TFRI-ONTARIO NODE RESEARCH SYMPOSIUM

MONDAY, DECEMBER 5, 2016

Innovating Towards Transformative Cancer Care

Collaboration Centre, MaRS Centre, South Tower,
1st Floor, 101 College St. Toronto, Ontario

Program & Abstracts

The Terry Fox Research Institute
www.tfri.ca

Welcome

The Terry Fox Research Institute's Ontario Node Research Symposium

Innovating towards transformative cancer care

Mike White, PhD

VP/CSO, Pfizer Inc.

Ugur Sahin, MD

Professor, Managing Director (Science & Research) of Translational Oncology at the University Medical Center of the Johannes Gutenberg University Mainz

Opening remarks from Dr. Robert Rottapel and Mr. Darrell Fox.

KEYNOTE SPEAKERS

Mike White & Ugur Sahin, with featured talks from Stephan Angers, Steven Jones, Shana Kelley, Anthony Nichols and Yonghong Wan.

Closing remarks from Dr. Victor Ling.

SYMPOSIUM HOSTS AND COMMITTEE MEMBERS

Marianne Koritzinsky – Princess Margaret Cancer Centre, University Health Network
Trevor Shepherd – Schulich School of Medicine & Dentistry, University of Western Ontario
Sheila Singh – McMaster Stem Cell and Cancer Research Institute, McMaster University
David Stojdl – CHEO Research Institute, University of Ottawa
Robert Rottapel – Princess Margaret Cancer Centre, University Health Network
Donna DeFrancesco – Princess Margaret Cancer Centre, University Health Network (Symposium Co-ordinator)

VOLUNTEERS

Sally Desilva, Marie Kumar, Julie Owen, Mary White

VOLUNTEER PHOTOGRAPHER

Cathy Feghali

Statement on Respect for Confidentiality of Unpublished Material:

The Institute has invited everyone attending this meeting because of their contribution, or potential for contribution, to the work of our research community. In building our community, we are committed to respecting the confidentiality of ideas and data that are unpublished at this meeting.

We request and require that all registrants refrain from recording such confidential information, and do not discuss such information with colleagues outside of this meeting. It is only in this way that we will collectively build the trust and respect that is necessary for effective collaborations.

We appreciate your respect for and compliance with this important request.

Agenda

TIME	ACTIVITY	SPEAKER
7:00am – 9:00am	**Early Registration**	
7:00am – 9:00am	**Poster Set Up** *(The Heritage Atrium, on the 1st floor of the MaRS Centre)*	*Note: All posters to be installed by 11am*
7:30am – 8:45am	**BREAKFAST**	
8:45am – 9:00am	**Welcome & Opening Remarks**	**Dr. Robert Rottapel, MD, FRCPC**, TFRI-Ontario Node, Toronto & **Mr. Darrell Fox**, Board of Directors, TFRI
SESSION I: CAPITALIZING ON THE CACOPHONY OF CANCER ETIOLOGY		
9:00am – 10:00am	**Keynote Speaker**	**Mike White, PhD**, VP/CSO, Pfizer Inc. *"Capitalizing on the Cacophony of Cancer Etiology"*
10:00am – 10:30am	**Invited Speaker**	**Stephan Angers, PhD,** Associate Professor, Faculty of Pharmacy & Department of Biochemistry, University of Toronto
10:30am – 11:00am	**BREAK**	
11:00am – 11:30am	**Rapid Fire Postdoc Talks** – 10 X 3 min	*Please see the names of these presenters on page vii*
11:30am – 1:00pm	**LUNCH & Poster Session** *(The Heritage Atrium, on the 1st floor of the MaRS Centre)*	
SESSION II:BIOMARKERS, TECHNOLOGIES & THERAPEUTICS		
1:00pm – 1:30pm	**Invited Speaker**	**Steven Jones, FRSC, FCAHS**, Co-Director and Head, Bioinformatics, Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency (Primary) Professor, Medical Genetics, UBC, Professor, Molecular Biology & Biochemistry, Simon Fraser University
1:30pm – 2:00pm	**Invited Speaker**	**Shana Kelley, PhD**, Professor, University of Toronto, Departments of Pharmaceutical Sciences, Chemistry, Biochemistry, Institute for Biomaterials and Biomedical Engineering
2:00pm – 2:30pm	**Invited Speaker**	**Anthony Nichols, MD**, Associate Professor, Director of Translational Head and Neck Cancer Research at London Health Sciences Centre
2:30pm – 3:00pm	**BREAK**	
SESSION III:IMMUNOTHERAPY		
3:00pm – 3:30 pm	**Invited Speaker**	**Yonghong Wan, MD**, Professor, Pathology and Molecular Medicine, McMaster Immunology Research Centre Cancer Division
3:30pm – 4:30pm	**Keynote Speaker**	**Ugur Sahin, MD**, Professor, Managing Director (Science and Research) of Translational Oncology at the University Medical Center of the Johannes Gutenberg University Mainz – TRON
4:30pm – 5:00pm	**Closing Remarks**	**Dr. Victor Ling, OC, OBC, PhD**, TFRI President & Scientific Director
5:00pm – 6:30pm	**RECEPTION & Poster Session** *(The Heritage Atrium, on the 1st floor of the MaRS Centre)*	

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** TFRI/TFF Supported*
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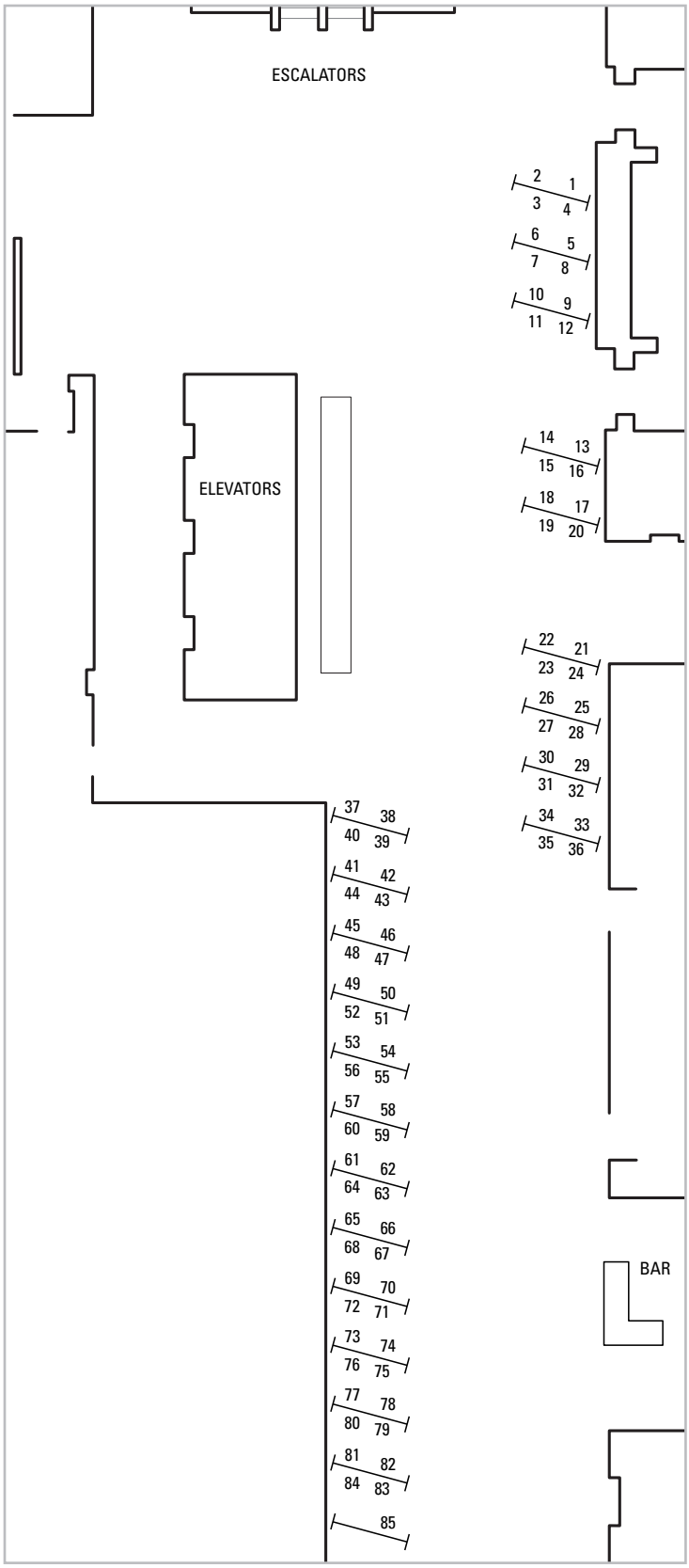
RAPID-FIRE POSTDOC TALKS PRESENTERS

We extend our congratulations to these 10 individuals who were selected by our judging panel to give presentations at today's meeting.

Adrian Vincent Buensuceso
Olga Gan
Abdi Ghaffari
Kara Harmatys
Yi Liang
Trevor McKee
Timothy McKinnon
Katherine Rowland
Nathan Schachter
Parvez Vora

Poster Set Up



TFRI POSTER BOARD SET UP:

Heritage Atrium
(accessible via the escalator from the lower concourse area; up one floor)

1. KDM1A MEDIATED EPIGENETIC REPROGRAMMING DRIVES CENPE EXPRESSION AND PROSTATE CANCER PROGRESSION

Yi Liang[1*], Musaddeque Ahmed[1], Haiyang Guo[1], Shuai Gao[2], Junjie Hua[1,3], Catherine Lu[1], Anthony Joshua[1], Changmeng Cai[2], Housheng Hansen He[1,3]

[1] Princess Margaret Cancer Center/University Health Network, Toronto, Ontario, Canada
[2] Department of Structural and Chemical Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[3] Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

** Post-doctoral fellow*

Androgen receptor (AR) signaling is a key driver of prostate cancer. For advanced cases, although androgen deprivation therapy initially works, relapse inevitably arises and results in castration-resistant prostate cancer (CRPC) that is often incurable. Novel therapeutic targets for the treatment of CRPC other than AR itself are needed to be identified. Previous studies showed that Lysine Specific Demethylase 1 (LSD1, KDM1A) associates with and coactivates AR on a large fraction of androgen-stimulated genes. However, the role of LSD1 in epigenetic regulation in CRPC is largely unknown. Here we performed comprehensive analyses to study the LSD1-mediated epigenetic reprogramming in both androgen-dependent prostate cancer and CRPC. Our results show that there is a shift of LSD1 target genes and genome-wide binding sites in CRPC, and that LSD1 activates a subset of cell cycle regulatory genes driven by LSD1 reprogramming in CRPC. We identify that centromere-associated protein E (CENPE), an LSD1 up-regulated target gene, is over-expressed in metastatic/CRPC clinical prostate cancer samples. The expression level of CENPE correlates with Gleason Score and clinical outcome of the patients with prostate cancer. CENPE is regulated by the co-binding of LSD1 and AR to its promoter region in CRPC specifically, and facilitates the cell proliferation of CRPC. Tumour suppressor retinoblastoma-associated protein 1 (RB1) may bind to CENPE promoter and repress the binding of LSD1/AR to prevent the transcription of CENPE in androgen-dependent prostate cancer cells in the presence of androgen. Furthermore, CENPE activating cell cycle gene signature predicts disease progression of prostate cancer. Disruption of CENPE by CRISPR-Cas9 gene editing system and small molecular inhibitor GSK923295 can significantly decrease tumour growth in mouse xenograft model of prostate cancer. Taken together, these findings demonstrate the LSD1-mediated epigenetic reprogramming driving CRPC, and provide a rationale for therapeutic CENPE targeting in CRPC patients.

2. CHROMATIN REARRANGEMENTS TYPICAL OF HEMATOPOIETIC STEM CELLS ARE CO-OPTED TO DRIVE LEUKEMOGENESIS IN NPM1c ACUTE MYELOID LEUKEMIA

Alex Murison[1*], Naoya Takayama[1,2*], Shin-ichiro Takayanagi[1], Sasan Zandi[1], Nadia Penrod[1], Amanda Mitchell[1], James Kennedy[1], Stanley Ng[1], John E. Dick[1], and Mathieu Lupien[1,3,4]

[1] Department of Molecular Genetics, Princess Margaret Cancer Centre, University Health Network, Toronto
[2] Department of Innovation Therapy, Chiba University Graduate School of Medicine, Chiba
[3] Department of Medical Biophysics, University of Toronto
[4] Ontario Institute for Cancer Research, Toronto

** Authors equally contributed to this work*

Acute Myeloid Leukemia (AML) is a myeloid malignancy that accounts for 1.2% of all cancer deaths in the USA. Though there are a variety of known risk factors affecting prognosis, overall survival rates are poor – below 50% for patients with an intermediate risk diagnosis - and the presence of quiescent, stem-like cells which are capable to evading chemotherapy and driving tumour re-initiation is a majorcomplicating factor in its treatment. Identifying the changes that occur during the earliest stages of normal hematopoiesis (particularly from hematopoietic stem cells (HSCs) to their earliest progeny, Multipotent Progenitors (MPPs)), and relating them back to leukemogenesis would represent a major advance. To date, however, only minor differences between these populations have been identified at transcriptomic or epigenetic levels, which do not explain the loss of self-renewal observed phenotypically.

In this study we have used ATAC-Seq to profie accessible chromatin in 96 AML fractions, sorted by CD34/CD38 markers from 24 bulk samples, whose ability to engraft a tumour in-vivo was used to determine the presence or absence of tumour initiating cells. We have compared this to ATAC-Seq performed on 13 sorted populations from human cord blood, including HSCs and MPPs.

We applied non-negative matrix factorization to identify chromatin accessibility signatures, which defined patterns of chromatin accessibility over hematopoiesis and leukemogenesis. This revealed one signature, which defined non-HSC progenitors only. Genomic regions associated with this signature reveal a role for modulation of the three-dimensional organization of the chromatin in defining stemness versus engagement of cell proliferation required for the commitment to cell fate commitment. These changes are paralleled in cancer, as a similar signature is observed in AML samples with mutations in Nucleophosmin1 (NPM1). Transfecting the recurrent NPM1c mutation into human cord blood is sufficient to induce changes to the chromatin accessibility reminiscent of the non-HSC progenitors chromatin signatures.

Taken together, our results highlight the direct contribution of a genetic event in reprogramming the chromatin accessibility in AML samples to induce a normal hematopoietic progenitor like signature.

3.

WITHDRAWN

4. EVALUATION OF EPIGENETIC TARGETS FOR TREATING GLIOBLASTOMA MULTIFORME USING CHEMICAL PROBES

Patty Sachamitr[*1,2,] Michelle Kushida[1], Maria Mangos[2], Joseph Vehyl[2], Fiona Coutinho[1], Cheryl Arrowsmith[2], Takis Prinos[2] and Peter Dirks[1]

[1] Developmental and Stem Cell Biology Program, Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Toronto ON, Canada
[2] Structural Genomics Consortium, University of Toronto, Toronto, ON, Canada

** Presenting author is a Post-doctoral Research Fellow.*

Glioblastoma (GBM) is the most aggressive and lethal brain malignancy. Adults affected with GBM have an extremely poor prognosis, with median overall survival of less than 15 months. The poor outcome of GBM has been attributed, in part, to the heterogeneous nature of the tumour and the presence of brain tumour stem cell population that drives the disease and promotes resistance to the therapy1. The current standard of care chemotherapy, temozolomide (TMZ), only extends the median survival rate by a few months and has been linked into increased frequency of mutations in patients2.

Epigenetic changes have been shown to play an important role in the development and progression of many cancers. Approximately 45% of GBM patients have been shown to harbour at least one mutation in genes that are functionally linked to chromatin organisation1, 3. We aim to identify epigenetic targets involved in the maintenance of GBM stem cell population.

To achieve this, we screened 20 patient-derived Glioma Neural Stem Cell lines (GNS) with 38 small molecule epigenetic probes from the Structural Genomics Consortium (SGC). Using a high throughput, live cell imaging-based screening platform, we identified two PRMT5 inhibitors, which inhibited the growth of GNS cells in vitro. Inhibition of PRMT5 using these probes reduced global levels of arginine symmetric demethylation, impaired cell proliferation and resulted in senescence of GNS cells. PRMT5 inhibitors were also found to reduce the sphere forming capability of both GNS cells lines and primary GBM tumour samples, indicating that it may hamper the maintenance of the stem cell populations in GBM. Knocking down PRMT5 gene corroborated the results obtained by target inhibition using the small molecule probes.

Our findings identified PRMT5 as a potential therapeutic target for GBM, whose pharmacological inhibition may be efficacious in the treatment of GBM.

References:

1. Brennan C. W. et al, Cell 155, 462-477 (2013)
2. Stupp R. et al, N Engl J Med 352, 987-996 (2005)
3. Verhaak R.G. et al, Cancer Cell 17(1), 98-110 (2010)

5. TARGETING PI3K/MTOR IN MOUSE RHABDOMYOSARCOMA MODELS DRIVEN BY FGFR4

Timothy McKinnon[1*], Rosemarie Venier[1], Marielle Yohe[2], Berkley E. Gryder[2], Brendan Dickson[3], Krista Schleicher[1], Dariush Davani[4], Winnie Wei[4], Cynthia Guidos[4], Abha Gupta[4], Javed Khan[2] and Rebecca Gladdy[1,5,6]

[1] *Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada*
[2] *Oncogenomics Section, Center for Cancer Research, National Cancer Institute, Gaithersburg, MD, USA*
[3] *Department of Pathology, Mount Sinai Hospital, Toronto, ON, Canada*
[4] *The Hospital for Sick Children, Toronto, ON, Canada*
[5] *Ontario Institute for Cancer Research, Toronto, ON, Canada*
[6] *Department of Surgery, Mount Sinai Hospital, Toronto, ON, Canada*

** Postdoctoral Fellow (Trainee)*

Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma and, while survival rates have increased over the past few decades, intermediate and high risk patients still have dismal outcomes. Genomic analyses have revealed that 93% of RMS have RTK/RAS/PI3K alterations and that fibroblast growth factor receptor 4 (FGFR4) is frequently mutated or overexpressed. The point mutation V550E constitutively activates FGFR4, stimulating downstream signaling pathways. We have demonstrated that $FGFR4^{V550E}$ is a potent oncogene in mouse models of RMS and that secondary tumour models can be generated following transplantation of $FGFR4^{V550E}$ overexpressing tumour cells into immunocompetent host mice. These models have been employed in a translational study to uncover the mechanism of transformation in $FGFR4^{V550E}$ driven RMS and to uncover experimental compounds that inhibit RMS growth. Immunoblot analysis using lysates from myoblasts overexpressing $FGFR4^{V550E}$ demonstrated activation of AKT and mTOR signalling pathways. Likewise, $FGFR4^{V550E}$ overexpressing tumours and tumour-derived cells also showed AKT and mTOR phosphorylation. Additionally, murine tumour cells overexpressing $FGFR4^{V550E}$ were subjected to a single agent, in vitro dose-response drug screen. Compounds were grouped by target class, and potency was determined using average percent area under the dose response curve (AUC). Using this technique, $FGFR4^{V550E}$ over-expressing tumour cells were highly sensitive to PI3K/mTOR inhibitors. In particular, GSK2126458 (omipalisib) was a potent inhibitor of $FGFR4^{V550E}$ tumour-derived cell and human RMS cell viability. $FGFR4^{V550E}$ overexpressing myoblasts and tumour cells had low nanomolar GSK2126458 EC_{50} values. Mass cytometry validated GSK2126458 specificity at single cell resolution, decreasing the abundance of phosphorylated Akt as well as decreasing phosphorylation of the downstream mTOR effectors 4ebp1, Eif4e and S6. In a preclinical study, GSK2126458 inhibited tumour growth in vivo. A statistically significant increase in disease specific survival (DSS) was observed in mice treated with GSK2126458 compared to mice treated with vehicle alone ($p<0.001$) or standard of care, vincristine ($p<0.05$). In summary, this functional genomics platform is amenable to study RMS driver mutations and as a preclinical tumour model to test therapeutic agents. Importantly, these results suggest a role for PI3K/mTOR inhibition in precision therapy regimens for RMS with FGFR4 mutations. This study provides further evidence for clinical studies involving mTOR inhibitors (e.g. temsirolimus).

