹

¹Dept. Lymphoid Cancer Research, BC Cancer Agency, UBC

Introduction: TMEM30A is involved in maintaining phosphatidylserine (PS), a phospholipid targeted by macrophages as an “eat me” signal, at the cytoplasmic side of the plasma membrane. A novel loss-of-function mutation in TMEM30A, unique to a subset of R-CHOP treated diffuse large B cell lymphoma (DLBCL) patients, results in improved prognosis.

Methods: We set out to recapitulate TMEM30A loss-of-function by generating CRISPR-Cas9 mediated biallelic frameshift mutations (TMEM30A-KO) in a DLBCL-derived cell line DOHH2, and, as a published control, the T cell lymphoblast cell line Jurkat. Changes in cell surface PS in untreated and R-CHOP treated cells were measured by annexin V binding via flow cytometry. Phagocytosis was monitored by co-culturing DOHH2 or Jurkat cells with a macrophage-derived cell line THP-1, measuring engulfment by flow cytometry using a pH-dependent cell surface dye.

Results: As previously reported in Jurkat cells, TMEM30A loss-of-function in DOHH2 resulted in increased extracellular PS, albeit to a much lower extent. Macrophages engulfed higher numbers of Jurkat TMEM30A-KO cells compared with DOHH2 TMEM30A-KO cells, suggesting a threshold of extracellular PS required for macrophage interaction. Administration of R-CHOP genotoxins further increased PS exposure in DOHH2 TMEM30A-KO cells relative to wildtype, suggesting an increased sensitivity towards these drugs.

Conclusions: From this study, TMEM30A loss-of-function in DLBCL increases PS externalization following treatment with R-CHOP relative to wildtype cells. This observation likely explains the improved prognosis in DLBCL patients with this mutation, as R-CHOP treatment likely induces increased phagocytosis and tumour clearance in these patients. Interestingly, untreated DOHH2 cells show minimal externalized PS compared with Jurkat cells, and show reduced interaction with macrophages in vitro. While the mechanism of this discrepancy has yet to be uncovered, this result may explain the unique presentation of this mutation in DLBCL, and not in other cancers to date.

Outcome/Impact: The favorable prognosis of DLBCL patients with reduced TMEM30A activity described in this study may outline wide-ranging potential for therapeutic intervention and companion diagnostic approaches.

Keywords: B cell lymphoma, microenvironment, phagocytosis, macrophages
CREATING AND CHARACTERIZING A TRANSPOSON-MUTEGENIZED LIBRARY OF VACCINIA VIRUS CLONES FOR THE TREATMENT OF HUMAN CANCER

Keller Brian A.1,2, Adrian Pelin1,2, Jiahu Wang2, Fabrice LeBoeuf2, Carolyn Nessim3, Carolina Ilkow2, Harry L. Atkins1,2, John C. Bell1,2

1Faculty of Medicine, University of Ottawa, Ottawa, Canada; 2Centre for Innovative Cancer Research, OHRI; 3Division of General Surgery, OHRI and University of Ottawa

Introduction: Oncolytic viruses (OVs) are an emerging class of multi-mechanistic biological cancer therapeutics designed to: (1) directly lyse cancer cells, (2) destroy tumour vasculature, and (3) induce anti-tumour immune responses. This latter effect has been demonstrated to have therapeutic efficacy on existing sites of disease, prevent further metastases, and provide long-lasting immune-mediated surveillance. Oncolytic poxviruses have demonstrated efficacy in a variety of clinical settings, however a systematic analysis of virus gene mutations that favour both oncolytic activity and immune stimulation remains to be completed.

Methods: We have utilized a next-generation sequencing (NGS)-based approach to identify the fittest clinical candidate strain of Vaccinia virus (VacV) based upon growth and spread in a variety of tumour cell systems. We then developed a transposon-based approach to comprehensively mutagenize the VacV genome.

Results: A unique 89-virus library of transposon-mutagenized VacV clones has been bio-selected in normal and tumour patient explants and in a variety of cell culture systems followed by NGS to identify insertion sites that favour selective OV growth. We are now identifying candidate viruses that enhance the anti-tumour immune characteristics of our lead candidate using a variety of human in vitro and ex vivo immune screens.

Conclusions: We have systematically generated a large library of viral clones that has allowed us to probe the VacV genome more comprehensively than previously possible. Using this tool, we expect to gain fundamental insights into the eradication and prevention of metastatic disease and recurrent cancers, which will result in the development of a more targeted and anti-tumour immunostimulatory OV clinical candidate.

Outcome/Impact: We aim to develop a novel VacV-based clinical candidate oncolytic to synergize with existing approved immunotherapies for the treatment of human cancers.

Keywords: Oncolytic virus, vaccinia, transposon, immunotherapy.
74. TRANSCRIPTIONAL REGULATION OF MIR-31 BY ONCOGENIC KRAS MEDIATES METASTATIC PHENOTYPES BY REPRESSING RASA1

Kent Oliver A.¹, Joshua T. Mendell⁴,⁵,⁶, and Robert Rottapel¹,²,³

¹Princess Margaret Cancer Centre, University Health Network, Toronto, ²Department of Immunology, ³Division of Rheumatology, St. Michael’s Hospital, Toronto, ⁴Department of Molecular Biology, Harold C. Simmons Comprehensive Cancer Center, ⁵Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas, USA

Introduction: Activating KRAS mutations are nearly ubiquitous in pancreatic cancer occurring in more than 95% of clinical cases. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by binding sequences within the 3'UTRs of target mRNAs. An integral role for miRNAs in cancer pathogenesis is well established; however, the role of miRNAs in KRAS-mediated tumorigenesis is poorly characterized.