6. FIRST KNOWLEDGE-BASED TREATMENT PLANNING MODEL FOR GLIOBLASTOMA TREATED WITH A HYPOFRACTIONATED SIMULTANEOUS INTEGRATED BOOST TECHNIQUE

Avishek Chatterjee, PhD; Monica Serban, MSc; Bassam Abdulkarim, MD, PhD; Valerie Panet-Raymond, MD; Luis Souhami, MD, FASTRO; George Shenouda, MBBCh, PhD, FRCP (C); Siham Sabri, PhD; Bertrand Jean-Claude, PhD; JAN SEUNTJENS, PhD. Presenting author is post-doctoral fellow. He is a STARS21 scholar, a program funded by TFRI.

Affiliation: McGill University Health Centre, Montreal, QC, Canada.

Acknowledgments: AC acknowledges partial support by the CREATE Medical Physics Research Training Network grant of the Natural Sciences and Engineering Research Council (Grant number: 432290). AC, BA, BJ, SS, and JS acknowledge being partially funded by Varian Research Grant titled "Registry Based Clinical and Genomic Research to Enable the 'Next Generation Radiation Therapy' in Glioblastoma Multiforme."

Purpose: Due to the presence of multiple serial organs-at-risk (OARs) in close proximity to the tumour, treatment planning for glioblastoma (GBM) is complex and time-consuming. The problem is compounded when there are two planning target volumes (PTVs), and when a hypofractionation schedule is used, which lowers OAR maximum dose constraints compared to standard fractionation. Clinical planning requires many dose control structures and multiple iterations to achieve acceptable dose distribution. Our goal was to leverage clinical best practice by creating a knowledge-based (KB) planning model using a commercial treatment planning software (CTPS). The model must yield plans with similar PTV coverage and OAR sparing as the clinical standard, without reliance on planner experience, and with significant reduction in planning time.

Methods and Materials: KB CTPS predicts dose-volume histogram (DVH) bands for each OAR (band width representing prediction uncertainty) using a linear regression relationship between dose and geometry. It provides optimization objectives for treatment planning. Our model training used 33 patients treated with 60 Gy to PTV1 (PTV60) and 40 Gy to PTV2 (PTV40) in 20 fractions. Key maximum dose constraints were: brainstem < 45 Gy, brainstem planning risk volume (PRV) < 49 Gy, optic apparatus < 42 Gy, and optic PRV < 46 Gy. PRV margins were 3 mm. Plans were created using either volumetric modulated arc therapy (VMAT) or Intensity Modulated Radiotherapy (IMRT). To improve DVH predictive ability and sparing efficacy, an intermediate model was created from a subset of plans that were best at OAR sparing. Subset selection was OAR-dependent, since a plan may optimally spare the brainstem but not the eyes. Using the intermediate model, all 33 cases were re-planned, resulting in the final model. The model was validated using an independent set of 19 patients with GBM who were treated with the same prescription as the training set.

Results: The plans created by the model had superior PTV40 coverage: $\Delta D99\% = 0.8 \pm 0.2$ Gy and $\Delta V95\% = 0.19 \pm 0.06\%$. For PTV60, KB planning had similar coverage and lower near maximum dose ($\Delta D1\% = 0.5 \pm 0.2$ Gy). Stated improvements have $p < 0.05$. Maximum dose to OARs were slightly lower for KB planning: 0.80 ± 0.81 Gy (brainstem), 0.91 ± and 0.65 Gy (optic apparatus), but not statistically significant. Mean dose to OARs were similar. Total planning time was typically 7 or 15 minutes, for IMRT and VMAT, respectively. It was dominated by optimization and calculation time, i.e., hardware-limited, and not requiring human intervention. This model is also applicable to a single hypofractionated prescription of 60 Gy, and to other brain tumours treated with the same prescription.

Conclusions: Our first-of-its-kind KB planning model for GBM with 2 PTVs yields plans that satisfy PTV coverage and OAR sparing requirements irrespective of tumour size and location within brain, in a time over an order of magnitude shorter than conventional planning. Our model will be made available for use by other institutions.

7. IDENTIFYING FUNCTIONAL LONG NONCODING RNA MODULATED BY RISK SNPS IN PROSTATE CANCER

Haiyang Guo[1], Musaddeque Ahmed[1], Yi Liang[1], Junjie Hua[1], Fraser Soares[1], Housheng Hansen He[1,2]

[1] Ontario Cancer Institute, Princess Margaret Cancer Center/University Health Network, Toronto, Ontario, Canada;
[2] Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada;

Haiyang Guo is a post-doctoral fellow in Dr. He's lab.

A recent analysis of over 7000 RNA-seq datasets identified 58,648 long noncoding RNAs (lncRNAs), twice as large as the number of protein coding genes. Although a few lncRNAs have well-characterized functions, the vast majority of this class of molecules remains functionally uncharacterized. Through integrative analysis of the lncRNA transcriptome with genomic and single nucleotide polymorphism (SNP) data from prostate cancer (PCa) genome-wide association studies (GWAS), 45 candidate lncRNAs were identified to be associated with risk to PCa. The mechanism underlying the top hit, PCAT1, was further evaluated. A risk-associated variant at rs7463708 increases binding of ONECUT2 at a distal enhancer that loops to the PCAT1 promoter. ONECUT2 recruits AR to PCAT1 enhancer resulting in upregulation of PCAT1 upon prolonged androgen treatment. Moreover, PCAT1 interacts with AR and lysine-specific histone demethylase 1 (LSD1) and is required for their recruitment to the enhancers of GNMT and DHCR24, two androgen late-response genes implicated in PCa development and progression. Importantly, PCAT1 is significantly upregulated in primary and metastatic PCa tumours and promotes PCa cell proliferation and tumour growth in vitro and in vivo. Our findings suggest that modulating lncRNA expression is an important mechanism for risk-associated SNPs in promoting prostate transformation.

8. SOX2+ CELLS STAND AT THE APEX OF GRANULE NEURON DEVELOPMENT AND ARE ABERRANTLY MAINTAINED IN MEDULLOBLASTOMA AFTER CONSTITUTIVE SHH ACTIVATION.

Hayden Selvadurai[1], Erika Luis[1,2], Ciaran Galvin[1], Rob Vanner[1,2], Lilian Lee[1], Heather Whetstone[1], Michelle Kushida[1], Peter Dirks[1,2].
[1]Developmental and Stem Cell Biology Program and Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Toronto.
[2]Department of Molecular Genetics, University of Toronto, Toronto.

Medulloblastoma (MB) is characterised as a disease of dysregulated cerebellum development. Consistent with this, recent work by us and others has demonstrated that tumours defined by high levels of Sonic Hedgehog (SHH) signalling grow hierarchically, with cells expressing the neural stem cell transcription factor Sox2 responsible for driving progression and recurrence. To test whether this pattern of growth recapitulates a normal developmental process, we assessed the extent to which Sox2 is expressed in the developing cerebellum and performed lineage tracing to define the output of Sox2 expressing cells over time. We found that Sox2 expression defines both the ventricular and rhombic lip embryonic germinal zones and that the granule neuron lineage is entirely derived from Sox2 expressing cells in these regions. We further identified the persistence of Sox2 expression in a rare fraction of granule neuron progenitors until the first days of postnatal cerebellum growth and show that these cells contribute extensively to the adult granule neuron population, suggesting expression of Sox2 defines a more primitive cell at the root of transient hierarchical growth. Constitutive activation of the SHH pathway in this rare cell fraction in new born mice caused aberrant maintenance of hierarchical growth leading to hyperplasia and rapid progression to MB. These findings provide a novel cellular mechanism for the initiation and progression of SHH-subtype MB.

9. IN VIVO IMAGING REVEALS THE RESPONSE OF PANCREATIC TUMOURS TO HIGH-DOSE IRRADIATION

Azusa Maeda[1,2], Yonghong Chen[1], Jiachuan Bu[1], Hilda Mujcic[1], Bradly Wouters[1,2], Ralph Dacosta[1,2]

[1]Princess Margaret Cancer Centre, University Health Network;
[2]Department 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: A BxPC3 pancreatic tumour xenograft was established in the dorsal skinfold window chamber (DSWC) model and a hind leg model. Tumours were irradiated with a single dose of 4, 12 and 24 Gy, and the following parameters were assessed using in vivo imaging techniques (fluorescence microscopy, Doppler ultrasound): tumour size, microvascular function, vascular permeability, platelets/ leukocytes adhesion and HIF-1α expression. Tumours were assessed histologically to validate the *in vivo* observations.

Results: Irradiation induced significant but transient vascular dysfunction in a dose-dependent manner, while maintaining most of the vascular structure intact. In the DSWC model, functional vascular density was reduced by approximately 65% by 10 days after irradiation compared to pre-irradiation when the tumours were irradiated with a single dose of 24 Gy. Similar trend was observed in the hindleg tumour model using Doppler ultrasound. In the DSWC model, platelet and leukocyte adhesion to the vascular endothelium was observed within hours to days after irradiation. Such acute reaction was followed by an increase in vascular permeability and HIF-1α expression occurring 1-2 weeks after irradiation. Significant tumour growth delay was observed in tumours irradiated with 24 Gy compared with non-irradiated control by 19 days after irradiation, followed by regrowth of irradiated tumours by 65 days after irradiation.

Conclusions: Irradiation of the BxPC3 tumours with a single dose of 24 Gy caused transient vascular dysfunction and increased HIF-1α expression. 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.

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10. RHAMM TRANSFORMS FIBROBLASTS THROUGH ERK1,2/RSK/FOS ACTIVATED NUCLEAR SIGNALING

K. Basu[1], P. Telmer[1], C. Tolg[1], S. Hamilton[1], S. Zhang[3], JB McCarthy[4], E. A. Turley[1,2]

(The presenting author is considered as a trainee (post-doctoral fellow))

[1]London Regional Cancer Program, London Health Sciences Centre, Victoria Hospital, London, Ontario, Canada; [2]Depts. Oncology, Biochemistry, Surgery, Schulich School of Medicine, Western University, London,
Ontario, Canada;
[3]Hospital for Sick Children, Division of Cardiovascular Research, Toronto, Ontario, Canada;
[4]Masonic Cancer Center, Minneapolis, Minnesota. Department of Laboratory Medicine and Pathology, Medical School, University of Minnesota, Minneapolis, Minnesota.

RHAMM/ HMMR (receptor for hyaluronan-mediated motility) is a multifunctional, centrosomal protein located in both extracellular and intracellular compartments that is required for efficient wound repair. It is poorly expressed during tissue homeostasis, transiently upregulated during response to injury and chronically overexpressed in most neoplasms in particular soft tissue sarcomas. RHAMM is subject to alternative splicing to generate at least 3 isoforms whose functions in sarcoma have remained largely unexplored.

We previously reported that an N terminal truncation of RHAMM (amino acids 1-163) is transforming in fibroblasts and that this oncogenic RHAMM isoform transforms through RAS signalling. Here, we report that truncated oncogenic isoforms can be generated by alternate start codon usage and document that the truncated protein products are highly motile within fibroblasts compared to full length RHAMM and that they uniquely accumulate in the nucleus. We further show that the nuclear localization of these oncogenic proteins is required for transformation. RHAMM mediated oncogenicity is associated with activation of the ERK1,2/ RSK/ FOS and transcription targets Elk-1/SRE and AP-1. Transcriptome and Q-PCR analyses show that nuclear RHAMM oncogenic proteins regulate the expression of a subset of Elk-1 and AP-1 targets notably AURKA along with TPX2 and FOXM1. *In silico* analyses of these genes in sarcoma clinical samples shows that RHAMM, TPX2, AURKA and FOXM1 are co-expressed and significantly associate with disease-free survival (DFS) ($p = 0.0048$) in soft tissue sarcomas (TCGA database). Our findings show that nuclear localization of oncogenic proteins such as RHAMM is essential for transformation and identify potential therapeutic targets in sarcomas.

11. LKB1 IS A CENTRAL STRESS METABOLISM MEDIATOR IN TUMOUR GROWTH AND CHEMO-RESISTANCE OF METASTATIC OVARIAN CANCER

Adrian Buensuceso[1,2], Yudith R. Valdes[1], Trevor G. Shepherd[1,2,4,5]

[1]Translational Ovarian Cancer Research Program, London Regional Cancer Program, London, Ontario, Canada
[2]Departments of Anatomy and Cell Biology,
[3]Biochemistry,
[4]Obstetrics and Gynecology, and
[5]Oncology, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, Ontario

Submitting author: Postdoctoral Fellow (trainee)

Epithelial ovarian cancer (EOC) is the most common gynecologic malignancy in the developed world. While the majority of patients with metastatic EOC respond initially to surgery and cytotoxic chemotherapy, almost all will ultimately relapse with chemo-resistant disease. Our recent data demonstrate that Liver Kinase B1 (LKB1), a key stress metabolism signalling protein, is necessary for promoting EOC cell survival in an in vitro spheroid model of metastasis. LKB1 signalling suppresses several anabolic biosynthetic pathways, while activating processes that generate ATP and recycle metabolites, such as autophagy. This enables cells to maintain viability and survive in metabolically-challenging environments like that encountered during EOC metastasis. While LKB1 may be considered a tumour suppressor in some instances, we have shown that EOC cells require intact LKB1 to promote spheroid cell survival and chemo-resistance. To further assess LKB1 function in EOC pathogenesis, we have established OVCAR8-*STK11*KO and HeyA8-*STK11*KO cell lines that lack LKB1.

Complete loss of LKB1 had little effect on proliferating cells in adherent culture. However, LKB1 loss sensitized EOC cells to the specific stresses of cell detachment, glucose deprivation, and exposure to the chemotherapeutic drugs carboplatin and paclitaxel. LKB1 loss impaired the spheroid-forming ability and viability of EOC cells in this non-adherent tumoursphere model. Interestingly, LKB1 loss in EOC spheroids had no effect on AMPK phosphorylation, indicating that LKB1 signalling in EOC cell survival and metastasis may act via alternative AMPK-independent pathways.

Reverse-phase protein array was used to identify differentially-expressed proteins that may mediate LKB1 effects in EOC. Dual-specificity phosphatase 4 (DUSP4) was elevated in OVCAR8-*STK11*KO and HeyA8-*STK11*KO cells, as well as EOC cell lines that carry endogenous LKB1-inactivating mutations (TOV21G and CaOV3). DUSP4 knockdown in LKB1-deficient HeyA8 and TOV21G cells rescued the impaired spheroid-forming ability caused by LKB1 loss. Furthermore, fibronectin (FN1) protein, an important extracellular matrix component, is significantly reduced in LKB1-deficient OVCAR8 spheroids. FN1 knockdown diminished compaction of parental OVCAR8 spheroids, which phenocopies the morphology of LKB1-deficient OVCAR8 spheroids. This suggests that LKB1 signalling may regulate FN1 to promote efficient spheroid formation and cell survival in these structures.

Lastly, we assessed the tumourigenic potential of HeyA8-*STK11*KO cells in an in vivo orthotopic metastasis model. Immunodeficient NOD-SCID mice inoculated i.p. with HeyA8-*STK11*KO cells exhibited significantly reduced intraperitoneal tumour burden compared to mice inoculated with LKB1-expressing HeyA8 control cells. This suggests that inhibition of LKB1 or its downstream targets involved in metabolic stress signalling in EOC cells may be a viable therapeutic strategy for metastatic EOC.

12. ENABLING PRIVACY-MAINTAINING NATIONAL ANALYSIS OF LOCALLY-CONTROLLED HEALTH DATA WITH THE CANDIG PROJECT

LJ Dursi (Centre for Computational Medicine, The Hospital for Sick Children), SJM Jones (Canada's Michael Smith Genome Sciences Centre, British Columbia Cancer Agency), G Bourque (McGill University and Génome Québec Innovation Center), TJ Pugh (Princess Margaret Cancer Centre, University Health Network), S Baker (Canada's Michael Smith Genome Sciences Centre, British Columbia Cancer Agency), Y Joly (Centre of Genomics and Policy, McGill University), PE Jacques, C Virtanen (Princess Margaret Cancer Centre, University Health Network), M Brudno (Centre for Computational Medicine, The Hospital for Sick Children)

TFRI/TFF Funding: None

In the research setting, identifying novel genes responsible for a genetic disorder often requires thousands of "cases" that are compared to healthy "controls" to identify genes enriched for functional variants. In the clinical setting, interpreting genetic variants of unknown significance is one of the major roadblocks for providing a definitive diagnosis for a disease. Interrogating the frequency of such variants across a large patient and population cohort is key to establishing their pathogenicity. In cancer research, specific mutations in a cancer may be targetable by matching them to therapeutic agents, however many patients with a mutation are typically required to power a clinical trial exploring the efficacy of a treatment. The ability to study these diseases grows with the number of samples, so that being able to inform research studies and clinical decision-making with data from cases across multiple hospitals and jurisdictions is thus important, especially for rare diseases and cancers.

To that end we are developing CanDIG, a national-scale fully distributed platform for privacy-maintaining analyses of locally-controlled data sets, connecting HPC4Health in Toronto, the McGill University and Genome Quebec Innovation Centre (MUGQIC) in Montréal, and Canada's Michael Smith Genome Science Centre (GSC) in Vancouver, and including representation from the three largest cancer centers in the country, with the BC Cancer Agency, Princess Margaret Cancer Center, and the Jewish Hospital of Montreal.

Our goal in developing CanDIG is to maximize the utility of genome sequences across Canada by building robust software for genomic analysis, connecting the computational infrastructure and data between Canadian genomics and HPC sites, and linking to additional data generated by clinicians and scientists around the world to allow Canadian scientists greater flexibility and power to analyze genomic data and generate testable biomedical hypotheses.

We describe our initial design of this national shared computation infrastructure that incorporates privacy controls, new databases, computational resources and tools to analyze and interpret massive sequence data to enable powerful new investigations of cancer and rare disease genomics, and how this underlying platform could support access to data and methods between partners within the TFRI and CanDIG network.

Conflicts of Interest: None

13. PSMA-TARGETED PHOTODYNAMIC LASER PRE-TREATMENT FOR FAST AND EFFICIENT NANOPARTICLE DELIVERY

Kara M. Harmatys[1-3], Marta Overchuk[1-3], Juan Chen[1-3], Martin Pomper[4], Gang Zheng[1-3]

[1]Princess Margaret Cancer Centre;
[2]University Health Network;
[3]University of Toronto;
[4]Johns Hopkins School of Medicine

Presenting author is a post-doctoral research fellow trainee. This work is supported by the TFRI.

One of the major barriers to effective cancer treatment is the inability to deliver sufficient amounts of drug to the tumour. Nanoparticles (NPs) provide a platform for high drug payload and delivery, but delivery is limited to the modest effects of the enhanced permeability and retention (EPR) effect. Recent developments for enhanced nano delivery employ a targeted monoclonal antibody conjugated to a photosensitizer.[1] When subjected to low-dose near-infrared (NIR) light, there is highly specific killing of perivascular cells, which increases vascular permeability and rapid drug leakage into the tumour interstitium. Antibody penetration into tumour tissue is limited due to its large size. Therefore, high-affinity small-molecule targeted photosensitizers could be more suitable agents due to their small size.

The Zheng lab has generated a small-molecule conjugate consisting of three building blocks: (1) A bacteriochlorophyll (BChl) NIR photosensitizer, (2) A peptide linker and (3) A high affinity anti-prostate specific membrane antigen (PSMA) inhibitor target ligand. PSMA is overexpressed in prostate cancer and widely explored in the clinic with an ^{18}F-labeled PSMA imaging agent currently in clinical trials.[2] BChl-peptide-PSMA was tested in combination with low-dose NIR laser treatment for the enhanced accumulation of nano agents to solid prostate tumours in mice. Initial studies using BChl-peptide-PSMA demonstrated significant in vitro specificity and selectivity as well as excellent tumour targeting to PSMA-positive tumours. A separate cohort of mice bearing dual PSMA-positive tumours was injected with BChl-peptide-PSMA probe 12 hours prior to low-dose light irradiation to one tumour. One hour after laser treatment, fluorescent lipid-containing NPs were injected intravenously and monitored for accumulation over time. Enhanced NP accumulation of the laser-treated tumour was observed in a rapid manner in comparison to the dark-control tumours, which rely strictly on the EPR effect.

The current demonstrated method can provide a solution for a more efficient delivery of nano agents that are unstable in vivo. Our lab recently published promising work on thermo-responsive photoacoustic NPs (J-porphysomes), which can serve as a model of a temperature-sensitive liposomal formulation.[3] The combination of the targeted Bchl-peptide-PSMA photosensitizer with low-dose laser treatment resulted in enhanced accumulation of intact J-porphysomes demonstrated by photoacoustic and fluorescence imaging. Future work will focus on more clinically-relevant NP drug delivery systems. Overall, enhanced NP delivery to solid prostate tumours was achieved using a small-molecule targeted photosensitizer in combination with low-dose NIR laser irradiation.

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14. CLINICAL STUDY AND ANALYSIS OF EX VIVO PHOTOACOUSTIC IMAGING IN ENDOSCOPIC MUCOSAL RESECTION TISSUES IN BARRETT'S ESOPHAGUS

Liang Lim[1], Catherine J. Streutker[2], Norman E. Marcon[2,3], Maria Cirocco[2], Alexandra Lao[2], Vladimir V. Iakovlev[2], Ralph Dacosta[1,3], Brian C. Wilson[1,3]

[1]Princess Margaret Cancer Centre, University Health Network, Toronto (Canada)
[2]St. Michael's Hospital, Toronto (Canada)
[3]University of Toronto, Toronto (Canada)

Presenting Author: Liang Lim is a postdoctoral fellow at Princess Margaret Cancer Centre / UHN.

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, and 850 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 concentration. 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. Based on our ex vivo data, changes in total hemoglobin content from intrinsic PAI (i.e. without exogenous contrast) can differentiate BE from squamous esophageal mucosa. However, most likely intrinsic PAI is unable to differentiate dysplasia from non-dysplastic BE with adequate sensitivity for clinical translation.

Conclusions: Intrinsic differences in microvascularity of dysplasia in BE does not allow adequate endoscopic detectability of intrinsic PAI, then the use of a photoacoustic contrast agent may be a useful alternative.

Keywords: Photoacoustic imaging, esophageal cancer, Barrett's Esophagus

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Conflict of Interest: The authors certify that they have NO affiliations or involvement in any organization or entity with any financial interest in the subject matter or materials in this manuscript.

15. REAL TIME IMAGING OF LYMPH NODE METASTASIS REVEALS EZRIN AS A TARGET IN SUPPRESSING INVASION AND IMMUNE EVASION IN CANCER

Abdi Ghaffari[1,4], Victoria Hoskin[1,4], Gulisa Turashvili[1], Sonal Varma[1], Jeff Mewburn[4], Graeme Mullins[1,4], Peter A. Greer[1,4], Friedemann Kiefer[2], Andrew G. Day[5], Yolanda Madarnas[3], Sandip SenGupta[1], and Bruce E. Elliott[1,4*]

[1]Department of Pathology and Molecular Medicine, Queen's University, Kingston, Canada
[2]Max Planck Institute for Molecular Biomedicine, Münster, Germany
[3]Department of Oncology, Queen's University, Kingston, Canada
[4]Queen's Cancer Research Institute, Division of Cancer Biology and Genetics, Kingston, Canada
[5]Kingston General Hospital Research Institute, Kingston, Canada

Note: Dr. Abdi Ghaffari, the presenting author, is a senior postdoctoral fellow.

The tumour-draining lymph node (TDLN) is the first site of metastasis in most cancers including breast (BC). However, the fate of cancer cells after arriving in the TDLN remains largely unknown partly due to a lack of adequate pre-clinical models of metastasis. Using a novel quantitative intravital microscopy (qIVM) model in lymphatic reporter mice (prox1-mOrange2-pA-BAC), we show that cancer cells (GFP-EO771^{lmv}) retain the ability to disseminate, primarily in a single-cell amoeboid-like mode of migration, after arrival at the inguinal TDLN. We demonstrate that inhibition of ezrin (NSC668394 inhibitor), a cytoskeletal-membrane linker and pro-metastatic protein, halts the invasion of motile cancer cells within the TDLN and reduces the metastatic load in the draining axillary node. In support of our preclinical finding, retrospective analysis of tumours in high-risk node positive BC patients revealed a significant association between elevated ezrin expression and reduced disease-free survival (HR=2.7, Cox Reg. p=0.025, adjusted for tumour size & grade). As TDLN plays a crucial role in mediating immune tolerance and evasion, we next explored the effect of ezrin-targeted therapy on T cell function in our intravital model. Mice treated with ezrin inhibitor displayed an increase in $CD8^+$ T cell activation and engagement with GFP-expressing cancer cells in the TDLN. T cell tracking, labeled by eFluor660-CD3 antibody, in lymphatic vessels and inguinal node revealed minimal effect on T cell homing and migration efficiency in mice treated with ezrin inhibitor. In conclusion, findings presented here provide evidence that ezrin inhibition suppresses cancer cell invasion and evasion from cytotoxic T cells and support the development of novel ezrin-targeted therapeutics to improve disease-free survival in high-risk BC patients with elevated tumour ezrin expression.

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16. HIGH EZRIN EXPRESSION IS ASSOCIATED WITH POOR RESPONSE TO CHEMOTHERAPY IN BREAST CANCER

Victoria Hoskin*, PhD [1,2], Abdi Ghaffari, PhD [1, 2], Sonal Varma, MD [2], Yolanda Madarnas, MD [3], Sandip SenGupta, MD [2], and Bruce E. Elliott, PhD[1,2].

[1]Division of Cancer Biology and Genetics, Cancer Research Institute;
[2]Department of Pathology and Molecular Medicine;
[3]Department of Oncology, Queen's University

** Victoria Hoskin is a Postdoctoral Fellow in Bruce Elliott's laboratory*

Resistance to chemotherapy is a significant clinical challenge which limits the efficacy of modern systemic treatments for cancer patients. Therefore, identifying markers of chemoresistance is important for the development of novel treatment strategies aimed at overcoming resistance. Ezrin is a metastasis-associated protein and is known to promote pro-survival signals in cancer cells. However, the role of ezrin in chemoresistance in breast cancer (BC) has not yet been elucidated. In this study, we show that altering ezrin expression changes the sensitivty of BC cells to the anthracycline, doxorubicin. Over-expression of ezrin in BC cells with endogenously low ezrin levels (MCF-7), markedly increased resistance, whereas depleting ezrin in BC cells with endogenously high ezrin levels (ZR-75-1), resulted in increased sensitivity to doxorubicin treatment. Using a novel ezrin inhibitor, NSC668394, we show that ZR-75-1 BC cells exhibit reduced cell viability when treated with NSC668394, and that ezrin inhibition synergistically enhanced cytotoxicity induced by doxorubicin in these cells. To assess whether ezrin expression is associated with response to chemotherapy in BC, we performed automated quantitiative analysis (Halo™ platform) of ezrin expression by immunohistochemistry staining (IHC) in a BC tissue microarray (TMA) consisting of a locally acrrued cohort (n=347). Multivariate Cox regression analysis revealed that elevated tumour ezrin expression is associated with increased risk of relapse (HR: 3.11, 95% CI 1.31-7.40, p=0.0099) and poor overall survival in (HR: 4.06, 95% CI: 1.41-11.66, p=0.0092) in BC patients who receive standard chemotherapy. Taken together, our results suggest that high ezrin levels are associated with chemotherapy resistance in vitro and that ezrin may hold promise as a potential predictive marker for response to chemotherapy in BC.

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17. CHARACTERIZATION OF A UNIQUE BMIC POPULATION IN HUMAN BRAIN METASTASES

Mohini Singh[1], Chitra Venugopal[1], Nicole McFarlane[1], Minomi Supapanditha[1], David Bakhshinyan[1], Maleeha Qazi[1], Parvez Vora[1], Chirayu Chokshi[1], Tomas Tokar[2], Igor Jurisica[2] and Sheila Singh.

[1]Stem Cell & Cancer Research Institute, McMaster University, Hamilton Ontario, Canada
[2]IBM Life Sciences Discovery Centre, Toronto Medical Discovery Centre, Toronto Ontario, Canada

** Presenting author is a trainee (Doctoral student)*

Brain Metastases (BM) are the most common adult cerebral tumour, occurring 10 times more than that of primary brain cancers. The inherent abilities of a primary tumour cell capable of initiating a BM resembles that of a cancer stem cell (CSCs). Previous work in our lab identified a population of CSCs within lung-derived brain metastases, termed brain metastasis-initiating cells (BMICs), that are responsible for the initiation of BM, identifiable by an exclusive subset of genes that regulate self-renewal and metastasis. Despite the prevalence and lethality of BM, there is no clinically relevant model that fully reflects metastasis in patients. We have generated a novel human patient-derived xenotransplantation (PDX) model of BM that allows for interrogation of the metastatic cascade from lung to brain, through injection of human GFP-expressing BMICs into immunocompromised mice via three routes: intracranial (ICr), intrathoracic (IT), and intracardiac (ICa). GFP+ cells were isolated from the lungs and/or brains from each injection route, and cells were minimally cultured *in vitro* for further characterization as well as RNA was submitted for microarray analysis to identify unique metastatic regulatory genes. *In vitro* these cells display characteristic differences for proliferation, sphere formation, migration and expression of CSC markers. Intriguingly, we identified substantial differential gene expression in the ITB BMICs as compared to BMICs collected from other sites. Future work will validate the role of select genes/metastatic signature in our established PDX models, and determine their potential as therapeutic targets and/or as predictive biomarkers of lung-to-brain metastasis in prospective cohorts of newly diagnosed lung cancer patients.

18. MODELLING OVARIAN SEROUS CARCINOMA IN VIVO USING FALLOPIAN TUBE EPITHELIAL CELLS

Ramlogan Sowamber[1], Sophia HL George[2], Noor Salman[1], Zahra Maamir[1], Nick Chauvin[1], Mahmoud Bitar[1]and Patricia Shaw[1].

[1]Campbell Family Institute for Breast Cancer Research , Princess Margaret Cancer Center, University Health Network, Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada,
[2]University of Miami, Sylvester Comprehensive Cancer Center, Miami, FL5

Introduction: Epithelial ovarian cancer consists of multiple histotypes with differing morphological, molecular genetics, and clinical features. The most common and aggressive histotype is High Grade Serous Carcinoma (HGSC) which accounts for 90% of deaths. Furthermore, the risk of developing HGSC is exacerbated by having a mutation in the BRCA1/2 gene. There is considerable evidence detailing the fallopian tube epithelium (FTE) as the site of origin of the disease yet the Ovarian Surface Epithelium (OSE) is still proposed as the site of origin by some. Few studies have modelled the disease *in vivo/xenograft*) using fallopian tube epithelial cells. We suggest that *in vivo/xenograft* models can prove as a useful tool for understanding the origin of the disease taking into account the genetic characteristics of the disease.

Methodology: Immortalized and transformed cell lines, from both FTE and OSE, were derived from both BRCA1 and BRCA2 mutation carriers and non-carriers. In particular, 16 OSE cell lines were created –3 from BRCA1 carriers and non-carriers. Cell lines were transfected with hTERT (ht) and SV40, and either vectors over-expressing cMYC, hRASV12 or PIK3CA-H1047R. *In vitro* assays were performed to compare proliferation, anchorage-independent growth and invasion between 2 BRCA1 (iOSE 267F and iOSE 592F) and 2 non-BRCA cell line over expressing hTERT and SV40 and either cMYC or hRASV12. All 12 lines showed proliferation and anchorage-independent growth. 8 of these lines with cMYC or hRASV12 were injected into the mammary fat pad of 6 week old female NSG mice (NOD.Cg-Prkdcscid). Similarly, 16 FTE lines were generated from 3-BRCA1/2 mutation and 3-non-BRCA carriers over-expressing hTERT and SV40 and either cMYC or hRASV12. As with iOSE lines, 8 of the FTE lines were injected in the mammary fat pad of NSG mice and were monitored for tumour development. In addition to FTE cells with SV40 hTERT, 4 additional FTE cell lines were generated with TP53-R175H, E7, and hTERT. These lines were subsequently transfected with either PIK3CA-H1047R, hRASV12, cMYC and/or CCNE1 and injected into NSG mice at the mammary fat pad and intraperitoneal.

Results: To date, tumors have developed from mice injected with ovarian surface epithelial cells and fallopian tube epithelial cells at the mammary fat pad and the intraperitoneal cavity. FTE cells injected intraperitoneally produced ascites and displayed tumors lining the abdominal cavity and major organs. Multiple tumour nodules were identified surrounding the uterine horn and within the mesocolon. The parental immortal lines of 4 cases were karyotyped: FTE-3437 and FTE-3619 (controls); FTE-3313 (BRCA2 mutant) and FTE-3798 (BRCA1 mutant). The FTE BRCA mutant cell lines demonstrated varying types of tetraploidy whilst the control (BRCA wild type) had fewer tetraploid chromosomes. It is hypothesized that SV40 alone is sufficient to stimulate genomic instability in the FTE cells. Several cell lines grown in culture with expression of hRASV12 demonstrated increased proliferation relative to normal control cells.

Conclusions: Our results suggest that tumour development occurs at different rates in-vivo and is dependent on multiple factors including genetic mutation, expression of oncogenes, and patient-derived characteristics. We demonstrate that cells grown in culture exhibit unstable chromosomal architecture characteristic of ovarian carcinomas which is one of many potential factors that promotes neoplastic change of the tissue. Understanding the role that these factors play promoting tumourigenesis will enable us to characterize the molecular changes that occur in-vivo prior to the development of ovarian serous carcinoma.

19. PROTEOMIC AND STRUCTURAL INVESTIGATION OF PHOSPHORYLATION-DEPENDENT INTERACTIONS IN THE CORE MAMMALIAN HIPPO PATHWAY

Amber L. Couzens[1*], Shawn Xiong[1,2,*], Daniel Y. Mao[1], James D.R. Knight[1], Sebastian Guettler[1,3], Sarah Picaud[4], Panagis Filippakopoulos[4,5], Anne-Claude Gingras[1,6,**] and Frank Sicheri[1,6,**]

[1]Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, Ontario, Canada, M5G 1X5
[2]Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada, M5S 1A8
[3]New address: The Institute of Cancer Research, Divisions of Structural Biology and Cancer Biology, London, UK, SW7 3RP
[4]Structural Genomics Consortium, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Oxford OX3 7DQ, U.K.
[5]Ludwig Institute for Cancer Research, University of Oxford, Oxford OX3 7DQ, U.K
[6]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada, M5S 1A8

** These authors contributed equally*

The Hippo tumour suppressor pathway functions to regulate organ size and tissue homoeostasis in response to diverse signal inputs. The core of the pathway consists of a short kinase cascade: MST1 and MST2 phosphorylate and activate LATS1 and LATS2 that in turn phosphorylate key transcriptional co-activators. Importantly, the scaffolding proteins MOB1A and MOB1B associate with both kinase families and participate in pathway activation. Recent structural studies have shed light on the mechanisms responsible for interactions between MST1 (or MST2), MOB1 and LATS family kinases, revealing the key contribution of phosphorylation-dependent associations, notably through a phosphopeptide-binding pocket on MOB1. Yet, the significance of these findings has not been fully explored in a cellular context. Furthermore, our previous interaction proteomics analysis revealed that besides associating with MST1 (and MST2), MOB1A and MOB1B can associate in a phosphorylation-dependent manner with at least two other signaling complexes, one containing the Rho guanine exchange factors (DOCK6–8) and the other containing the serine/threonine phosphatase PP6. Whether these complexes are recruited through the same mode of interaction as MST1 and MST2 remains unknown. Here, we performed a comprehensive series of biochemical, proteomics, biophysical and structural analyses on MOB1 and its protein kinase interactors. These studies revealed an unsuspected robustness in the autophosphorylation-driven recruitment of MST1 and MST2 to MOB1. Our studies also uncovered novel details of the mechanism of activation of LATS kinases by MOB1 and a feedback loop that causes the subsequent dissociation of the upstream kinases MST1 and MST2 from MOB1. Lastly, using mutational analysis and interaction proteomics, we discovered that the phosphorylation-dependent recruitment of PP6 phosphatase and Rho guanine exchange factor protein complexes differs in key respects from that elucidated for MST1 and MST2. Taken together, our studies confirm and extend previous work to define intricate regulatory steps in a critical tumour suppressor pathway.