Methods: Cell culture, northern blot, quantitative PCR, luciferase promoter assay, chromatin immunoprecipitation, scratch wound assay, and invasion assay.

Results: Here it is demonstrated that expression of miR-31 is coupled to expression of oncogenic KRAS and activity of the MAPK pathway. MiR-31 is highly expressed in patient derived xenografts and a panel of pancreatic and colorectal cancer cells harboring activating KRAS mutations. The miR-31 host gene is a large non-coding RNA that correlates with miR-31 expression and enabled identification of the putative miR-31 promoter. Using luciferase reporters, a minimal RAS responsive miR-31 promoter was found to drive robust luciferase activity dependent on expression of mutant KRAS and the transcription factor ELK1. Furthermore, ELK1 interacts directly with the endogenous miR-31 promoter in a MAPK-dependent manner. Expression of enforced miR-31 significantly enhanced invasion and migration of multiple pancreatic cancer cells resulting from activation of RHOA through regulation of the miR-31 target gene RASA1. Importantly, acute knockdown of RASA1 phenocopied enforced miR-31 expression on the migratory behavior of pancreatic cancer cells through increased RHOA activation.

Conclusions: Oncogenic KRAS can activate RHO through the miR-31 mediated regulation of RASA1 indicating miR-31 acts as a KRAS effector to modulate invasion and migration in pancreatic cancer.

Outcome/Impact: The data suggest that miR-31 may be a biomarker for highly invasive KRAS-mutant cancers.

Keywords: Pancreatic, KRAS, miR-31, invasion-migration.

75. HYPOXIA INDUCES CONTEXTUAL ‘LOSS-OF- HETEROZYGOSITY’ AND PROMOTES PARPi SENSITIVITY

Mahamud O1, Lo W2, Chua ML3, Zafarana G3, Bristow RG1,2,3

Departments of 1Medical Biophysics; 2Radiation Oncology, University of Toronto; 3Radiation Medicine Program, Princess Margaret Cancer Centre, University Health Network

Introduction: Intratumoural hypoxia leads to decreased expression in DNA damage response (DDR) and repair pathways. Given the ‘two-hit’ model for loss of gene function, we hypothesize that hypoxia-mediated loss of gene expression and function, coupled with an already inactive allele (shallow deletion or inactivation) may ultimately give rise to an exploitable contextual ‘loss-of- heterozygosity’ phenotype.

Method: To interrogate this relationship, isogenic DLD-1 cells heterozygous and homozygous null for BRCA2 were placed under 21% or 0.2% O2 gas for 72 hours. Hypoxia-mediated changes in DDR response and DNA repair were evaluated by cell proliferation, western blots, qPCR, immunofluorescence and clonogenic assays.

Results: No differences in proliferation were observed between oxic and hypoxic cells over the course of 72 hours. Under hypoxic conditions, confirmed by mRNA and protein expression of hypoxia-inducible genes VEGF and HIF1α, decreased expression of homologous-recombination genes (HR) (BRCA1, BRCA2, RAD51) was observed at the mRNA and protein level. Profound sensitivity to PARP inhibition (PARPi), under oxic and hypoxic conditions was seen in the BRCA2−/− cells. Conversely, BRCA2+/− cells exhibited modest PARPi sensitivity under oxic conditions but were 2-fold more sensitive to PARPi under hypoxic conditions.

Conclusions: Herein we illustrate through a novel mechanism of contextual ‘loss-of-heterozygosity’, which marries the tumour microenvironment and innate genetic alterations, increased PARPi sensitivity in DNA repair deficient cells.

Outcome/Impact: Intratumoral hypoxia is associated with many solid cancers. Furthermore many solid tumours exhibit innate genetic defects in DNA repair genes (ex. BRCA2+/− in 11% of sporadic prostate cancers). By identifying these individual patients we may ultimately broaden the clinical utility of PARP inhibitors.

Keywords: BRCA2, Homologous-recombination, Hypoxia, Microenvironment
76. MANTLE CELL LYMPHOMA SEQUENCING IMPLICATES NOVEL GENES IN MALIGNANCY

Pararajalingam Prasath¹, Sarah Arthur¹, Arezoo Mohajeri¹, Bruno Grande¹, Miguel Alcaide¹, Barbara Meissner², Pan Qiang Hammarstrom³, Randy D. Gascoyne², Joseph Connors², Ryan Morin¹,².

¹Simon Fraser University, ²BC Cancer Agency, ³Karolinska Institute

Introduction: Mantle Cell Lymphoma (MCL) is an aggressive Non-Hodgkin's Lymphoma that is incurable with standard cancer therapies and identification of driver mutations is complicated by genomic complexity and tumour and inter-patient heterogeneity. This study aims to survey the mutational landscape of MCL by leveraging public and private MCL sequencing data in order to identify driver mutations and mutations relevant to treatment and relapse.

Method: Tumour-normal pairs (n=98) and trio (n=37) MCL samples from two published cohorts (n=59) and an two unpublished cohorts (n=76) were mapped to the latest reference genome, GRCh38. Somatic variants (SNVs), copy number variants (CNVs), structural variants (SVs) were identified using Strelka, TITAN, Sequenza and Manta. Driver mutations were identified using OncoDriveFM and OncoDriveFML and recurrent CNVs were called using GISTIC.