20. IDENTIFYING GENES THAT COOPERATE WITH MUTANT TRP53 AND PIK3CA IN MOUSE MAMMARY TUMORS

Nathan F. Schachter[1,2], Jessica R. Adams[2], Katelyn Kozma[2], Patryk Skowron[2], Sorana Morrissy[2], Livia Garzia[2], Adam J. Dupuy[4], Michael D. Taylor[2,3], Sean E. Egan[1,2]

[1]Molecular Genetics, University of Toronto, Toronto, ON, Canada;
[2]Developmental & Stem Cell Biology,
[3]Arthur and Sonia Labatt Brain Tumour Research Center, SickKids Hospital, Toronto, ON, Canada;
[4]Anatomy & Cell Biology, University of Iowa, IA, United States of America.

Introduction: In Canada, Breast cancer (BC) is the most frequently diagnosed malignancy and second leading cause of cancer-related deaths in women. Studies show that *TP53* (which codes for the p53 transcription factor) and *PIK3CA* (coding for the p110α catalytic subunit of PI3K) are the most frequently mutated genes in BC. However, genetic alterations that cooperate with these driver events remain largely unknown.

Methods: To identify cooperating mutations, we performed a transposon-based Sleeping Beauty (SB) cancer gene discovery 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 *Trp53*$^{LSL\text{-}R270H}$, *Rosa26*$^{LSL\text{-}Pik3ca(mut)}$, or neither allele, we sought to distinguish genes preferentially altered by transposons in the presence of mutant-p53 or hyperactive-PI3K. Thus far, over 400 tumors have been collected for analysis. Ligation-mediated PCR combined with next-generation sequencing has identified genes repeatedly targeted for mutagenesis by transposons.

Results: SB mice with Trp53$^{LSL\text{-}R270H}$ or R26$^{LSL\text{-}Pik3ca(mut)}$ alleles displayed significant acceleration of tumor development and increased penetrance relative to controls. Using transposon insertion site analysis, we identified 40 and 84 genes that were recurrently, clonally altered in p53 and PI3K-mutant SB mammary tumors, respectively. Of note, there was considerable dissimilarity between genes targeted for mutagenesis in the presence of mutant *Trp53, Pik3ca*, and control SB tumors. This supports the notion that driver mutations select for highly specific secondary cooperating alterations. In addition to many background-specific mutations, insertion site analysis also revealed several genes that were targeted for mutation in multiple environments (e.g. *Rasa1, Met*). Interestingly, these data suggested *Notch1* activation cooperates with both *Pik3ca* and *Trp53* mutations. This result was validated *in vivo* using transgenic *R26L*$^{SL\text{-}Notch1(ICD)}$ mouse strains.

Conclusions: SB mutagenesis has identified candidate cooperators of mutant *Trp53* and *Pik3ca* mutation in mammary tumorigenesis. Guided by these results, we have demonstrated that elevated *Notch1* signaling cooperates with both driver mutations *in vivo*.

Outcome/Impact: Validating other cooperative mutations uncovered by our screen is expected to yield novel therapeutic targets for treatment of aggressive BCs.

21. OVERCOMING THE COMPLEXITY OF MOLECULAR TRANSPORT IN NEOPLASTIC TISSUE: USING A DYNAMIC ANALYSIS OF FAZA-PET IMAGING TO QUANTIFY HYPOXIA IN HUMAN TUMOURS

E. Taylor[1,2*], J. Gottwald[1,3], I. Yeung[1,4], H. Keller[1,4], M. Milosevic[1,4], N. C. Dhani[1,5], D. W. Hedley[1,3,5], D. A. Jaffray[1,2,3,6]

[1]Princess Margaret Cancer Centre, University Health Network, Toronto, Canada
[2]Techna Institute, University Health Network, Toronto, Canada
[3]Department of Medical Biophysics, University of Toronto, Toronto, Canada
[4]Department of Radiation Oncology, University of Toronto, Toronto, Canada
[5]Division of Medical Oncology and Hematology, Princess Margaret Cancer Centre, Toronto, Canada
[6]Institute for Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada

**Presenting author is a postdoctoral fellow*

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There is undisputable clinical evidence that hypoxia is a major determinant of patient outcome following radiation treatment, chemotherapy, and surgery. Despite this, hypoxia-targeted therapies including molecular agents and intensity-modulated radiation therapy have not yet entered widespread clinical practice in large part because there is no widely accepted method to reliably detect and locate hypoxia non-invasively in human tumours. Positron emission tomography (PET) imaging of ^{18}F-labeled hypoxia-sensitive tracer molecules such as fluoroazomycinarabinoside (FAZA) is a promising way of accomplishing this. These tracers are slow to bind, however, resulting in a much smaller uptake than arises in FDG-PET. This means that FAZA uptake is sensitive not only to hypoxia in the tumour, but also physiological features—collagen content, perfusion, and non-vascular drug efflux channels—that impact transport of these molecules. Recent work [1, 2] carried out at Princess Margaret Cancer Centre on hypoxia detection in ductal pancreatic adenocarcinomas has discovered complex tracer kinetics in these tumours, confounding the quantification of hypoxia using standard nuclear medicine metrics (e.g., SUVs). To overcome this, we have developed a novel dynamic PET imaging scheme that corrects for the transport properties of pancreatic tumour tissue and hence, we believe, is more sensitive to hypoxia. Future work will further validate our approach by comparing with histological assessments of hypoxia in resected patient tumours.

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22. EPR MEDIATED TUMOUR HYPOXIA REDUCTION VIA PFOB NANOEMULSION ADMINISTRATION WITHOUT CARBOGEN BREATHING

Yun Xiang[1](PDF), Nicholas Bernards[2], Bryan Hoang[1,2], Jinzi Zheng[2,3], and Naomi Matsuura[1,3]

[1]*Department of Medical Imaging, University of Toronto, Ontario, Canada*
[2]*TECHNA Institute for the Advancement of Technology for Health, University Health Network, Toronto, Ontario, Canada*
[3]*Institute of Biomaterials and Biomedical Engineering /Materials Science & Engineering, University of Toronto, Ontario, Canada*

Abstract: Hypoxia is a common feature of prostate cancer and is correlated with increased tumour aggressiveness and reduced response to radiotherapy (RT). Therefore, the reoxygenation of hypoxic tumours can offer an effective way to increase the efficacy of RT. A direct method to oxygenate hypoxic tumours using perfluorocarbon (PFC) droplets as non-toxic oxygen carriers in the blood circulation was evaluated in phase I/II trials in the 1990s. However, no clear benefit was observed, potentially compromised by the use of these non-optimal first-generation PFC agents in combination with the inability to independently assess tumour hypoxia in real-time. Later, second-generation PFC agents composed of perfluorooctylbromide (PFOB, $C_8F_{17}Br$) with higher oxygen capacity and emulsion stability demonstrated increased radiosensitization in preclinical hypoxic tumours in combination with carbogen breathing.

Recent studies suggest that increased PFC droplet radiosensitization depends on the timing of RT delivery. We hypothesize that RT delivery should coincide with the maximum PFC accumulation in the tumour and that a simple model can predict the increased tumour radiosensitivity through trapped PFC. Here, we used Krogh cylinder model to simulate the oxygen diffusion from the microvasculature into the tumour tissue. The influence of PFC agents in circulation, the carbogen breathing, and the PFC agents accumulated in the tissue were evaluated by this model. To compare the results of this model with experiment, PFOB nanoemulsions were prepared and injected in a radioresistant mouse model with xenograft DU145 prostate tumours. A validated positron emission tomography (PET) agent, [^{18}F]-FAZA, was used to non-invasively assess the hypoxia level of the tumours inoculated in the same mouse model.

The mathematical model shows that the accumulation of PFOB nanoemulsions in the tumour tissue increases the effective diffusivity of oxygen, pushing the boundary of radial oxygen diffusion distance further into the tumour. Moreover, this improved diffusion distance is comparable to the one achieved by carbogen breathing. In the experimental set-up, nanosized, stable PFOB nanoemulsions were produced with/without fluorescent labeling and gradually accumulated in tumour tissues over a 24 hours period following the *i.v.* injection, while in other organs the PFOB concentration stabilized after 6 hours. [18F]-FAZA-PET imaging 24 hours post-injection of the PFOB emulsion showed a significant reduction in tumour hypoxia levels. The level of hypoxia reduction was the same as that achieved by animals breathing carbogen alone. This result was consistent with the prediction from the mathematic model, and proved the potential of PFOB nanoemulsions to improve the radiotherapeutic effect when accumulated in the tumours, without the use of administration of carbogen.

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23. LYSINE-52 STABILIZES THE MYC ONCOPROTEIN THROUGH A SCFFBXW7-INDEPENDENT MECHANISM

Jason De Melo[1*], Sam Kim[1], Corey Lourenco[1,2], Linda Z. Penn[1,2].

[1]Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada
[2]Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

** Jason De Melo is a post-doctoral fellow in the laboratory of Dr. Linda Penn*

The oncogenic transcription factor c-MYC (MYC) is deregulated, and often overexpressed, in more than 50% of cancers. MYC deregulation is associated with poor prognosis and aggressive disease, suggesting that the development of therapeutic inhibitors targeting MYC would dramatically impact patient care and outcome. MYC is a highly regulated transcription factor, with a protein and mRNA half-life of approximately 30 min. The most extensively studied pathway regulating MYC protein stability involves ubiquitylation and proteasomal degradation mediated by the E3-ligase, SCF^{Fbxw7}. Here we provide evidence for a SCF^{Fbxw7} independent regulatory mechanism centered on Lysine 52 (K52) within MYC Box I (MBI) of the MYC protein. This residue has been shown to be post-translationally modified by both ubiquitylation and SUMOylation, hinting at the interplay of post-translational modifications at this site, and the importance of this residue. We demonstrate that mutation of K52 to arginine (R) renders the MYC protein more labile. Mechanistically, we show that the degradation pathway regulated by K52 is independent of the Cullin-Ring-Ligase (CRL) family of E3-ligases, which includes not only the canonical SCFFbxw7, but also a number of other known MYC-targeting E3-ligases, such as SCF^{Skp2}, $SCF^{\beta TCRP}$, $SCFF^{bxo28}$ and DCX^{TRUSS}. Taken together, our data identifies a novel regulatory pathway centred on K52 that may be exploited for the development of anti-MYC therapeutics.

Key Words: MYC, Fbxw7, Post-translation Modification, Degradation, Cancer,

24. PLERIXAFOR ABROGATES TREATMENT–INDUCED INCREASES IN CXCL12 SIGNALING AND IMPROVES PRIMARY TUMOUR RESPONSE IN CERVICAL CANCER TREATED WITH RADIO-CHEMOTHERAPY

Naz Chaudary PhD [1*], Melania Pintilie MSc[2,4], Pratibha Thapa MSc[1],Magali Lecavalier MD[3,5,6], Kathy Han MD[3,5,6], Richard P. Hill PhD[1,3,4,5], and Michael Milosevic, MD[3,5,6]

[1]University Health Network, Princess Margaret Cancer Centre and Campbell Family Institute for Cancer Research,
[2]Department of Biostatistics, University Health Network and Princess Margaret Cancer Centre,
[3]Radiation Medicine Program, University Health Network and Princess Margaret Cancer Centre,
[4]Department of Medical Biophysics, University of Toronto,
[5]Department of Radiation Oncology, University of Toronto,
[6]Institute of Medical Sciences, University of Toronto

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Purpose: There is an important need to improve the effectiveness of radio-chemotherapy (RTCT) for cervical cancer. The CXCL12/CXCR4 pathway may be upregulated by RT and contributes to both treatment resistance and the development of metastases. The objectives of this study were: 1. To explore different ways of sequencing RTCT and the CXCR4 inhibitor Plerixafor to maximize efficacy, building on our previous work that showed enhanced tumour growth delay with RTCT and Plerixafor administered concurrently; and 2. To evaluate biomarkers of response to RTCT and Plerixafor.

Methods: Primary cervical cancer xenografts (OCICx 20) were implanted in the cervixes of mice and treated with RTCT (30 Gy in 15 daily fractions + weekly cisplatin 4 mg/kg) with or without Plerixafor (5 mg/kg/day). This RTCT protocol was designed to mirror the clinic as closely possible. Plerixafor was administered concurrently with RTCT for 3 weeks, adjuvantly after RTCT for 3 weeks or continuously (concurrently and adjuvantly) for 6 weeks. Mice were followed after treatment with serial CT imaging to monitor tumour regrowth. In a separate experiment, mice were treated in the same way with RTCT and concurrent Plerixafor, but sacrificed immediately at the end of treatment for biomarker assessment.

Results: The combination of RTCT and Plerixafor produced substantial tumour growth delay compared to RTCT alone regardless of sequencing. However, the continuous and adjuvant Plerixafor arms were associated with significantly longer growth delay than the concurrent only arm. RT alone and RTCT alone caused significant increases in tumour pCXCR4 expression relative to controls, consistent with a treatment-induced increase in signaling via the CXCL12/CXCR4 pathway. Plerixafor had no effect on pCXCR4 expression in untreated tumours. However, the addition of Plerixafor to RTCT significantly reduced pCXCR4 levels and abrogated the treatment-induced increase in CXCL12/CXCR4 signaling seen with RTCT alone. There also was a reduction in pERK expression with RTCT + Plerixafor relative to RTCT alone, consistent with a downstream effect of CXCL12/CXCR4 inhibition.

Conclusions: RTCT increases signaling via the CXLC12 pathway in cervical cancer. The addition of Plerixafor concurrent with RTCT offsets this increase and improves primary tumour control. Adjuvant Plerixafor after RTCT may further improve disease control. Plerixafor is commercially available for other indications, which will facilitate rapid translation of these findings to phase I/II clinical trials.

25. ANTI-CANCER DRUG DEVELOPMENT TO MODULATE HIPPO PATHWAY FUNCTION

Dohee Lee[1], Daniel Mao[1], Gerald Gish[1], Rima Al-awar[4], David Uehling[4], Shawn Xiong[1,2], Alessandro Datti[1], Jeff Wrana[1,3] and Frank Sicheri[1,2,3]

[1]*Lunenfeld-Tanenbaum Research Institute, Toronto, Canada*
[2]*Department of Biochemistry, University of Toronto, Canada*
[3]*Department of Molecular Genetics, University of Toronto, Canada*
[4]*Ontario Institute for Cancer Research, Toronto, Canada*

Under normal conditions, a cell receives signals from its surrounding cells that give instructions on when to start or stop growing. This process is tightly regulated and is controlled by the Hippo pathway. The Hippo pathway comprises a conserved kinase cassette in which MST1/2 activate Lats1/2 that in turn phosphorylate YAP1/TAZ to drive their cytosolic sequestration. This inhibits their transcriptional regulatory activity. When the Hippo pathway is off, YAP1/TAZ translocate into the nucleus to interact with their transcription factor, the TEAD family of DNA binding proteins and drive a pro-tumourigenic transcriptional program.

In most cancers, YAP1/TAZ is either amplified or activated due to inactivation of the Hippo pathway. Thus, the reduction of YAP1/TAZ activity has been recognized as a potential anti-cancer therapy. Currently, no effective and target specific drugs are available. Therefore, our aim is to develop an effective, safe and target specific drug that inhibits the YAP1-TEAD interactions to stop the cancer.

We developed a stable cyclic peptide (LTRI10) that effectively inhibits the YAP1-TEAD interaction with an IC_{50} of ≈10uM. Efforts at optimizing this cyclic peptide for potency are ongoing. Also, we are using the SMART robotics platforms at the LTRI to conduct high-throughput screening to discover novel compounds that interfere with YAP1-TEAD interaction. Our early stage efforts have identified hits that are being confirmed using orthogonal assays.

Ultimately our effort will guide medicinal chemistry efforts aimed at designing and optimizing the small molecule modulators of Hippo pathway function.

The presenting author, **Dohee Lee** is a post-doctoral fellow at LTRI.
This project is supported by TFRI.