Results: Pooled analysis of these cohorts has increased our statistical power to uncover recurrently mutated genes that appear to act as drivers of this malignancy. In addition to previously described driver mutations in ATM, CCND1, TP53 and WHSC1, S1PR1 (a gene not associated with any NHL to date) exhibited recurrent deletions indicative of a tumour suppressor role in MCL. Integrative analysis of genes affected by point mutations and significant targets of CNVs is further informing on the potential role of individual mutated genes in this cancer. Analysis of trios shows evidence for branched clonal evolution and may further inform on the mechanism of relapse in these patients.

Conclusion: Mutational analysis of MCL tumours from four cohorts indicate that mutations in S1PR1 may play a role in MCL and further analysis is likely uncover additional genes contributing to oncogenesis and/or treatment resistance of MCL.

Outcome/Impact: Findings of this study will provide a comprehensive view of the genetic factors involved in MCL oncogenesis and relapse.

Keywords: Mantle cell lymphoma, meta-analysis, S1PR1, tumour suppressor

Study is supported by TFRI New Investigator grant (grant #1043)
77. TRANSLATIONAL CONTROL OF THE TUMOUR MICROENVIRONMENT

Robichaud Nathaniel¹, Rapita Sood¹, Qianyu Guo², Sonia del Rincon², Wilson H. Miller², Nahum Sonenberg¹

¹Department of Biochemistry and Goodman Cancer Research Centre, McGill University; ²Lady Davis Institute for Medical Research, Segal Cancer Centre, Jewish General Hospital, Montreal, Quebec, Canada.

Introduction: Translational control has emerged as a critical determinant in tumourigenesis. The mRNA cap-binding protein eIF4E is an oncoprotein that plays a key role in cancer initiation and progression. We recently demonstrated that its phosphorylation on serine 209 by the Map Kinase Integrating Kinases (MNK1/2) promotes epithelial to mesenchymal transition, invasion and metastasis. Here, we describe the unsuspected critical importance of eIF4E phosphorylation in the tumour microenvironment (TME) for mammary tumour development and metastasis.

Methods: To investigate the role of eIF4E phosphorylation in the TME, we utilized a syngeneic model in which a murine mammary tumour cell line (66c14) was orthotopically injected into mice bearing the non-phosphorylatable eIF4E S209A mutation and their WT counterparts. Tumour growth was monitored by caliper measurements, and tissues were collected for histological assessment and immunohistochemistry.

Results: The onset of tumour formation was significantly delayed in eIF4E S209A mice. Established tumours showed no differences in growth, proliferation, apoptosis or vascularization, but tumours in WT mice were characterized by cancer-associated fibroblast (CAF) infiltration. This may be due to underlying metabolic profiles, which were distinct in WT and eIF4E S209A fibroblasts. Importantly, co-injection of these CAFs with 66c14 cells abrogated the difference in tumour onset between WT and eIF4E S209A mice. In addition, the effect of phosho-eIF4E in the TME on the metastatic process was investigated. Strikingly, eIF4E S209A mice were nearly completely resistant to the formation of lung metastases from primary mammary tumours, as well as to lung colonization following tail vein injection of 66c14 cells. Furthermore, treatment of tumour-bearing WT mice with an inhibitor of MNK1/2 significantly reduced lung metastasis without affecting primary tumour growth.

Conclusions: Phosphorylation of eIF4E in the TME promotes tumour development and metastasis, potentially by promoting CAF activation.

Outcome/Impact: These findings suggest that MNK inhibitors be used to prevent metastatic progression in breast cancer patients.

Keywords: Translation, tumour microenvironment, metastasis, metabolism
IDENTIFYING GENES THAT COOPERATE WITH MUTANT P53 & ACTIVATED STAT3 IN BREAST CANCER

Schachter Nathan F.1,2, Jessica R. Adams2, Katelyn Kozma2, Patryk Skowron2, Sorana Morrissy2, Livia Garzia2, Adam J. Dupuy4, Michael D. Taylor2,3, Sean E. Egan1,2

1Molecular Genetics, University of Toronto2 Developmental & Stem Cell Biology, Sickkids Hospital, Toronto 3Arthur and Sonia Labatt Brain Tumour Research Center, SickKids Hospital 4Anatomy & Cell Biology, University of Iowa, USA.

Introduction: Breast cancer (BC) is one of the leading causes of death in women worldwide. Tumours with poor prognosis often display two characteristics; mutation of TP53 (which codes for the p53 transcription factor), and constitutive activation of the Stat3 transcription factor. Currently, genetic alterations that cooperate with mutant-p53 or elevated Stat3 signaling in BC remain largely unknown.

Methods: To identify genes that cooperate with mutant-p53 or activated-Stat3, we performed a Sleeping Beauty (SB) screen in mice. SB screens involve mobilizing transposons engineered to activate, repress, or truncate genes depending on site of insertion and orientation. By generating SB mice carrying Trp53LSL-R270H, Rosa26LSL-Stat3C, or neither allele, we have been able to distinguish genes preferentially altered in the presence of mutant-p53 or activated-Stat3. Thus far, >350 tumours have been collected for analysis. Using ligation-mediated PCR and next-generation sequencing, we have identified genes repeatedly targeted for mutagenesis by transposons.

Results: SB mice with Trp53LSL-R270H or R26LSL-Stat3C alleles displayed significant acceleration of tumour onset and penetrance relative to controls. Sequencing analysis identified 39 and 113 genes recurrently altered in p53-mutant and Stat3-activated SB mammary tumours, respectively. Of note, there was significant dissimilarity between mutations found in the presence of mutant Trp53, activated Stat3, and control SB tumours, suggesting primary oncogenic mutations select for specific, secondary cooperating alterations. Interestingly, Met was identified as the most frequently mutated gene in both p53-mutant (p=8.5x10^-45) and Stat3-activated tumours (p=0). Indeed, IHC confirmed wide-spread Met over-expression in these SB tumours. Additionally, genes encoding proteins known to interact with or control p53 or Stat3 activity (such as Phf2 and Il6st, respectively) were also altered in mutant backgrounds.