26. β-CATENIN/WNT ACTIVATION AS A MECHANISM FOR SELF-RENEWAL: A MODEL FOR UNDERSTANDING INTER- AND INTRA-TUMOURAL HETEROGENEITY IN GLIOBLASTOMA

Katherine J. Rowland[1,2], Nishani Rajakulendran[5], Hayden J. Selvadurai[1,2], Nicole Park[1,2,4], Milly So[1,2], Ryan J. Ward[1,2], Lilian Lee[1,2], Renee Head[1,2], Michelle Kushida[1,2], Ian Clarke[1,2], Nizar Batada[6], Stephane Angers[5], Peter B. Dirks[1,2,3,4]

[1]Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, Ontario, Canada.
[2]Arthur and Sonia Labatt Brain Tumour Research Center, Hospital for Sick Children, Toronto, Ontario, Canada.
[3]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.
[4]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada,
[5]Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada,
[6]Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

Glioblastoma (GBM) is the most common adult brain tumour and continues to be a significant cause of mortality among affected patients due to ineffective therapies. The poor prognosis is due in part to a propensity for tumour recurrence despite conventional treatment. Such relapse may be attributed to residual cancer stem cells, a subpopulation of GBM cells with stem-like characteristics exclusively capable of tumour initiation and maintenance. Activating mutations in the canonical Wnt/β-catenin (cWnt) pathway leading to dysregulated cWnt signaling has been described in various human cancers. Despite studies that examine cWnt signaling pathway in glioblastoma stem (GNS) cells, few extensively report on its regulation of self-renewal, differentiation and tumourigenicity in human GNS cells. To that end, we have isolated and characterized GNS cells from numerous human GBM samples. To determine the requirement of autocrine cWnt signaling for GNS cell renewal we inhibited Wnt secretion, using the porcupine inhibitor LGK974, and measured self-renewal using a limited dilution assay. There was a significant reduction in GNS cell frequency with LGK974 (1uM) in four out of eight lines tested and RNAseq analysis suggests that GBMs that fall under the Proneural subtype may be sensitive to Wnt inhibition. To consider the heterogeneity of cells within a given tumour, GNS cells were transduced with a cWnt/β-catenin activity reporter (BAR) construct and mice were engrafted orthotopically. GBM cells with the highest cWnt pathway activity had increased self-renewal and proliferation as compared to GBM cells with lowest cWnt activity. Subsequent analyses were performed to assess self-renewal following treatment with Wnt activators (Wnt3a, GSK3 inhibitors) on freshly dissociated cells from patient GBMs and GNS cell-derived xenografts. Surprisingly, Wnt3a treatment enhanced self-renewal of cWnt pathway-high xenograft cells, while BIO and other GSK3 inhibitors reduced self-renewal, relative to vehicle-treatment. Lastly, xenografts derived from GNS cells transduced with a dominant-negative mutant of *TCF7L2* showed reduced tumourgenicity and self-renewal as compared to control xenograft-derived cells. Therefore, targeting cWnt signaling may represent an effective therapeutic approach to suppress tumourigenicity in a subset of GBM.

Presenting author is a post-doctoral fellow.

27. IMAGE-GUIDED FOCAL PHOTOTHERAPY OF PANCREATIC CANCER USING PORPHYRIN-LIPID NANOPARTICLES

Christina M. MacLaughlin,[a,b,c] Lili Ding,a Cheng Jin,[a,b,d] Pinjiang Cao,[a] Iram Siddiqui,[a] David M. Hwang,[e] Juan Chen,[a] Brian C Wilson,[a,b,c] David W. Hedley,[a,b,c] Gang Zheng[a,b,d]

[a]*University Health Network, Princess Margaret Cancer Centre, Toronto ON*
[b]*Department of Medical Biophysics, University of Toronto, Toronto ON*
[c]*Department of Medical Oncology and Hematology, Princess Margaret Hospital, Toronto ON*
[d]*Department of Pharmaceutical Sciences, University of Toronto, Toronto ON*
[e]*Department of Pathology, University Health Network, Toronto ON*

**Corresponding author: gang.zheng@uhnres.utoronto.ca*

Administering safe and effective therapy is a major challenge in pancreatic cancer due to the frequent location of tumours in the head of the pancreas and resultant juxtaposition with major vasculature, nerves, and vital organs. First line treatments including surgical resection and radiotherapy are frequently not possible due to the risk of nonspecific damage to surrounding tissues. In fact, >30% of pancreatic cancer-related mortality result from an inability to control primary tumour burden, highlighting the need for novel focal treatment strategies. Herein, we investigate the feasibility of porphyrin-lipid nanoparticle-mediated photothera pies for focal ablation of pancreatic tumours, evaluated using a clinically-relevant primary orthotopic pancreas xenograft model. Porphyrin-lipid nanoparticles have been shown to specifically accumulate in tumours via the enhanced permeability and retention effect. In this study we investigated two porphyrin-lipid nanoparticles: porphysomes for photothermal therapy (PTT) and porphyrin lipoproteins for photodynamic therapy (PDT). Porphysomes are ~120 nm diameter liposome-like nanoparticles that are composed of ~80,000 porphyrin-lipids. Their large NIR absorption cross-section and physical stability enabled local generation of heat, raising the pancreatic tumour tissue temperature above ~55-70°C to cause coagulative necrosis within seconds. Interestingly, we observed a strong "heat-sink effect" in the well-perfused pancreatic tumours during PTT, which may prove important for planning thermal dosimetry. Porphyrin lipoproteins are small ~30 nm diameter HDL-mimetic nanoparticles loaded with porphyrin-lipid, that are readily taken up by pancreatic tumour cells. The released monomeric porphyrin-lipid is highly fluorescent and a strong photosensitizer, which enabled PDT of the pancreatic tumours under intraoperative fluorescence endoscopy guidance. In both PDT and PTT studies, porphyrin-lipid nanoparticles were shown to specifically accumulate within pancreatic tumour tissue, thereby reducing tumour burden without damaging surrounding normal tissues. Porphyrin-lipid nanoparticle-mediated photothera pies were demonstrated to be feasible strategies for managing primary tumour burden in pancreatic cancer.

This project is supported by a Terry Fox New Frontiers Program Project Grant, *Nanoparticle-enhanced photoacoustic imaging for cancer localization and therapeutic guidance*, led by Dr. Brian Wilson.

28. IDENTIFICATION OF GENES & PATHWAYS THAT UNDERLIE RESISTANCE TO ONCOLYTIC VIRUS INFECTION & KILLING THROUGH A GENOME WIDE LENTIVIRAL CRISPR SCREEN

Larissa Pikor[1], Jason Moffat[2], John C. Bell[1]

[1]Department of Cancer Therapeutics, Ottawa Hospital Research Institute, Ottawa, ON, K1H 8L6
[2]Donnelly Center for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, M5S 3E1

*** Presenting author is a trainee (Post Doctoral Fellow)*

Oncolytic virotherapy uses replication competent viruses to selectively kill tumour cells without damaging normal tissues. Oncolytic viruses (OVs) act through multiple mechanisms, including tumour cell lysis, destruction of tumour vasculature and perhaps most importantly, stimulation of long lasting anti-tumour immune responses. Despite the clinical successes of OVs, like the majority of cancer therapies efficacy is only achieved in a subset of patients, limiting their widespread application. In an attempt to better understand the intrinsic cellular factors that promote resistance to viral infection and/or limit anti-tumour responses, we performed a negative selection genome-wide lentiviral CRISPR-Cas9 knockout screen followed by treatment with Maraba-MG1 in a panel of five highly resistant lung and pancreatic cancer cell lines and normal bronchial epithelial cells. CRISPR library infected cells treated with either a low (0.1) or high (3) MOI were significantly more susceptible to MG1 infection and exhibited increased cell death relative to mock treated controls. Next-generation sequencing of surviving cell populations revealed depletion of numerous guide-RNAs (gRNAs) in OV treated cells and the MAGeCK-VISPR and BAGEL algorithms were used to identify genes specifically depleted following MG1 treatment in each cell line. This study will generate fundamental knowledge regarding the mechanisms that underlie the susceptibility of cancer and normal cells to OV infection. Importantly, these findings can be immediately applied to engineer more effective oncolytics or improve patient selection, leading to improved response rates and patient survival.

29. A DIGITAL PATHOLOGY ANALYSIS PIPELINE THAT BRIDGES HISTOLOGICAL MORPHOMETRIC ANALYSIS WITH IMAGING MASS CYTOMETRY AND MASS SPECTROMETRY TO CHARACTERIZE THE MOLECULAR COMPOSITION OF THE TUMOUR MICROENVIRONMENT

Trevor D. McKee1*, Jade Bilkey[1], Qing Chang[2#], Ravi Vellanki[1], Sehrish Butt[1], Mark Zaidi[1], Arash Zarrine-Afsar[3], Iram Siddiqui[1], David Hedley[1*], Bradly G. Wouters[1*], David A. Jaffray[1,3*].

*[1]Departments of Radiation Oncology, Medical Biophysics, and the STTARR Innovation Centre, Princess Margaret Cancer Centre, and [2]Fluidigm Canada Inc., Markham, Canada*Funded by TFRI PPG #COI Declaration: Dr. Chang is employee of Fluidigm Canada Inc. and [3]Techna Institute, University Health Network (UHN), Toronto, Canada*

Histological staining, interpreted by a pathologist, has remained the gold standard for cancer diagnosis and staging for over 100 years. The advent of molecular medicine has led to the use of companion diagnostics to direct the application of targeted therapeutics. These diagnostics require information on the multi-species molecular composition of the solid tumour, to identify tumour molecular subclasses that may benefit from such tailored therapeutic approaches.

Here, we discuss developments in digital pathology image analysis utilized for quantitative assessment of molecular species, including: brightfield immunohistochemical (IHC) and immunofluorescence (IF) staining, imaging mass cytometry (IMC), and desorption electrospray ionization mass spectrometry (DESI-MS) imaging, and combinations thereof. We have developed a quantitative analysis pipeline for IHC staining that includes colour deconvolution, cellular segmentation and stain quantification. This IHC analysis pipeline provides the advantage of preserved morphology of tissue, but at the expense of limited bandwidth, staining one species per section. Utilizing IF staining techniques, 3 to 6 co-stained species can be simultaneously assessed. IF analysis methods pair cellular segmentation with multiplexed cell co-localization, as well as an assessment of distance relationships between subspecies. IF imaging provides detailed molecular assessment of cellular phenotype, but lacks morphology information found in brightfield techniques. New developments in imaging mass cytometry have increased the bandwidth of simultaneous co-stained molecular species for routine assessment to 15-40 molecular species, using metal isotope-labeled antibodies and a commercial system for laser ablation and isotope quantification. This technology provides a useful balance between multiplexed molecular information, and morphological assessment accessed via total ion current and metal-containing histological staining preparations. It also provides highly quantitative counts of the uptake of isotopologous heavy metal-tagged probes for assessment of tumour hypoxia and drug delivery. We have developed novel analysis algorithms that take advantage of the detailed co-occurrence of multiple molecular species to build a hierarchical assessment of the cellular and tissue sub-compartments. We have also developed methods to better understand the basis for variations in the abundance of cancer biomarker ions detected with DESI-MS imaging. Serial sections of hematoxylin and eosin (H&E) stained sections and unstained sections subjected to DESI-MS imaging, when aligned with one another, showed a correlation between measures of tissue density and DESI-MS image intensity. We discovered that we could utilize one specific measure of tissue density: cytoplasmic area – to normalize the effect of tissue density on the DESI-MS images, thus utilizing detailed cellular morphometric analysis from the H&E image to better interpret subtle heterogeneities in the abundance of cancer biomarkers within MS images. This set of quantitative analytical tools establish the foundation for a bridge between traditional morphometric assessment of tumour biopsies, and the detailed spatially resolved chemical and molecular content maps of each tumour, providing an invaluable toolkit for the discovery of cancer molecular subtypes and development of therapeutic interventions.

30. A COMPREHENSIVE MAP OF CRITICAL PATHWAYS AND NETWORKS IN CANCER STEM CELLS

Jeff C. Liu, Veronique Voisin, ChangJiang Xu, Ruth Isserlin, Gary D. Bader

Toronto Donnelly Centre, University of Toronto, 160 College Street, Toronto, ON, M5S 3E1

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.

31. EXPANSION OF BioID TOOLBOX FOR STUDY OF PROTEIN INTERACTOME IN PRIMARY CELLS

PAYMAN SAMAVARCHI-TEHRANI*, Anne-Claude Gingras

**postdoctoral fellow*

Supported by TFRI

The genesis of the hundreds of different cell types in the human body is the result of a highly orchestrated transcriptional program, which establishes a unique gene expression signature in each cell. This signature is a result of combinatorial binding of transcription factors (TF) to specific genomic loci and recruitment of epigenetic readers, writers and remodelers. These in turn result in the establishment of a cell type specific chromatin landscape with distinct loci of transcriptional activity or suppression. The application of genomic techniques is providing insight into overall chromatin architecture and TF genomic occupancy patterns. However, due to challenges in biochemical purification of intact chromatin associated complexes, there is limited understanding of the full gamut of proteins and complexes implicated in gene regulation.

A recently developed technique that can offer advancement in our study of transcriptional proteome is the proximity biotinylation approach, BioID. In this technique, a promiscuous biotin ligase (BirA*) fused to a bait protein can carryout in vivo biotinylation of the closely associated proteins. In the context of chromatin biology, this enables capturing interactions that occur in the nucleoplasm and on chromatin (an accompanying abstract by Lambert et al. demonstrates the application of BioID to defining complexes involved in acetyl lysine recognition). Yet, while BioID overcomes a number of technical hurdles associated with traditional biochemical purification techniques, it has so far been primarily used in Flp-In T-REx cell lines or immortalized cancer cell lines. However, there are various biological questions in which use of other cell types, or primary cells, will provide a unique opportunity to study specific biological processes. To facilitate our specific study of TF interactome in primary cells, we set out to expand the BioID toolbox to allow for its implementation in the diverse cell types of interest.

To enable these studies, we have added to the BioID toolbox a set of lentiviral vectors that will broaden the application of BioID to other cell models. We demonstrated that these viral BioID vectors are efficient in determining the proximity proteome across different primary cells and immortalized cell lines. We sought to benchmark the approach using a number of well-studied bait proteins such as LMNA, TUBB, nucleosomal protein H2B, and basal transcription factor TBP. We demonstrate that when used in the same cellular contexts as standard BioID vectors, they recover similar sets of proximity interactors, highlighting the utility of the powerful BioID approach to a wide range of new biological questions. This approach now allows us to address our specific biological questions regarding TF interactome implicated in cellular reprogramming and lineage specification.

32. SINGLE-CELL ASSAY OF HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPC) REVEALS DRAMATIC HETEROGENEITY OF HSC PROLIFERATIVE POTENTIAL AND IMPLICATES SPHINGOSINE-1-PHOSPHATE SIGNALING IN HSPC FATE DETERMINATION.

Olga I. Gan[1], Stephanie Xie[1], Kerstin B. Kaufmann[1], Jenny Ho[1], Changjiang Xu[2], Veronique Voisin[2], John E. Dick[1]

[1]University Health Network, Toronto, Canada
[2]D. Donelly Centre, University of Toronto

Better understanding of normal blood differentiation programs during ontogeny was achieved recently (F.Notta et al, Science, 2016) by applying single-cell differentiation assay of highly purified HSPC populations. Deciphering the mechanisms and functional impact of the key genes involved in HSPC development or disease progression requires their introduction into the specific cell populations through gene transfer. We apply single-cell erythro-myeloid stroma-based differentiation assay to interrogate differentiation and proliferative potentials of human HSPC following introduction of a neutral marker gene and sphinogosine -1 – phosphate receptor 3 (S1PR3) - a gene involved in bioactive sphingolipid signaling.

The major differentiation characteristics of HSC (CD34+CD38-CD45RA-CD90+CD49f+) and multipotent progenitors (MPP - CD34+CD38-CD45RA-CD90-CD49f-), i.e. their ability to produce myeloid, erythroid, erythro-myeloid and erythro-myelo-megakaryocitic colonies were not significantly changed by the procedures accompanying marker gene transfer. The investigation of the proliferative potential of HSC revealed their huge heterogeneity in cell production after 16-17 days of cell culture. Thus, the size of the colonies produced by single cells varied from several dozens to 180 000 cells pointing out that HSC and its progeny might proliferate with the rate of more than 1 division per day. Such heterogeneity of HSC proliferative potential raises the question about phenotypic HSC functional homogeneity. The interrogation of differentiation and proliferation potentials of HSPC subpopulations following the introduction of S1PR3 revealed that its overexpression led to a significant dramatic loss of the ability of single HSC and MPP to produce erythroid cells in sharp contrast to progenitor populations (common myeloid progenitor and myelo-erythroid progenitor) in which the effect of overexpression of S1PR3 gene was much less evident. These data show the differential response of stem and progenitor cell subpopulations to forced expression of S1PR3 and underscore the unexpected importance of bioactive lipid signaling for the differentiation properties of human stem and progenitor cells.

This work was supported by The Terry Fox Research Institute.

33. THE PRINCESS MARGARET LIVING BIOBANK (PMLB) FACILITY PATIENT-DERIVED TUMOR XENOGRAFT (PDX) CORE

Nhu-An Pham, The Princess Margaret Living BioBank

The Princess Margaret Living Biobank (PMLB) Patient-Derived tumor Xenograft (PDX) core facility is a collaboration between PM researchers and the UHN Biospecimen Core. PDX models are increasingly being recognized as clinically more relevant models to identify phenotype-genotype association in patient tumors, and to discover biomarkers of drug response and resistance. During early passages in immune deficient mouse hosts, PDXs mirror closely the histopathological features and genetic profiles of the original patient tumors. Due to the high cost of model establishment, experimentation, and quality control protocols, there are ongoing efforts to unify PDX resources and processes by forming consortiums. Examples include EurOPDX, NCI-DCTD Repository, Cancer Research UK. Other labs have partnered with industry, e.g. Jackson Lab and Horizon Discovery.

The PMLB PDX aims to: 1) create a UHN repository of PDX models for use by researchers or public-private partnerships; 2) develop an integrated data portal linking PDX models with patient banked tumor samples, pathology, clinical annotation, genomics, and experimental data; 3) establish standard operating protocols to implement best practices for PDX model establishment and conducting experiments using PDX models; 4) offer core services to conduct PDX-based experiments. Proposals will be reviewed by the Scientific Board. Project specific experiments will require animal use protocols approved by Animal Resources Centre. Access to model-patient matched clinical data will require Research Ethics Board approval. We are in the process of centralizing the PDX bank for tumor models including lung, pancreas, ovarian, colon, head & neck, cervix, esophageal and mesothelium. In addition, we will create a PMLB Data Portal for users to access model specific data, perform feature specific searches, upload/download data will be supported by the Cancer Informatics team. Currently, only some of lung, pancreas and ovarian models have been characterized with various molecular platforms including whole exome next generation sequencing, gene mutation panels, copy number variants, and gene expression profiles. Consideration is being made to extend/complete limited molecular profiling on all models (>652) available in PMLB.

34. THE EFFICACY OF CD133 BITES AND CAR-T CELLS IN PRECLINICAL MODEL OF RECURRENT GLIOBLASTOMA.

Parvez Vora[1*], Chirayu Chokshi[1], Mohini Singh[1], Maleeha Qazi[1], Chitra Venugopal[1], Sujeivan Mahendram[1], Jarrett Adams[2], David Bakhshinyan[1], Max London[2], Minomi Subapanditha[1], Nicole McFarlane[1], James Pan[2], Jonathan Bramson[1], Sachdev Sidhu[2], Jason Moffat[2], Sheila Singh[1].

[1]McMaster University, Hamilton, ON, Canada;
[2]University of Toronto, Toronto, ON, Canada

**trainee presenting author*

Glioblastoma (GBM) is a uniformly fatal primary brain tumour, characterized by extensive cellular heterogeneity. Numerous studies have implicated CD133+ brain tumour initiating cells (BTICs) as drivers of chemo- and radio-resistance in GBM. We have recently demonstrated that a CD133-driven gene signature is predictive of poor overall survival and targeting CD133+ treatment-refractory cells may be an effective strategy to block GBM recurrence.

Chimeric antigen receptors (CARs) and bispecific T-Cell engaging antibodies (BiTEs) present promising immunotherapeutic approaches that have not yet been validated for recurrent GBM. Using CellectSeq, a novel methodology that combines use of phage-displayed synthetic antibody libraries and DNA sequencing, we developed the CD133-specific monoclonal antibody 'RW03'. We constructed CD133-specific BiTEs that consist of two arms; one recognizes the tumour antigen (CD133) and the second is specific to CD3 antigen that bind to human GBMs and PBMC-derived T cells, respectively. We observed BiTEs redirecting T cells to kill GBMs, with greater efficiency observed in $CD133^{high}$ GBMs, validating BiTE target specificity. Incubating T-cells with BiTEs and the $CD133^{high}$GBMs resulted in increased expression of T cell activation markers. In parallel, we derived the single chain variable fragment (scFv) from RW03, added a myc-tag and generated a second-generation CAR with ΔNGFR as a selection marker. CD133-specific CAR-T cells were cytotoxic to CD133+ GBMs. Co-culturing CD133 CAR-T cells with GBMs triggered T cell activation and proliferation. Treatment of GBM tumour-bearing mice with CD133-specific CAR-T cells yielded extended survival in mice and significant reductions in brain tumour burden.

Furthermore, we uniquely adapted the existing chemoradiotherapy protocol for GBM patients for treatment of immunocompromised mice engrafted with human GBMs. Within this model, we have initiated treatment of recurrent GBM directed against CD133+ BTICs, to allow for a direct prospective comparison of toxicity and efficacy of BiTEs and CAR T cell strategies.

PROJECT FUNDED BY: TERRY FOX RESEARCH INSTITUTE (TFRI) AND BIOCANRX

35. COMBINATION OF ITRACONAZOLE AND AUTOPHAGY INHIBITORS: A NOVEL THERAPEUTIC APPROACH FOR THE TREATMENT OF CANCER

Stefano Marastoni[1] (Post-doctoral fellow), Aleksandra Pesic[1], Ali Madani[1,2], Mark Freeman[1], Petr Smirnov[1], Benjamin Haibe-Kains[1,2,3,4], Robert Rottapel[1,4,5], Bradly G. Wouters [1,2,6], Anthony M. Joshua[1,7].

[1]Princess Margaret Cancer Centre, University Health Network, Toronto, Canada;
[2]Department of Medical Biophysics, University of Toronto, Toronto, Canada;
[3]Department of Computer Science, University of Toronto, Toronto, Canada;
[4]Ontario Institute of Cancer Research, Toronto, Canada;
[5]Department of Medicine, University of Toronto, Toronto, Canada;
[6]Department of Radiation Oncology, University of Toronto, Toronto, Canada;
[7]Institute of Medical Science, University of Toronto, Toronto, Canada;

Autophagy is a process whereby cellular proteins and organelles are recycled to sustain cellular metabolism. This phenomenon is frequently related to drug resistance, thus the employment of autophagy inhibitors may have a clinical benefit by sensitizing cancer cells to drug therapy. In a drug screening aimed at identifying toxic compounds to prostate cancer cells in combination with autophagy inhibitors, we found Itraconazole (Itra) as one of the top hits. Itra is an antifungal drug that has been reported to alter cholesterol trafficking and to have anticancer activity.

The autophagy inhibitor chloroquine (CQ) sensitized a variety of androgen-sensitive, castration-resistant and enzalutamide resistant LNCaP prostate cancer cell lines to Itra. Using filipin staining for free cholesterol, in cells co-treated with CQ and Itra we detected a dramatic increase of free cholesterol in the late endosomes as defined by LAMP1 co-staining.

To determine whether the treatment of Itra and CQ has potential therapeutic utility in other cancer cell lines, we examined the activity of Itra/CQ a panel of 32 ovarian cancer cells. Interestingly, activity was cell line dependent with differing sensitivity to both Itra alone and Itra plus CQ. A multivariate biomarker discovery analysis based on the correlation between the expression profile in the ovarian cancer cells and their response to the drugs identified the homotypic fusion and protein sorting (HOPS) - tethering complex as a top hit.

HOPS is generally important for vacuolar/lysosomal fusion, playing an important role in the last stages of endosome and autophagosome dynamics. Our data suggest that the combination of Itra and CQ induces accumulation of cholesterol and affects endosomal traffiking and autophagy flux, resulting in a stressing condition that is lethal for the cells. Further work is in progress to validate this hypothesis.

In conclusion, this combination of drugs could represent a novel therapeutic approach in the treatment of different types of cancer.

36. NUAK1 HAS POTENTIAL GROWTH SUPPRESSIVE ACTIVITY IN METASTATIC EPITHELIAL OVARIAN CANCER

Parima Saxena[1,2], Yudith Ramos Valdes[1], Adrian Buensuceso[1,2], Kyle Francis[6], Kevin Brown[6], Karen Colwill[7], Anne-Claude Gingras[7], Robert Rottapel[6], Gabriel E DiMattia[1,3,4,5], Trevor G Shepherd[1,2,4,5]

[1]*Translational Ovarian Cancer Research Program, London Regional Cancer Program; Departments of*
[2]*Anatomy & Cell Biology,*
[3]*Biochemistry,*
[4]*Oncology,*
[5]*Obstetrics & Gynaecology, Schulich School of Medicine & Dentistry, Western University, London ON, Canada;*
[6]*University of Toronto;*
[7]*Lunenfeld-Tanenbaum Research Institute*

Epithelial ovarian cancer (EOC) cells form multicellular aggregates, or spheroids, and enter a dormant state during intraperitoneal spread. Defining features of dormant EOC spheroids are reduced anabolic metabolism and cell proliferation due to metabolic stress signalling activation, thereby facilitating to chemo-resistance. It is widely established that LKB1 phosphorylates the downstream substrates AMP-activated protein kinase (AMPK) and AMPK-related kinases (ARKs) to mediate metabolic stress signalling. Further to this point, we have demonstrated that Liver kinase B1 (LKB1) activity is up-regulated in EOC spheroids and is required for cell survival while in suspension. Using OVCAR8 and HeyA8 cells lacking LKB1 generated via CRISPR/Cas9 genome-editing technology, we have made a surprising recent observation that increased phospho-AMPK is still maintained in EOC spheroids regardless of LKB1 expression. This result implicates other key effectors of LKB1 survival stress signalling in EOC cells and spheroids independent from its classical target, AMPK. To discover potential dysregulated kinases mediating this response, we performed multiplexed inhibitor beads-tandem mass spectrometry (MIBs/MS) using OVCAR8 cells and spheroids, with and without intact LKB1. Out of the 12 different ARK genes in humans, only NUAK1 was identified as significantly decreased in OVCAR8 cells and spheroids due to LKB1 loss. NUAK1 has been shown to negatively control cell growth and proliferation by direct regulation of cell cycle checkpoint proteins in several cell systems; however, knowledge regarding NUAK1 function in EOC is currently unknown. The *NUAK1* gene is infrequently altered (<2%) in both copy-number and mutation status in serous adenocarcinomas (provisional TCGA dataset). NUAK1 protein is generally expressed at low levels in proliferating EOC cell lines, but it is detectable in immortalized human fallopian tube epithelial cell lines. We performed *NUAK1* siRNA knockdown in several EOC cell lines to assess its requirement in adherent proliferating EOC cells and spheroids. Surprisingly, our results indicate that loss of NUAK1 expression increases spheroid cell viability, size, and reattachment potential. Knockdown of *NUAK2*, the most closely-related ARK family member to *NUAK1*, has little to no effect on EOC cells and spheroids. In fact, *NUAK2* knockdown in EOC cells results in a compensatory increase in *NUAK1* mRNA expression. Finally, treatment of EOC cell lines with the NUAK1/2 small molecule inhibitor WZ4003 increases EOC cell number and clonogenic capacity. Taken together, our results indicate that NUAK1 has important growth suppressive effects in EOC cells that may be critical to mediate LKB1 signalling on the dormancy phenotype of EOC spheroids, which are entities implicated in ovarian cancer metastasis and emergence of chemo-resistance.

The presenting author (PS) is a graduate student in the Department of Anatomy and Cell Biology at Western University.

37. DNA DEMETHYLATING AGENTS AND INTERFERONS AS MODULATORS OF WNT/B-CATENIN SIGNALING IN COLORECTAL CANCER

Ettayebi I[1], Mehdipour P[1], Roulois D[1], Medina T[1], Singhania R[1], O'Brien C[1], De Carvalho DD[1]

[1]Princess Margaret Cancer Centre, University Health Network, Toronto, ON M5G 2M9, Canada.

Colorectal cancer (CRC) is the second leading cause of death from cancer in men and the third in women in Canada. There is accumulating evidence suggesting that CRC is organized in a hierarchical manner, where Cancer-Initiating Cells (CIC) have the capacity for self-renewal and differentiation into the bulk tumour. Furthermore, results from xenograft models and human clinical trials indicate a selective enrichment of CICs in tumours that are resistant to therapy, suggesting that targeting CICs may represent a new paradigm in cancer treatment. We have recently reported the treatment with low dose of the DNA demethylating agent, 5-Aza-2'-deoxycytidine (5-AZA-CdR), can target CICs through the activation of the RIG1-MDA5 viral sensing pathway, leading to an anti-viral response in the cancer cells. Although it is now known that 5-AZA-CdR induces a state of "viral mimicry" in these cancer cells, the mechanism by which 5-AZA-CdR can specifically target CICs is not well understood. In this study, we propose a novel intersection between RIG1-MDA5 and Wnt/β-catenin pathways. We found that treatment of CIC enriched cancer cells with both 5-AZA-CdR and type I and III interferons reduces canonical Wnt/β-catenin signaling, leading to a decrease in expression of Wnt target genes. Furthermore, treatment with type I and III interferons showed a reduced CIC frequency in colorectal cancer cells *in vitro*. These findings may explain a novel mechanism by which 5-AZA-CdR can specifically target CIC enriched populations in colorectal cancer and highlights the importance of immune pathways in epigenetic therapy.

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38. THERAPEUTIC TARGETING OF TUMOURIGENIC EPHA2+/EPHA3+ BRAIN TUMOUR INITIATING CELLS WITH BI-SPECIFIC ANTIBODY IN GLIOBLASTOMA.

Maleeha A. Qazi[1], Parvez Vora[1], Chirayu Chokshi[1], Chitra Venugopal[1], Max London[2], Amy Hu[2], Minomi K. Subapanditha[1], Mohini Singh[1], Jarrett Adams[2], Jason Moffat[2], Sachdev Sidhu[2], Sheila K. Singh[1].

[1]Department of Surgery, Faculty of Health Sciences, McMaster University,
[2]Donnelly Centre for Cellular & Biomolecular Research, Banting and Best Department of Medical Research, University of Toronto.

Glioblastoma (GBM), the most aggressive primary human brain tumour, carrier a dismal prognosis and is increasingly characterized by cellular and genetic intra-tumoral heterogeneity (ITH). Many of the 14 members of the erythropoietin-producing hepatocellular carcinoma receptor (EphR) family and their ephrin ligands are expressed in GBM cells and constitute potential molecular targets for novel therapeutic agents. We hypothesize that multiple members of the EphR family play a critical role in orchestrating the clonal evolution of GBM progression. Individual Eph receptor targeting strategies have shown only modest pre-clinical success, likely because single agent therapy cannot target the degree of ITH in GBM. Using a highly specific human Eph receptor monoclonal antibody (mAb) panel (EphR profiler), we identified five Eph receptors with dysregulated expression in recurrent GBM as compared to primary GBM. With our unique chemoradiotherapy-adapted, patient-derived xenograft model of GBM, we identified EphA2 and EphA3 expression to be upregulated after therapy. Here we show that EphA2 and EphA3 co-expression marks a highly tumorigenic cell population in recurrent GBM with higher *in vitro* and *in vivo* self-renewal and proliferation capacity as compared to EphA2+/EphA3-, EphA2-/EphA3+ or EphA2-/EphA3- cells. Lentiviral mediated knockdown of EphA2 and EphA3 blocks this self-renewal and proliferation capacity in recurrent GBM. Through further characterization using mass cytometry (CyTOF) assay, we find that EphA2 and EphA3 is co-expressed with multiple brain tumour initiating cell (BTIC) markers (CD133, CD15, Bmi1, Sox2, Integrin α6 and FoxG1). Considering the important role of EphA2+/EphA3+ cells in GBM tumorigenesis and recurrence, we generated a bi-specific antibody (bsIgG) that co-targets EphA2 and EphA3. *In vitro* treatment of GBM with bsEphA2/A3 IgG led to pharmacological blockade of phosphorylated EphA2. We then assessed the *in vivo* efficacy of the bsEphA2/A3 IgG to block GBM tumour growth in our PDX model, and found that treatment with intracranial bsIgG resulted in non-invasive and significantly smaller lesions. The striking reduction in tumour burden in recurrent GBM after co-targeting of EphA2 and EphA3 validates the premise of our therapeutic strategy of targeting multiple EphRs. Discovering the clonal composition of recurrent GBM will enable us to target cellular subpopulations, and this ITH, with selective compounds that inhibit BTIC and Eph receptor activity with minimal off-target effects. Comprehensive Eph receptor profiling of individual patient-derived GBM will allow us to develop a therapeutic strategy for each patient's tumour, employing polytherapy with mAbs against Eph receptors expressed at recurrence.

The presenting author is a PhD student.
This project is funded by the 2016 TFRI PPG grant.

39. E3 UBIQUITIN LIGASE CBL-B AS A POTENTIAL THERAPEUTIC TARGET FOR CD4+ T CELL IMMUNOTHERAPY

JAMES SEONGJUN HAN[1,2], Douglas Chung[2], Michael St. Paul[1,2], Alisha Elford[2], Laurence Chapatte[2], Pamela Ohashi[1,2]

[1]Department of Immunology, Faculty of Medicine, University of Toronto, Ontario M5S 1A1, Canada
[2]Campbell Family Institute for Breast Cancer Research, Princess Margaret Cancer Centre, Toronto, Ontario M5G 2C1, Canada

Adoptive T-cell therapies (ACT) are reaching its potential in multiple malignancies including melanoma and leukemia; however, one of the limitations is the presence of Treg cells, defective $CD4^+$ T cells and subsequent lack of IL-2 in tumours. To address the challenge, adoptive transfer of modified $CD4^+$ T cells has been recently explored to restore a robust pro-inflammatory $CD8^+$ T cell response. Lymphocytes deficient in E3 ubiquitin ligase, Cbl-b, displays hyper-inflammatory phenotype and resistance against immunosuppression, therefore serving as a potential target in the development of ACT. Thus, the research specifically investigated the cellular mechanisms behind Cbl-b deficiency in $CD4^+$ T cells for future therapeutic applications. $CD8^+$ or $CD4^+$ T cells were negatively selected from spleens of C57BL/6 and Cbl-$b^{-/-}$ mice (2 to 4 months) and were (co-) stimulated in different combinations for 1) flow cytometry-based immunophenotyping and 2) cytokine secretion profiling using CBA, ELISA and ICS. Treg cells were isolated using FACS for Treg suppression assay and cytokine stimulation panel. Lastly, Treg suppression assays of $CD4^+$ and/or $CD8^+$ was performed using exogenous IL-2, anti-IL2 Ab and anti-CD25 Ab, as well as IL-2R$\alpha^{-/-}$ condition. Cbl-$b^{-/-}$ $CD4^+$Foxp3- T cells, all displaying enhanced Th1 surface phenotype, secreted high quantity of IL-2, IFN-γ and IL-17A, as previously illustrated. The pro-inflammatory cytokines secreted by Cbl-$b^{-/-}$ CD4+Foxp3- T cells, more specifically IL-2, induced significantly elevated 41-BB, ICOS, GITR and CD25 on CD8+ T cells, and mediated significantly decreased % suppression in both $CD4^+$Foxp3- and $CD8^+$ T cells specifically through IL-2Rα. Accordingly, modulation of IL-2 signaling using IL-2, anti-IL-2 and PC61 regulated $CD8^+$ T cell function as well as % suppression. Overall, we demonstrate that specifically Cbl-$b^{-/-}$ CD4+FoxP3T cells' IL-2 hyper-secretion serve as an important mechanism to 1) boost pro-inflammatory $CD8^+$ T cell function and 2) mediate $CD4^+$ and $CD8^+$ T cell's resistance against IL-2 deprivation by regulatory T cells.

Note: The project is not supported by TFRI or TFF, and there is no conflict of interests.
Presenting author: Graduate student

40. IDENTIFICATION OF ONCOGENIC AND METASTATIC DRIVER GENES THAT COOPERATE WITH P53 OR P53/RB-LOSS TO INDUCE TRIPLE-NEGATIVE BREAST CANCER

Ronak Ghanbari-Azarnier[1, 2], Philip.E Chung[1, 2], Zhe Jiang[1], Eldad Zacksenhaus[1, 2].

[1]Division of Advanced-Diagnostics, Toronto General Research Institute, Toronto, ON and
[2]Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON.

**Presenting Author: Graduate Student/ Grant Acknowledgments: TFRI & CIHR*

Introduction: Triple-negative breast cancer (TNBCs) is an aggressive subtype with poor prognosis; metastasis is highly lethal. The tumour suppressor *TP53* is frequently lost together with *RB1* in TNBCs. Our lab demonstrated that deletion of Rb and p53 in mouse mammary epithelium, using MMTV-Cre promoter, induced tumours with features of TNBCs but with limited capacity to metastasize.

Hypothesis: I hypothesize that sleeping beauty (SB) mutagenesis will identify oncogenic networks that drive primary and metastatic TNBCs, and that these oncogenic networks may serve as therapeutic targets for this aggressive disease.

Methods: To identify oncogenic events that cooperate with Rb and p53 loss in TNBCs, I initiated a SB screen by generating mice in which p53 or p53 plus Rb are disrupted and the SB transposon is mobilized from 2 different chromosomes (T2/Onc3a & 3b).