Conclusions: SB mutagenesis has identified Met pathway activation and other mutation-specific alterations that cooperate with mutant-p53 and activated-Stat3 in BC pathogenesis.

Outcome/Impact: Validating the cooperative oncogenic networks uncovered by our screen is expected to yield novel targets for treatment of aggressive BCs.

Keywords: Breast Cancer, Sleeping Beauty Mutagenesis, p53, Stat3 Signaling
79. IDENTIFICATION OF PACLITAXEL RESPONSE MEDIATORS IN BREAST CANCER USING AN IN VIVO GENOME-WIDE KNOCKDOWN SCREEN

Sultan M¹, TT Huynh ¹,², E Lamoureux³, ML Thomas ¹, KM Coyle¹, CA Giacomantonio¹,⁴, MG Langille³, P Marcato¹,²

Departments of Pathology¹, Microbiology and Immunology², Pharmacology³, and Surgery⁴, Dalhousie University, Halifax, NS, Canada

Introduction: Treatment for breast cancer often involves the use of paclitaxel as a first line chemotherapy to target tumour cells; however, some patients do not respond to treatment and would be better treated with an alternative drug. Thus, being able to identify the genes which when expressed in a tumour predict paclitaxel sensitivity or resistance prior to administration would improve treatment efficacy and patient survival.

Methods: A genome-wide RNAi screen was performed with MDA-MB-231 tumour xenografts in female NOD/SCID mice. This allowed the identification of enriched and depleted shRNA sequences that theoretically target paclitaxel sensitivity and resistance genes, respectively. The effect of successful individual gene knockdown on paclitaxel sensitivity was assessed in vitro using different cell viability assays. Additionally, using genes identified in the screen a robust gene signature was a created and tested in large patient data sets via machine learning methods.

Results The screen identified 26 and 16 putative paclitaxel sensitivity genes and resistance genes, respectively for breast cancer. In vitro confirmation assays of several potential resistance genes revealed a significant decrease in MDA-MB-231 cell viability under paclitaxel treatment when they were individually knocked down. Additionally a gene signature, derived from the screen-identified genes, achieved 82-85% accuracy in predicting breast cancer patient outcome to paclitaxel-containing treatment regimens across multiple patient tumour gene expression data sets.

Conclusions: A genome-wide shRNA knockdown screen has led to the identification of novel mediators of paclitaxel sensitivity and resistance in breast cancer, and the generation of gene signature for the prediction of patient response.

Outcome/Impact: Additional confirmation experiments will help enhance the accuracy of the genetic profile which can be used to identify candidate breast cancer patients who would most benefit from paclitaxel treatment as opposed to treatment with other drugs. Furthermore, some of the identified genes are potentially novel drug targets that can be used to sensitize resistant patients to taxane treatment.

Keywords: Breast cancer, paclitaxel, gene signature, genome-wide RNAi screen
80. VALIDATION OF THE PROGNOSTIC VALUE OF KI-67 AND P27 IN PROSTATE CANCER: THE CANADIAN PROSTATE CANCER BIOMARKER NETWORK (CPCBN) EXPERIENCE

Bergeron Alain¹, Hélène Hovington¹, Hervé Brisson¹, Molière Nguilé Makao¹, Véronique Ouellet², Vincent Fradet¹, Martin Gleave³, Armen Aprikian⁴, Neil Fleshner⁵, Fred Saad², Louis Lacombe¹.

¹CRCHU de Québec-Université Laval, ²CRCHUM, ³VPC, ⁴RC-MUHC, ⁵UHN

Introduction: The CPCBN is a network of researchers that aims to validate prognostic biomarkers that could have an impact on the management of prostate cancer (PCa). We report here on the first step of the validation of two biomarkers associated with cell proliferation, namely the Ki-67 nuclear antigen and the cyclin-dependant kinase inhibitor p27.

Methods: To validate biomarkers, a cohort composed of 1508 PCa patients treated by radical prostatectomy (RP) was assembled. Adjacent benign and tumoural tissues from RP specimens were sampled on tissue microarrays (TMAs). In a first step, a TMA displaying tissues from 250 of the 1508 patients was tested. Immunohistochemical stainings were performed on a Link 48 autostainer (DAKO). Four-microns sections of the TMA were submitted to heat-induced antigen retrieval in a PT-Link and then stained with antibodies against Ki-67 (clone MIB-1) or p27 (clone SK53G8) using the EnVision-Flex45 protocol (DAKO). Percentage of stained cells was determined by a trained technician and correlation with tumours characteristics and biochemical recurrence were assessed.

Results: Both gain of Ki-67 and loss of p27 nuclear antigen expression were observed in some benign glands but much more frequently in tumour glands. Ki-67 protein and loss of p27 protein expression were detected in 72% and 4% of the tumours, respectively. Multivariate Cox regression analysis of the square root of the percentage of Ki-67-stained tumour cells (cut-off=1.36 [1,85%]) and of the inverse function of the percentage of p27-stained tumour cells (cut-off=0,0289 [35%]) were associated with biochemical recurrence (Ki-67; HR: 1.9, 95% CI 1.03-3.4, p=0.038 and p27; HR: 1.8, 95% CI 1.0-3.4, p=0.034).