Forty primary tumours and forty metastases from each cohort will be harvested to identify common integration sites followed by deep sequencing. Then, the top 5-10 candidate oncogenes/tumour suppressors will be validated to determine their effect on tumorigenesis/metastasis.

Results: So far, 6 mice developed mammary tumours in cohort 3a (average latency - 112 days post first pregnancy) and 3 in cohort 3b (average latency - 121 days); however, macro-metastases are not frequent. Therefore, we decided to perform a survival surgery on mice with oversized tumours to remove the primary tumours and follow them up longer for developing lung metastases. With this method, we are able to detect macro-metastases.

Conclusion: SB mutagenesis can promote metastasis in mouse models with limited dissemination potential such as the model proposed herein. The proposed experiments will identify novel oncogenic events that cooperate with p53-loss or combined p53/Rb-loss to induce metastatic TNBCs. I have generated 2 sets of cohorts of SB mutagenesis to identify these oncogenic events. Some mice have developed tumours, which are currently being analyzed in details.

41. INTERFERON INDUCED STAT1 ASSOCIATES WITH DIFFERENTIAL CHEMOTHERAPY RESPONSE IN HIGH-GRADE SEROUS OVARIAN CANCER

Gillian Reid-Schachter (graduate student trainee), Peter Truesdell, Nichole Peterson, Charles Graham, Julie-Ann Francis, Andrew Craig, Madhuri Koti.

Department of Biomedical and Molecular Sciences/Cancer Biology and Genetics Division/Queen's Cancer Research Institute/Queen's University, Department of Pathology and Molecular Medicine & Department of Obstetrics and Gynecology/Kingston General Hospital

Purpose: High-grade serous ovarian cancer (HGSC) is the most prevalent and fatal histological subtype of ovarian cancer. Unfortunately, 70% of HGSC patients show resistance to chemotherapeutic drugs, and clinical management is challenged by a lack of accurate prognostic and predictive biomarkers of chemotherapy response. It is now established that immune cells within the tumour microenvironment (TME) significantly contribute to tumor cell death or survival following exposure to chemotherapy. We previously reported the presence of a distinct, pre-existing T-helper Type I associated immune TME, mediated by Signal Transducer and Activator of Transcription Factor 1 (STAT1), that predicted chemotherapy response in HGSC. Current work builds on our previous findings and investigates the mechanisms linking STAT1 to a pre-existing inflammatory TME and differential response to chemotherapy.

Experimental Procedures: Tumour STAT1 expression and intra-epithelial CD8+ T-cells were confirmed as prognostic and predictive biomarkers in a second independent biomarker validation study. Immunohistochemistry and a custom NanoString platform, composed of 34-target genes of STAT1 and immune phenotypic markers, were used on fresh frozen chemo-naïve HGSC tumour samples. Based on the findings from these profiling studies, a subset of samples with high and low STAT1 expression levels were subjected to further gene profiling analysis to correlate the immune transcriptomic landscape and its association with chemotherapy response. Stable silencing of the STAT1 gene was performed by lentiviral transduction of murine ovarian cancer cells (ID8) with STAT1 short hairpin RNA (shRNA) to create STAT1-knockdown (KD) cells and STAT1-non targeting (NT) control cells. Ongoing in vivo experiments will define the contribution of STAT1 in modulating; the TME, tumor progression, the recruitment of tumor infiltrating lymphocytes, and response to chemotherapy in HGSC.

Results: Gene expression analysis revealed significant differential expression of genes involved in the cellular Type I Interferon pathway, notably STAT1 and its target genes (such as ISG15, DDX58, IFIT1, CXCL10, CXCL11), between chemosensitive and resistant groups. Preliminary in vitro analysis has revealed proliferative and migratory advantages to STAT1-KD cells compared to the STAT1-NT cells; indicating that STAT1 plays a role in the regulation of cell survival and proliferation in the absence of the immune TME.

Conclusion: STAT1 expression significantly associates with progression free survival and response to chemotherapy in HGSC. High levels of STAT1 and its target genes potentially contribute to CD8+ T-cell recruitment and immune mediated chemosensitivity in HGSC. Ongoing in vitro and in vivo studies will confirm the mechanisms underlying variations in the interferon induced STAT1 pathways in the TME of HGSC. Elucidating the mechanisms underlying differential STAT1 expression in HGSC primary tumours will contribute to better patient stratification for informed biomarker guided immunotherapies.

This research is supported by the TFRI.

42. ASSESSING THE UTILITY OF CIRCULATING TUMOUR DNA AS A SURVEILLANCE TOOL FOR LI-FRAUMENI SYNDROME USING A MURINE MODEL

Sangeetha Paramathas [1,4], Nathan Lewis [4], Tanya Guha [2,4], Zainab Motala [2,4] and David Malkin[1,2,3,4,5]

[1]*Department of Medical Biophysics, University of Toronto; Toronto ON, Canada*
[2]*Institute of Medical Sciences, University of Toronto; Toronto ON, Canada*
[3]*Department of Pediatrics, University of Toronto; Toronto ON, Canada*
[4]*Genetics and Genome Biology, The Hospital for Sick Children; Toronto ON, Canada*
[5]*Department of Haematology/Oncology, The Hospital for Sick Children; Toronto ON, Canada*

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**Abstract present (Sangeetha Paramathas) is a graduate student (trainee).*

Li-Fraumeni Syndrome (LFS) is a hereditary cancer predisposition syndrome commonly characterized by the presence of inherited mutations in the tumour suppressor gene *TP53*. This leads to an early onset of a wide spectrum of tumours in multiple organ systems. Current surveillance protocols for early tumour detection include biochemical screening, MRI and ultrasound scans, colonoscopy and mammography (for adults). While early detection is associated with improved survival, the complexity of testing, potential to 'misdiagnose' tumours (false positive/negative) and requirement for multiple imaging modalities makes clinical surveillance challenging to implement and interpret. Circulating tumour DNA (ctDNA) is a recently described diagnostic and prognostic tool that takes advantage of fragments of the genome that are released into the blood from dying tumour cells. In this study we use xenograft tumour models to assess the correlation of tumour burden and ctDNA levels. We have conducted preliminary studies that have successfully shown obvious increases in ctDNA with increased tumour burden and a complete clearance of ctDNA after tumour resection. These studies will provide the foundation for our future work where we will use *Trp53* mutant mice that develop tumours spontaneously to analyze ctDNA and better model clinical LFS while assessing the clinical utility of ctDNA.

43. OLIGOMERIZATION STATUS MODULATES CELL FATE DECISIONS BETWEEN GROWTH, ARREST AND APOPTOSIS

Nicholas W. Fischer[1,2], Aaron Prodeus[1,2], David Malkin[1,3,4], Jean Gariépy[1,2,5]

[1]*Department of Medical Biophysics , University of Toronto , Toronto , Ontario , Canada.*
[2]*Physical Sciences , Sunnybrook Research Institute , Toronto , Ontario , Canada.*
[3]*Department of Paediatrics , University of Toronto , Toronto , Ontario , Canada.*
[4]*Division of Haematology/Oncology, Hospital for Sick Children , Toronto , Ontario , Canada.*
[5]*Department of Pharmaceutical Sciences , University of Toronto , Toronto , Ontario , Canada.*

Mutations in the oligomerization domain of p53 are genetically linked to cancer susceptibility in Li-Fraumeni Syndrome. These mutations typically alter the oligomeric state of p53 and impair its transcriptional activity. Activation of p53 through tetramerization is required for its tumor suppressive function by inducing transcriptional programs that lead to cell fate decisions such as cell cycle arrest or apoptosis. How p53 chooses between these cell fate outcomes remains unclear. Here, we use 5 oligomeric variants of p53, including 2 novel p53 constructs, that yield either monomeric, dimeric or tetrameric forms of p53 and demonstrate that they induce distinct cellular activities and gene expression profiles that lead to different cell fate outcomes. We report that dimeric p53 variants are cytostatic and can arrest cell growth, but lack the ability to trigger apoptosis in p53-null cells. In contrast, p53 tetramers induce rapid apoptosis and cell growth arrest, while a monomeric variant is functionally inactive, supporting cell growth. In particular, the expression of pro-arrest CDKN1A and pro-apoptotic P53AIP1 genes are important cell fate determinants that are differentially regulated by the oligomeric state of p53. This study suggests that the most abundant oligomeric species of p53 present in resting cells, namely p53 dimers, neither promote cell growth or cell death and that shifting the oligomeric state equilibrium of p53 in cells toward monomers or tetramers is a key parameter in p53-based cell fate decisions.

44. MicroRNA-106A CONFERS RADIOTHERAPY RESISTANCE AND TUMOUR AGGRESSION BY TARGETING LITAF IN PROSTATE CANCER

Christianne Hoey[1,2], Jessica Ray[1,2], Samira Taeb[2], Xiaoyong Huang[2], Nancy Yu[1,3], Paul Boutros[1,3], and Stanley K. Liu[1,2,4]

[1]*Dept. of Medical Biophysics, University of Toronto*
[2]*Sunnybrook Research Institute, Sunnybrook Health Sciences Centre*
[3]*Ontario Institute for Cancer Research, University Health Network, Toronto, Canada*
[4]*Dept. of Radiation Oncology, University of Toronto*

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Introduction: Prostate cancer (PCa) is the most prevalent cancer affecting Canadian men. PCa recurrence is a major clinical problem with up to a 40% biochemical recurrence rate at five years after external beam radiotherapy (ionizing radiation, IR). This significantly decreases patient outcomes and poses a serious burden on Canada's healthcare system. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and their expression is dysregulated in cancer. Specifically, miR-106a is overexpressed in many cancers, and we hypothesize that miR-106a confers IR resistance and tumour aggression in PCa.

Materials and Methods: To determine whether miR-106a is enriched in PCa samples, bioinformatic analysis was performed using The Cancer Genome Atlas Data Portal in R statistical environment.Clonogenic survival assays were used to assess cancer cell survival following IR treatment in PC3 and DU145 prostate cancer cell lines overexpressing miR-106a (by miR-106a mimic transfection). Proliferation assays were used to quantify number of viable cells with and without IR between miR-106a overexpressing cells and normal control cells. Gene array analysis was used to identify possible targets of miR-106a. Cells were stained for β-galactosidase expression following IR to assess senescence, as a mode of cell death following IR. In order to assess tumour growth delay, athymic nude mice were injected with DU145-control and DU145-miR-106a stable cells subcutaneously in the right flank. When tumours reached a volume of > 100mm3, they were irradiated and tumour volume was measured by calipers.

Results and Discussion: MiR-106a was significantly overexpressed in PCa samples relative to normal prostate samples, suggesting that miR-106a is involved in prostate carcinogenesis. Clonogenic assays displayed increased survival after IR treatment with cells overexpressing miR-106a compared to control cells. Proliferation assays showed that miR-106a-overexpressing cells had a higher proliferation rate than control cells in both unirradiated and IR-treated cells. We identified lipopolysaccharide-induced TNFα factor (LITAF) as a putative downstream target of miR-106a. LITAF knockdown lead to increased proliferation and clonogenic survival following IR. We assessed miR-106a and LITAF's effects on cellular senescence, as senescence is the predominant mode of cell death following IR in prostate cancer. We found that both miR-106a overexpression and LITAF knockdown resulted in significantly fewer senescent cells post-IR. This suggests that miR-106a inhibits LITAF, which increases survival following IR by reducing senescence in PCa cells. We found that miR-106a overexpression and LITAF knockdown increases expression of ATM mRNA and protein. Upregulation of ATM is associated with radiation resistance, explaining how miR-106a and LITAF are involved in radioresistance. Tumour xenograft experiments confirmed that miR-106a increases proliferation compared to control tumours. This trend was seen without IR, in addition to following radiation treatment. Thus, these *in vitro* and *in vivo* experiments show that miR-106a is involved in PCa aggression and confers a radiation-resistant phenotype, by targeting the novel radiation response gene LITAF.

45. MECHANISMS IN COLORECTAL CANCER TUMORIGENESIS

Christina Oatway[1,2] (trainee), Calley Hirsch[1], Alex Gregoiroff[1], Jeff Wrana[1,2]

[1]*Centre for Systems biology, Lunenfeld-Tanebaum Research Institute, Mount Sinai Hospital*
[2]*Department of Molecular Genetics, University of Toronto*

Colorectal cancer (CRC) is the third leading cause of cancer-related death, with an average of 25, 000 Canadians being diagnosed each year. The study of CRC is therefore critical in order to further our understanding of not only how these tumours are initiated but also how they develop. In terms of CRC, there are a number of oncogenes and tumour suppressors that are known to be mutated and lead to disease development. One of the most common mutations is in the adenomatous polypsis coli (APC) gene, which is found mutated in over 80% of CRC and is thought to be a very early mutation.

We want to use intestinal organoids that are mutated for APC in order to discern novel genes that are crucial to the survival of these mutated organoids. We devised a CRISPR-Cas9 based screen in which kinases will be knocked out in APC-/- organoids and next generation sequencing will be done in order to determine drop-out hits (genes crucial to the survival of the mutated organoids, or oncogenes) and drop-in hits (genes that when knocked out increase the stability of cancerous cells, or tumour suppressors). This screen will be carried out in mouse organoids with further validation being done in human organoids with the hopes that these hits could potentially be novel targets of drug therapies for the treatment of CRC.

Human organoids are derived from human embryonic stem cells, however the differentiation process doesn't yield a high enough volume of organoids for our purposes. We therefore have devised a second screen to identify small molecule inhibitors that increase the generation of human intestinal organoids. We have found potential hits that could help further our understanding of intestinal development.

Funded by TFRI

46. ACTIVATION OF THE MST-LATS-YAP/TAZ HIPPO PATHWAY

Shawn Xiong, PH.D. [A], Dan Mao [B], Frank Sicheri[A, B, &C]

[A]*The University of Toronto, Department of Biochemistry;*
[B]*Lunenfeld-Tanenbaum Research Institute;*
[C]*The University of Toronto, Department of Molecular Genetics*

Shawn Xiong, Ph.D. student, supported by TFRI,

The Hippo pathway was first identified in *drosophila*, where mutations in proteins Hippo, Warts, or Salvador led to uncontrollable growth resulting from excessive cell proliferation. Classically, the core Hippo pathway in mammals consists of a short kinase cascade: the activated MST1/2 kinases (Hippo in flies) phosphorylate and activate LAST1/2 kinases (Warts in flies), and in turn LATS1/2 phosphorylate and inhibit YAP/TAZ transcription co-activators activities. The activation of the pathway is further aided by Sav1 (Salvador in flies) binding to MST1/2 and MOB1 binding and co-activating LAST1/2.

Upstream MST1/2 kinases can be activated by oxidative stress, chemical toxins, heat shock, disruption of cytoskeleton, as well as phosphorylation by Tao kinases. Activated MST1/2 kinases can then phosphorylate many substrates in addition to LATS1/2, suggesting the kinases function beyond Hippo pathway. For instance, MST1/2 are known to phosphorylate NDR1/2 to regulate centrosome duplication, FOXO1 to promote apoptosis, Beclin1 and LC3 to regulate autophagy. This raises the question how MST1/2 can selectively act on LATS1/2-YAP/TAZ Hippo signalling without triggering a multitude of other downstream effectors?

Here, I report the key to selectively activate the MST1/2-LATS1/2-YAP/TAZ signalling pathway lies in the phosphorylation of the small co-activator protein, MOB1. Phosphorylated MOB1 acts as a central adaptor to connect MST1/2 kinases specifically with LATS1/2 kinases, thus activating LATS1/2 kinases towards the YAP/TAZ phosphorylation in the cytoplasm.

47. PRINCESS MARGARET LIVING BIOBANK (PMLB) ORGANOID CORE

Nikolina Radulovich and Ming-Sound Tsao

Recently, 3D organoid culture of resected patient material has gained an unprecedented advantage over cell lines and patient-derived xenograft (PDX) models for treatment discoveries within the framework of personalized medicine. Patient-derived mini organoids grown from tissue residing stem cells very closely resemble tissues found inside a human body and maintain the mutational status of high frequency gene alterations found in primary tumors.

Princess Margaret Living Biobank (PMLB) organoid core is a joint three-phased project between Princess Margaret researchers and University Health Network (UHN) BioBank to develop 3D organoid cell line models across multiple tumor histologies that will benefit the entire UHN research community and leverage resources to support investigator-driven science. In the Phase I project, PMLB set out to optimize protocols and establish propagable 3D organoid cell lines from lung, pancreas, colon, prostate, ovary and breast tumors tissues obtained by surgical resection at UHN. Our PMLB organoid models are being deposited at UHN BioBank and linked to other patient banked materials, clinical and molecular profiling data including genomic profiling. PMLB performs extensive identification and characterization testing for all submitted organoid models including Short Tandem Repeat (STR) genotyping for parental cell line identity matching, mycoplasma testing, pathohistological identification of organoid tissues using a panel of tissue specific biomarkers reviewed by site specialist UHN pathologist and growth analysis. Currently we are successfully establishing organoid cultures from colon, pancretas and lung tumors and are optimizing culturing conditions for organoids grown from breast, prostate and ovary tumors.

We are working on the development of a web-based searchable database of available organoid models to begin distribution for sponsored research projects between UHN and outside entities, including commercial companies. Serving the entire University Health Network research community, the PMLB aims to provide our customers with tools to answer many basic oncology research questions in hopes to positively impact cancer treatment.

48. RHAMM LOSS INCREASES MMTV-PYMT MAMMARY TUMOR METASTASIS

Cornelia Tolg[1], Alanna Edge[4], Maja Milojevic[4], Han Yuan[2], Jenny Ma[1], Mary Cowman[2], Jim Mc Carthy[3], Kathleen Hill[4], Eva Turley[1]

[1]*London Regional Cancer Program*
[2]*New York University*
[3]*Masonic Cancer Center, Minneapolis*
[4]*Western University*

Breast cancer (BCa) progression is driven by both a tumor-guided remodelling of the host microenvironment and tumor cell genomic instability. RHAMM is a dual function, intracellular mitotic spindle and extracellular hyaluronan (HA) co-receptor. Since decreased expression of RHAMM results in aberrant chromosome segregation in cultured cells, we predicted that RHAMM loss will propel tumor aggression by contributing to genomic instability.

Rhamm$^{-/-}$MMTV-PymT females were generated by crossing *Rhamm*$^{-/-}$mice with wild type (WT) MMTV-PymT mice. RHAMM loss had no effect on primary tumour initiation or size but resulted in significantly more metastatic lung nodules than in WT MMTV-PymT mice. CNV analysis using a mouse genotyping array did not detect increased length copy number losses or increased numbers in CNVs linked to RHAMM loss that are consistent with mitotic spindle defects.