Conclusions: Both Ki-67 and p27 were shown to be independent predictors of biochemical recurrence. Extending these analyses to the whole TMA (1508 patients) will allow correlation with other clinical issues, such as PCa-specific death and provide more powerful analyses of their prognostic value.

Outcome/Impact: Validated prognostic biomarkers used alone or in combination to identify tumours at higher risk of progression may have a great impact in PCa management.

Keywords: Prostate cancer, Ki-67, p27, Immunohistochemistry.
PLERIXAFOR INHIBITS MYELOID CELL RECRUITMENT AND IMPROVES THE RADIOCURABILITY OF CERVICAL CANCER

Chaudary Naz 1 PhD, Melania Pintilie3 MSc, Richard P. Hill1,4,5 PhD and Michael Milosevic2,4 MD

1Ontario Cancer Institute and Campbell Family Institute for Cancer Research, 2Radiation Medicine Program and 3Department of Biostatistics, Princess Margaret Cancer Centre and University Health Network, Toronto, Canada; Departments of 4Radiation Oncology and 5Medical Biophysics, University of Toronto, Toronto, Canada

Purpose: There is an important need to improve the effectiveness of radio-chemotherapy (RTCT) for cervical cancer. These tumours recruit myeloid cells from the bone marrow via the CXCL12/CXCR4 pathway, which in turn influence vascular function and radiotherapy response. The objective of this study was to explore combined treatment with Plerixafor (a CXCL12/CXCR4 inhibitor) and standard RTCT on primary tumour control and the development of metastases, using orthotopic primary xenografts derived directly from patients with cervical cancer.

Methods: Two primary cervix (OCICx13 and OCICx20) were grown in the cervices of immune deficient mice. These tumour models have been shown to mirror the clinical and biological behavior of cervical cancer in patients. To simulate clinical treatment, image-guided radiotherapy (30 Gy in 15 daily fractions) and concurrent weekly cisplatin (4 mg/kg) were administered, with or without Plerixafor (5 mg/kg/day). The primary endpoints were tumour growth delay, the frequency of lymph node metastases and animal survival. Chemokine expression and neutrophil recruitment were evaluated by immunohistochemistry. Acute gut toxicity was assessed using the crypt cell assay. Blood and normal organs were examined for late toxicity.

Results: The combination of RTCT and Plerixafor produced substantial tumour growth delay, reduced metastases and improved survival compared to standard RTCT alone in patient-derived xenograft models. There was a reduction in chemokine signaling (CXCL12/CXCR4) and myeloid cell infiltration (GCSF, CD11b) with combination treatment compared to RTCT alone. There was no effect of Plerixafor on acute GI toxicity, nor were there changes in blood counts or organ morphology to indicate increased late hematological or normal tissue toxicity.

Conclusion/Impact: This preclinical study demonstrates that the addition of Plerixafor to standard RTCT for cervical cancer improves local tumour control and reduced metastases with no increase in toxicity. Plerixafor is commercially available for other indications, which will facilitate translation of these findings to phase I/II clinical studies.

Keywords: orthotopic cervix xenografts, metastasis, chemokines, radiochemotherapy, Plerixafor
82. FER-ACTIVATED ANDROGEN RECEPTOR (pY223AR): A PREDICTIVE BIOMARKER OF PROSTATE CANCER PROGRESSION

Turki Altaylouni, Fatima Zouanat, Eleonora Scarlata, Lucie Hamel, Fadi Brimo, Armen Aprikian, Simone Chevalier

Urologic-Oncology Research Team, Cancer Research Program, Research Institute of McGill University Health Centre, Montreal, Qc.

Introduction: Prostate cancer (PCa) figures among leading causes of cancer deaths in North America. To date, androgen-deprivation therapy (ADT) is the gold standard treatment for patients experiencing a recurrence after radical prostatectomy or radiation therapy, but ADT invariably fails and is followed by castration resistance (CRPC) and further progression of the disease. The host lab reported that the androgen receptor (AR) and the signal transducer and activator of transcription (STAT)3 are substrates of the Fer tyrosine kinase, become its partners once activated (AR/Y223; STAT3/Y705) and accumulate into the PCa cell nucleus (Zoubeidi et al 2009; Rocha et al 2013). Pathological findings revealed that Fer, AR and pSTAT3 (Y705) are at highest levels in the tumortumour cell nucleus when patients received ADT or progress beyond CRPC (unpublished). The aim was to assess the fate of activated pY223AR in PCa tissues using specific homemade polyclonal rabbit antibodies.

Method: Immunohistochemistry (IHC) was performed on sections from human prostates and metastases (tissue microarrays/TMA) covering the whole spectrum of prostatic diseases. Levels and intracellular distribution of pY223AR were quantified in percentages (%) and H scores.

Results: Epithelial cells of both healthy and pure benign (BPH) cases expressing AR but not Fer were 100% negative for pY223AR. Negative cells increase with progression, up to 16% in ADT/CRPC. The intensity of positive cells and H score increased with Gleason in primary and with progression, most elevated nuclear levels being in ADT/CRPC patients (H score=211) and in metastases: Lymph Nodes ≥ Seminal Vesicle ≥ Bone > Kidney > Bladder (p<0.01). Kaplan-Meier (survival) analysis validated that nuclear pY223AR levels correlate with biochemical recurrence (log rank, p<0.05 at H score>160).

Conclusion: The Fer-activated form of AR follows Fer expression patterns in the prostate and in PCa, being not detectable in epithelial cells of both healthy and pure benign cases and in AR positive cells increasing in nuclear intensity with disease progression. High H score of pY223AR is associated with biochemical recurrence.

Outcome: Fer-activated AR represents a novel prostate cancer biomarker with prognostic value in predicting survival probability.

Supported by McGill Uro-Oncology Research Funds.
83. MULTI-OMICS-BASED APPROACH TO IDENTIFY BIOMARKERS OF THERAPEUTIC RESISTANCE IN COLORECTAL CANCER PATIENTS THROUGH ANALYSES OF SEQUENTIAL METASTATIC TISSUE AND LIQUID BIOPSIES; Q-CROC-01: NCT00984048

Couëtoux du Tertre Mathilde¹, Ryan Morin², Suzan McNamara¹, Maud Marques¹⁵, Benoit Samson³, Bernard Lespérance⁴, Thierry Alcindor⁵, Yoo-Joung Ko⁶, Richard Dalfen⁷, Eve St-Hilaire⁸, Lucas Sideris⁹, Felix Couture¹⁰, Sabine Tejpar¹¹, Ronald Burkes¹², Mohammed Harb¹³, Francine Aubin¹⁴, Errol Camlioglu¹⁵, Simon Turcotte¹⁴, Adriana Aguilar¹⁵, Adrian Gologan¹⁵, Petr Kavan¹⁵, Gerald Batist¹⁵

¹Q-CROC, Montreal, Qc; ²SFU, Vancouver BC; ³Charles LeMoyne, Montreal, Qc; ⁴Sacré Cœur de Montreal, Montreal, Qc; ⁵MUHC, Montreal, Qc; ⁶Sunnybrook Health Science Centre, Toronto, Ont; ⁷St. Mary’s Hospital, Montreal, Qc; ⁸Georges Dumont, Moncton, NB; ⁹Hôpital Maisonneuve Rosemont, Montreal, Qc; ¹⁰Hôtel-Dieu, Qc; ¹¹Katholieke Universiteit, Leuven, Belgium; ¹²Mount Sinai, Toronto, Ont; ¹³Moncton Hospital, Moncton, NB; ¹⁴CHUM, Montreal, Qc; ¹⁵Segal Cancer Centre-JGH, Montreal, Qc

Introduction: The overall objective of the Canadian Colorectal Cancer Consortium is to establish a molecular-based approach to cancer care to improve the outcome of colorectal cancer (CRC). This study builds on the Q-CROC-01 study established by the Quebec Clinical Research Organization in Cancer (Q-CROC). The main objective is to identify biomarkers that can predict molecular signatures linked to therapeutic resistance.

Methods: Patients with metastatic CRC receiving first-line treatment, FOLFOX or FOLFIRI +/- bevacizumab, consent to biopsies and blood sampling prior to treatment and at resistance. Tissue was profiled using exome sequencing and RNAseq. Serial monitoring of plasma ctDNA and proteomics were also evaluated. Clinical data was collected until progression of disease.

Results: Expression profiles on paired pre-post biopsies identified genes that are significantly induced at resistance. Proteomic analysis identified 151 proteins differentially expressed in resistant patients compared to responders. Genomic sequencing revealed both depletion and enrichment in different somatic mutations over time of treatment. ctDNA detected the mutational status during treatment and correlate with their relative levels in biopsies. Immune response was also explored through clustering analysis.

Conclusions: Our study, using a multi-omic strategy and integration of independent results, validates a new approach to biomarker discovery.

Outcome/Impact: These findings may hold clues to optimize current therapeutic decision making which may identify potential target pathways for second-line stratification of patients.

Keywords: colorectal cancer, biomarkers, multi-omic, sequential biopsies
84. USING DNA BARCODING TO ELUCIATE COLORECTAL CANCER CELL HETEROGENEITY AND CLONAL DYNAMICS AT BASELINE AND IN RESPONSE TO CHEMOTHERAPY

Haynes Jennifer¹, Allison Nixon², Nicholas Pedley¹, Kevin Brown², Yadong Wang¹, Jason Moffat², Catherine O’Brien¹

¹Princess Margaret Cancer Centre, University Health Network; ²Donnelly Centre, University of Toronto, Toronto, Ontario.

Introduction: The molecular basis of cellular heterogeneity in colorectal cancer (CRC) and the relative contribution of different clones to tumour growth both at baseline and in response to therapy are not well understood. Cellular DNA barcoding is a highly sensitive and unbiased tool to track the number and size of multiple subclones within a single tumour xenograft, and can identify clones that display differential responses to therapy, including those that are genetically identical.

Methods: We transduced patient-derived CRC cells with a high-complexity, lentiviral-based library of unique DNA sequences (barcodes) and injected barcoded cells subcutaneously into immunodeficient mice. To determine the tumour-initiating cell frequency and clonal composition of human CRC xenografts, we performed in vivo limiting dilution assays (LDAs). To investigate clonal dynamics in response to therapies, we treated mice with different chemotherapies starting when tumours were ~100 mm³ in size. For all experiments, tumours were collected when they reached 500-1000 mm³ in size, and the barcode populations within each tumour were quantified.

Results: DNA barcode analysis of LDA tumours revealed new insights into the clonal composition of CRC xenografts and clonal dynamics upon serial passage. In both primary and serial LDAs, we observed less barcode diversity when fewer numbers of cells were injected, with dominant barcode clones appearing only at lower numbers of cells injected. Unexpectedly, both mono- and poly-barcoded tumours formed from cells injected at the limiting dose. Upon serial passage, we found some barcode clones enriched across most tumours, whereas others were depleted. Interestingly, some barcode clones not enriched in the parental tumour appeared in serially transplanted tumours. DNA barcode analysis of clonal dynamics in response to different chemotherapies will be discussed.

Conclusions: Our data demonstrate that DNA barcoding is a useful tool to study tumour heterogeneity at the single cell level and to uncover clonal dynamics in human CRC xenografts.

Outcome/Impact: Understanding how subclonal heterogeneity contributes to tumour growth and therapeutic response is key to identifying drivers of therapeutic resistance and improving outcomes for CRC patients.

Keywords: colorectal cancer, DNA barcoding, clonal dynamics, therapeutic response
A COMPREHENSIVE MAP OF CRITICAL PATHWAYS AND NETWORKS IN CANCER STEM CELLS

Liu Jeff C. 1, Veronique Voisin1, ChangJiang Xu1, Ruth Isserlin1, Gary D. Bader1
1Toronto Donnelly Centre, University of Toronto, Toronto, ON

Introduction: It has been shown that a hierarchy exists in cancer where cancer stem cells (CSC) are at the apex with the ability to regenerate the disease and resistant to chemo- and radiation- therapy. Here, we are showing an example of how pathway and network analysis is used to extract common CSC features knowing that finding CSC-driving mechanisms is critical in the fight against cancer.

Method: RNA-Seq datasets comparing CSC and normal stem cells (NSC) from multiple tissues were processed using the STAR alignment software with the latest genome assembly (GRCh38). Differential Expression and Gene Set Enrichment Analysis generated the lists of significance in genes and pathways. An Enrichment Map (EM) and a GeneMANIA network both created using Cytoscape combined and summarized the genes and pathways into networks of interactions.

Results: Next-Generation Sequencing such as RNA-Seq have enabled us to get a systemic understanding of the dynamic cellular processes through monitoring gene expressions, epigenetic changes, and genetic alterations. However, these “omic” approaches generate tremendous amount of data that makes interpretations a difficult challenge. A network map of the pathways different between CSC and NSC was created, which simplified patterns and revealed some relationships in stem cells.

Conclusion: Normal cells contain multiple dynamic and interlocking systems that can respond to external and internal cues to tightly control self-renewal/proliferation, survival, metabolism, and differentiation. During cancerous transformation, especially in the CSCs, this balance is disrupted and leads to unrestrained cell division, survival, and invasion. GeneMANIA and EM provide the tools to discover these dynamic pathways and networks and produce a comprehensive map of CSC. The relationship among the pathways will provide information for developing specific anti-CSC strategies.

Impact: Summarizing and collapsing larger amount of CSC data into pathways and interacting networks is designed to highlight hallmarks of stem cells that could be used in a global therapeutic strategy. It also could reveal tissue specific and individual properties that would facilitate the identification of therapeutic targets for personalized medicine.

Keywords: Cancer Stem Cell, Next-Generation Sequencing, RNA-Seq, Gene Set Enrichment Analysis, Enrichment Map, GeneMANIA, Cytoscape.
86. QUALITY ASSESSMENT OF THE CANADIAN PROSTATE CANCER BIOMARKER NETWORK (CPCBN) PLATFORM

**Ouellet Véronique** ¹, Andrée-Anne Grosset¹, Gabriela Fragoso¹, Véronique Barrès¹, Nathalie Delvoye¹ N, Mathieu Latour¹, Dominique Trudel¹, Armen Aprikian², Alain Bergeron² A, Fadi Brimo², Robert Bristow⁴, Simone Chevalier², Darrel Drachenberg⁵, Ladan Fazli⁶, Neil Fleshner⁴, Martin Gleave⁶, Pierre Karakiewicz¹, Laurence Klotz⁷, Louis Lacombe³, Jean-Baptiste Lattouf¹, Theodorus van der Kwast⁴, Anne-Marie Mes-Masson¹, and Fred Saad¹.

¹CHUM/CRCHUM; ²MUHC/RC-MUHM; ³CHU de Québec-Université Laval; ⁴UHN; ⁵MPC; ⁶VPC; ⁷Sunnybrook;

**Introduction:** The CPCBN is a program that gathers researchers from several institutions from four different Canadian provinces. This network assembled a validation tissue-microarray (TMA) resource composed of 1508 prostate cancer patients treated by radical prostatectomy. Researchers who wish to access a large cohort to validate prognostic biomarkers can access this richly annotated resource. Over the last decade, we and others have uncovered a robust association between the nuclear localisation of nuclear factor-kappa B (NF-kB) p65, prostate cancer aggressiveness and biochemical recurrence (BCR). Thus, we chose to confirm these results with the CPCBN series partly as a validation exercise.

**Methods:** Automated immunohistochemistry staining of p65 was performed using the CPCBN test-TMAs. This test series contains a minimum of 3 cores of tumour tissues and 2 cores of adjacent benign tissues from 250 RP specimens. Two independent observers scored percent of nuclear staining. Statistical analyses were performed using SPSS software.

**Results:** Demographic analyses showed that the test-TMA series is representative of the prostate cancer population in Canada with a median age at diagnosis of 62 years. With a 94 months follow-up the cohort presented 30% of biochemical relapse and 4% of bone metastasis development. By Kaplan-Meier analysis, we validated the significant association between an increase in nuclear frequency of NF-kB p65 and biochemical relapse (log rank, p=0.05, cut off of 3%) while Cox regression analyses showed a trend (dichotomized p65: p=0.06, Exp (B) 1.58, CI 95% 0.99-2.53). Nuclear frequency of p65 was also associated with increase risk of developing bone metastasis (Cox regression, p=0.03, Exp(B) 1.06, CI 95% 1.006-1.117) although this will need to be confirmed on the larger TMA series.

**Conclusions:** Our study recapitulates previous observation linking NF-kB p65 with disease progression and also highlight its role as a predictor of bone metastasis.

**Outcome/Impact:** The CPCBN test-TMA series represent an appropriate first-step in biomarker validation. Meta-analysis of multiple markers can ultimately define a nomogram of combined biomarkers for early treatment decisions in men newly diagnosed with prostate cancer.
87. MODULATING NANOPARTICLE DRUG DELIVERY USING RADIATION AND HEAT

Stapleton Shawn¹, Ali Vedadi¹, Michael Dunne², Michael Milosevic¹, Christine Allen², David Jaffray¹

¹Radiation Medicine Program, Princess Margaret Hospital, Toronto, Canada
²Pharmaceutical Sciences, University of Toronto, Toronto, Canada

Introduction: Nanomedicine drug delivery systems can deliver large doses of drugs specifically to cancer, however only a modest improvement in anti-tumour efficacy has been observed. The limited efficacy is due in part to substantial intra-tumoural heterogeneity of drug delivery system (e.g. liposomes). Here we investigate the ability for radiation (RT) and heat (HT) to improve the intra-tumoural distribution of liposomes and the resulting effect on efficacy.

Methods: Micro-Computed tomography (CT) imaging was used to quantify the bulk accumulation and intra-tumoural distribution of a liposome contrast agent (CT-liposome). Measurements were performed in an orthotopic MDA-MB-231 mouse xenograft. Tumours were pre-treated with 15 Gy of radiation (n=5), mild-hyperthermia (42°C; n=5), or untreated (n=5) prior to CT-liposome administration. To investigate mechanism, interstitial fluid pressure (IFP), perfusion, vascular permeability, vascular volume fraction and interstitial volume fraction were measured. Efficacy was evaluated by pre-treating tumours prior to the administration of liposome doxorubicin (Doxil).

Results: Pre-treatment with RT and HT resulted in a 1.6 and a 1.9 fold peak increase in bulk accumulation of liposomes, respectively. Furthermore, pre-treatment substantially improved the intra-tumoural distribution of liposomes, specifically increasing accumulation in the tumour centre. Both RT and HT significantly decreased IFP. However, only HT had a substantially effect on tumour vascularity, by increasing perfusion and vascular volume. No changes to permeability or interstitial volume was observed. Pre-treating with RT and HT prior to Doxil significantly increased tumour growth delay compared to controls.

Conclusions: We demonstrate that RT and HT improves the bulk accumulation and intra-tumoural distribution of liposomes. A significant drop in IFP, following RT and HT, appears to be the dominant mechanism that drives the improved intra-tumoural distribution of liposomes. The improved of accumulation and intra-tumoural distribution of liposomes by RT and HT significantly enhanced the efficacy of Doxil.

Outcome/Impact: RT and HT are viable strategies to improve the accumulation of liposomes in solid tumours, which result in increased delivery of drug and increased tumour growth delay.
The Terry Fox Research Institute from coast to coast

TFRI is an Institute without walls linking the capabilities of 73 leading cancer care and cancer research institutes and universities organized through six regional “nodes”.

NATIONAL PARTNERS
Canadian Institutes of Health Research
Canadian Tumour Repository Network (CTRnet)
Genome Canada
Lung Cancer Canada
The Terry Fox Foundation

BRITISH COLUMBIA
BC Cancer Agency
BC Cancer Foundation
The Centre for Drug Research and Development
Genome British Columbia
St. Paul’s Hospital (Providence Health)
Simon Fraser University
Team Finn Foundation
University of British Columbia
Vancouver Coastal Health Research Institute

ALBERTA
Alberta Cancer Foundation
Alberta Health Services
Alberta Innovates – Health Solutions
Cross Cancer Institute
Genome Alberta
Tom Baker Cancer Centre
University of Alberta
University of Calgary

PRAIRIES
CancerCare Manitoba
Children’s Hospital Research Institute of Manitoba
Saskatchewan Cancer Agency
Research Manitoba
University of Manitoba
University of Saskatchewan

ONTARIO
BioCanRx
Brock University
Children’s Hospital of Eastern Ontario
Hospital for Sick Children
Juravinski Cancer Centre
London Health Sciences Centre
McMaster University
Mount Sinai Hospital
Ontario Cancer Institute
Ontario Institute for Cancer Research
Ottawa Hospital Research Institute
Queen’s University
Sunnybrook Research Institute
Thunder Bay Research Institute
University Health Network (Princess Margaret Cancer Centre)
University of Guelph
University of Ottawa
University of Toronto

QUEBEC
Centre hospitalier de L’Université de Montréal
Centre hospitalier Universitaire du Québec
CHU Sainte-Justine Foundation
Institut Universitaire de Cardiologie et de Pneumologie de Québec
Jewish General Hospital
Institut de recherche en Immunologie et cancérologie
Fonds de recherche Québec – Santé
Fondation centre de cancérologie Charles-Bruneau
L’Institut de Recherches Cliniques de Montréal
McGill University Goodman Cancer Centre
McGill University Health Centre
McGill University
Québec Breast Cancer Foundation
The Cole Foundation
Université de Montréal
Université Laval
Université Sherbrooke

ATLANTIC
Atlantic Cancer Research Institute (Moncton)
Capital District Health Authority
Dalhousie University (Halifax)
Isaac Walton Killam Health Centre
New Brunswick Health Research Foundation
Memorial University of Newfoundland (St John’s)
New Brunswick Cancer Network
QEI Health Sciences Centre (Halifax)
The University of New Brunswick
The University of Prince Edward Island