RHAMM interacts with BRCA1, a breast tumour suppressor gene that regulates homologous double strand break repair. Further CNV analysis detected an increase of small CNV deletions in RHAMM$^{-/-}$ primary tumours and lungs consistent with a defect in double strand break repair. In addition, RHAMM loss is associated with a significant increase in active TGFß-1 in lung tissue. IPA analysis of genes affected by the small CNV deletions predicts that RHAMM ablation results in a loss of ERK1/2 hubs, which affects TGFß-1 signaling outcome. These results predict that alteration of RHAMM expression has consequences to both the tumor microenvironment and tumor cell genome that impact mammary tumor progression.

49. COMPETITIVE IN VIVO GAIN-OF-FUNCTION SCREEN IDENTIFIES NOVEL REGULATORS OF NORMAL AND MALIGNANT HEMATOPOIETIC STEM CELLS

Kerstin B Kaufmann[1,2,7], Stanley WK Ng[1,3], Shin-ichiro Takayanagi[1,2], Peter van Galen[4], Erno Wienholds[1,2], Stephanie Z Xie[1,2], Amanda Mitchell[1,2], Igor Jurisica[1], Jean CY Wang[1,2,6], John E. Dick[1,2]

[1]Princess Margaret Cancer Centre, University Health Network,
[2]Department of Molecular Genetics, University of Toronto, 3Institute of Biomaterials and Biomedical Engineering, University of Toronto,
[4]Massachusetts General Hospital
[5]Campbell Family Institute for Cancer Research, Ontario Cancer Institute, University Health Network and Department of Medical Biophysics, University of Toronto
[6]Department of Medicine, University of Toronto
[7]Postdoctoral Fellow

A growing body of evidence indicates that leukemia stem cells (LSC) can survive chemotherapy and initiate relapse in acute myeloid leukemia. For development of novel therapies it is crucial to understand the mechanisms underlying LSC survival and self-renewal, stem cell properties shared with normal hematopoietic stem cells (HSC). To identify the key regulators of LSC/HSC self-renewal we assessed the potential of 64 candidate genes to enhance self-renewal in an advanced competitive *in vivo* screen.

Candidate genes were selected based either on high expression in functionally validated LSC vs. non-LSC fractions (*Ng et al., accepted*) or densely interconnected genes derived from protein interaction analysis of LSC genes. In two parallel screens we transduced HSC and progenitor enriched fractions with 64 lentiviral vectors to assemble 16 pools, each consisting of 8 individual gene-transduced populations, 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 gene pool and cell subsets (CD34+) out of mice by digital droplet PCR.

We observed multiple diverse patterns of competition and robustness across cell subsets, individual mice, pools and both screens. Based on a scoring algorithm that considers these parameters the 9 highest scoring genes were selected for individual evaluation *in vivo*. This short list comprises genes known to be involved in either transcriptional regulation, cellular metabolism, adhesion, extracellular matrix remodelling or are protease and kinase inhibitors. For the majority of them distinct phenotypes on engraftment, lineage output and migration have been confirmed and their roles in stem cell maintenance and commitment on the molecular level are currently under investigation. Of note, in the progenitor screen *HOXA4*, a known positive regulator of murine repopulating cells, received the highest score. In a first *in vivo* validation assay transducing $CD34^{+}CD38^{+}$ cells *HOXA4* outperformed all other candidates analyzed highlighting the power of our screening approach to find stemness regulators.

Thus, we successfully developed an advanced competitive *in vivo* gain of function screen and thereby extracted potential regulators of self-renewal. Detailed functional studies on the candidate genes under investigation will uncover new therapeutic targets, setting the stage for eradication of LSC in AML.

This work was supported by The Terry Fox Research Institute, Ontario.

50. GENETIC MODIFIERS OF RADIATION RESPONSE IN LI-FRAUMENI SYNDROME

Katherine Sauberli-Stewart[1,2*], Nardin Samuel[1,2,3,4], Ilana Geist[5], Benjamin Brew[2], Lauren Erdman[2,6], Anna Goldenberg[2,6], David Malkin[1,2,7,8].

[1]*Institute of Medical Sciences, University of Toronto, Toronto, Canada*
[2]*Genetics and Genome Biology Program, The Hospital for Sick Children, Toronto, Canada*
[3]*Department of Medical Biophysics, Faculty of Medicine, University of Toronto, Toronto, Canada*
[4]*Ontario Institute for Cancer Research, Toronto, Canada*
[5]*University of Guelph, Guelph, Canada*
[6]*Department of Computer Science, University of Toronto, Toronto, Canada*
[7]*Division of Hematology/Oncology, The Hospital for Sick Children, Toronto, Canada*
[8]*Department of Pediatrics, University of Toronto, Toronto, Canada*

**Graduate Student*

Funded by the Terry Fox Research Institute

Li-Fraumeni Syndrome (LFS) is an inherited cancer predisposition disorder commonly associated with a wide spectrum of germline *TP53* mutations in affected individuals. These mutations result in aberrant p53 protein expression and defective DNA damage response. It has been suggested that defective DNA repair may increase the risk of therapeutic or even diagnostic radiation-induced second malignant neoplasms (SMN) in LFS patients. However, aside from anecdotal reports, there is little reported empiric evidence to support this premise and no pre-clinical data to predict response or transformation potential in individual patients. Utilizing our rich resource of LFS patient samples, we set out to develop a pre-clinical model of radiation exposure to determine the effects of acute and chronic exposure. We exposed skin-derived fibroblasts from LFS patients harbouring a spectrum of heterozygous *TP53* mutations to either acute (0.5Gy or 8Gy) or chronic (0.5Gy daily, four days) γ-irradiation. Preliminary data suggest a variable response in induction of downstream p53 targets including p21 and cell growth response suggesting possible mechanistic differences in response to varying radiation dose and schedule. RNA-seq is being performed on these fibroblast cell lines pre- and post- acute and chronic, low-dose γ-irradiation to identify important modifiers of radiation response. Results of these studies will inform predictive algorithms to predict radiation-response and SMN risk in LFS patients.

51. AN INVASIVE CELL SUBPOPULATION AS A MODEL FOR TRIPLE NEGATIVE BREAST CANCER

Tahereh Vakili[1], Cornelia Tolg[2], K.A. Hill[2] and Eva A. Turley[1,2,3]

The presenting author is considered a trainee (Graduate student)

[1]Department of Biochemistry,
[3]Department of Oncology, Western University,
[2]London Health Sciences Centre-London Regional Cancer Program
[2]Department of Biology, Western University, London, ON, Canada

Intra-tumour heterogeneity is a major hurdle in the diagnosis and therapy of triple-negative breast cancer (TNBC). Currently, there is no approved targeted therapy for this BCa subtype. Hyaluronan (HA) is a prognostic factor for tumour progression and poor outcome in TNBC. Therefore, we designed an ALEXA468-HA probe to isolate HA^{high} subsets of TNBC MDA-MB-231 cells using FACS.HA^{high} subpopulations uniquely phenocopy the aggressive and resistant traits of TNBC as they are more invasive, metastatic, slow-growing, and chemoresistant (e.g. Doxorubicin) than the parent line. We therefore used this subset as a model to identify therapy targets. We first assessed the sensitivity of these tumour cells to MEK1 inhibition as a candidate for targeted therapy because hyper-activation of this pathway predicts metastasis and poorer outcome in TNBC. We showed that HA^{high} subsets are more sensitive to MEK1,2 inhibition than their parental and HA^{Low} counterparts. The HA receptor RHAMM is involved in MEK1,2 signaling and is displayed to a greater extent in HA^{high} than HA^{Low} subsets. We therefore used CRISPR/Cas gene editing to assess the role of RHAMM in the sensitivity of HA^{high} cells to MEK1,2 inhibition. RHAMM deletion significantly decreased the sensitivity of MDA-MB-231 cells to MEK1,2 inhibition, identifying this protein as key factor in regulating MEK signaling pathways. Our findings suggest RHAMM is a biomarker for identifying TNBC patients who are sensitive to MEK inhibitors. Our results also suggest that HA^{high} subsets are a relevant TNBC model for identifying additional therapy targets.

52. INHIBITING MITOCHONDRIAL RNA POLYMERASE AS AN ANTICANCER STRATEGY FOR ACUTE MYELOID LEUKEMIA USING RIBONUCLEOSIDE ANALOGUE 4'-AZIDOCYTIDINE

Noor-Ul-Ain Khalid[1], Sanduni U. Liyanage[2], Yeojin Park[3], Darius Babusis[3], Adrian S. Ray[3], Joy Y. Feng[3], Aaron D. Schimmer[2], Leonardo Salmena[1,2] and Rebecca Laposa[1]

[1]Department of Pharmacology and Toxicology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
[2]Princess Margaret Cancer Centre, University Health Network, Toronto, Canada
[3]Gilead Sciences Inc. Foster City, California, USA

Acute myeloid leukemia (AML) cells exhibit dysregulated mitochondrial function in the form of high oxidative phosphorylation (OXPHOS), an increased mitochondrial mass and low respiratory chain spare reserve capacity. This deregulation in AML cells renders them vulnerable to agents that target mitochondrial function. We reasoned that a possible therapeutic strategy would be to inhibit mitochondrial RNA polymerase (POLRMT) since it indirectly controls OXPHOS, it has been considered a "metabolic oncogene", and it is druggable. POLRMT transcribes the mitochondrial genome, which codes for 13 essential subunits in the mitochondrial electron transport chain. We hypothesized that AML cells have an elevated rate of transcription of the mitochondrial genome, and this could point to a therapeutic window relative to normal blood cells. Technical validation of the approach was conducted in OCI-AML2 cells; nascent RNA was pulse-labeled using 4-thiouridine (s^4U) and nascent RNA was collected. The label is cell-permeable and readily incorporated into newly transcribed RNA, and the sulfur moiety provides a handle for biochemical coupling. Biotinylation of the thiol group allows for the pull-down of an enriched s^4U labeled RNA fraction through the use of streptavidin beads.Metabolic labeling of cellular RNA in OCI-AML2 cells, followed by biochemical purification, indicated that 3-4% of the RNA pool contains s4U, providing yields appropriate for downstream analysis by RT-PCR and RNA-seq.

Our goal was to functionally validate POLRMT as a target in AML models. We have previously demonstrated that OCI-AML2 cells are sensitive to the POLRMT inhibitor 2'-C-methyladenosine (2'-CM), also a ribonucleoside analogue inhibitor. Here we took advantage of an improved POLRMT inhibitor, 4'-azidocytidine, which is more selective and potent than 2-CM (IC50 of 4'-AC-triphosphate for POLRMT is 4 μM, while the IC50 of 2'CM-triphosphate is 230 μM); both function as a chain terminators of mitochondrial transcription. In order to assess the effects of 4'-AC on growth and viability, a number of leukemia cell lines (OCI-AML2, U937, K1a, OCI-LY17 and NB4) were treated with concentrations up to 100 μM of 4'-AC for 10 days and assessed by cell counts and ATP concentration. Dose-dependent and time-dependent decreases in growth and viability were observed with an EC50 of 50 μM. To assess the effect of 4'-AC on mitochondrial gene expression, OCI-AML2 cells were treated with up to 50 μM of 4'-AC for 10 days and 4'-AC caused a concentration-dependent and time-dependent decrease in the expression of mitochondrial genes ATP8, CO2, ND1 and ND6. In order to determine whether 4'-AC decreases oxidative phosphorylation (OXPHOS), we measured basal oxygen consumption in intact cells using the Seahorse XF^e96 Analyzer. In OCI-AML2 cells treated with up to 50 μM of 4'-AC, 4'-AC decreased OXPHOS in a concentration- and time-dependent manner.

Lastly, we addressed the intracellular activation of 4'-AC in OCI-AML2 cells by measuring the conversion of the parent compound to its active triphosphate metabolite in cells treated with 10 μM 4'-AC for 24 hours. Triphosphate levels were measured by ion-pairing liquid chromatography-tandem mass spectrometry (LC-MS/MS). These studies confirmed formation of pharmacologically active 4'-AC-TP in OCI-AML2 cells.

Taken together, the data indicate that 4'-AC has on-target efficacy as a POLRMT inhibitor in leukemia cell culture models.
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53. RECOVERY OF QUALITY OF LIFE (QOL) AND PHYSICAL FUNCTION OVER THREE YEARS IN ADULT SURVIVORS OF ACUTE MYELOID LEUKEMIA (AML) AFTER INTENSIVE CHEMOTHERAPY (IC)

N. Timilshina (graduate student), H Breunis, GA Tomlinson, SMH Alibhai

Author Affiliations: Toronto General Research Institute, and University of Toronto, ON.

Introduction: The treatment of AML with IC is associated with significant toxicities. The aim of the current study to describe QOL and physical function recovery over 3 years from diagnosis compared with normative data.

Methods: Adult AML patients (age 18+) undergoing IC without stem cell transplant were enrolled in a prospective, longitudinal study. Assessments were done at baseline (pre-IC) and at 11 time points over the next 3 years. At each visit, patients completed the EORTC QLQ-C30 and the FACT-Fatigue questionnaires to measure global QOL and fatigue, respectively, in addition to 2 physical performance measures (PPMs; grip strength and 6-minute walk test (6MWT)). We compared results with age- and gender-stratified population normative data to determine the proportion of patients whose scores were less than the minimum clinically important difference from normal on each outcome.

Results: A total of 237 eligible patients (median age 57, 56% male) were enrolled and followed for up to 3 years. At baseline, compared to normative data global QOL and QLQ-C30 subscales were low in both genders. During recovery over 3 years, a higher proportion of male survivors returned to normal in global QOL and fatigue compared to female survivors. A large majority of male survivors returned to normal fatigue levels (91.3%) whereas only 2/3 of female survivors (65.6%) returned to normal at 3 years. Performance on PPMs was poor on average in both males and females, with only 44% in grip strengths and less than 25% of patients in 6MWT returning to normalin both genders over 3 years.

Conclusions: The vast majority of survivors of AML after successful conventional IC achieve significant improvements in global QOL and most QOL domains as well as fatigue with values similar to normative data by 3 years after diagnosis, but recovery was lower in women than men and physical recovery remained blunted. Further research is needed to understand apparent gender differences in recovery.

Table 1: Age-adjusted QOL and physical function from baseline to 3-year by gender compared with population normative value

MEASURES	MALE (%)				FEMALE (%)			
	BASELINE	1 YEAR	2 YEAR	3 YEAR	BASELINE	1 YEAR	2 YEAR	3 YEAR
GLOBAL QOL	21	76	69	87	29	67	65	72
PHYSICAL FUNCTIONING	52	52	69	88	50	59	56	71
ROLE FUNCTIONING	21	63	54	83	27	52	69	68
EMOTIONAL FUNCTIONING	49	69	73	71	44	64	59	75
COGNITIVE FUNCTIONING	57	71	80	58	39	43	49	56
SOCIAL FUNCTIONING	20	63	69	83	22	44	53	68
FACT-FATIGUE	26	70	75	91	28	53	57	66
GRIP STRENGTHS	40	43	60	44	29	38	36	44
6MWT	3	8	9	16	4	9	14	19

54. TARGETING ER, JNK, AND MTOR PATHWAYS AS A NOVEL THERAPEUTIC APPROACH TO TREAT RHABDOMYOSARCOMA

Zainab A. Motala[1,2], Kevin M. Chen[3], Adam D. Durbin[4,5], Gino R. Somers[6], David Malkin[1,2,7,8]

[1]Department of Pediatrics, The Hospital for Sick Children, Toronto, ON, Canada
[2]Institute of Medical Science, University of Toronto, ON, Canada
[3]MD Program, University of Toronto, ON, Canada
[4]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
[5]Division of Pediatric Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA
[6]Division of Pathology, The Hospital for Sick Children, Toronto, ON, Canada.
[7]Division of Hematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada
[8]Department of Pediatrics, University of Toronto, Toronto, ON, Canada.

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood, with an estimated five-year overall survival of less than 25% in metastatic patients. Despite refinements in risk stratification and multimodality chemotherapy, improvements in RMS survival have been minimal and no molecularly targeted therapies are currently available. Previously, we demonstrated that RMS cells express estrogen receptor (ER) and are sensitive to 4-hydroxytamoxifen (4OHT), an active metabolite of tamoxifen. Here, we investigate the mechanisms by which 4OHT exert these effects. We show that 4OHT-induced apoptosis involves estrogen receptor-dependent phosphorylation of c-Jun N-terminal kinase (JNK), as well as inhibition of the anti-apoptotic AKT/mTOR pathway. These mechanisms can be exploited by combining 4OHT with other drugs acting on the JNK and AKT/mTOR pathways: vincristine (a core RMS chemotherapy agent) enhances JNK phosphorylation, while temsirolimus (an emerging RMS drug currently in phase III trials) inhibits mTOR complex 1; combining tamoxifen with either of these drugs led to increased efficacy. Finally, we show that tamoxifen causes significantly reduced tumour growth in an RMS xenograft mouse model. Taken together, our work offers *in vivo* results (supporting our previous *in vitro* data) showing that tamoxifen may be effective against RMS, and provides a mechanistic framework that explains the anti-RMS effects of tamoxifen and its ability to potentiate vincristine and temsirolimus.

55. ROLE OF HIGH MOLECULAR WEIGHT HYALURONAN IN ULTRAVIOLET B LIGHT-INDUCED TRANSFORMATION

Katelyn Cousteils[1], Dr. Argang Yazdani, Dr. Eva Turley[1,3]

[1]Department of Biochemistry, Western University
[2]Division of Plastic and Reconstructive Surgery, Western University
[3]London Regional Cancer Program, London Health Sciences Centre

Presenting author is a trainee (graduate student).

Skin cancer comprises one in three of all cancers diagnosed annually in North America. The majority of these are basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs), which arise from the keratinocyte layer. The incidence of both of these skin cancers is strongly linked to UVB exposure. Though rarely lethal, BCCs are not prevented by sunscreen and are associated with significant patient morbidity. Here, we investigated the use of enhancing high molecular weight hyaluronan (HA) in the keratinocyte layer to address the clinical need for a preventative agent in BCC initiation.Our rationale is based upon recent reports linking an absence of skin and keratinocyte tumorigenesis in the skin of naked mole rats to elevated high molecular weight (HMW) HA (Tian et al., 2013) and in culture evidence that high molecular weight HA prevents transformation with UVB of a human keratinocyte cell line (Hašová et al., 2011). We formulated HMW HA-phosphatidylehthanolamine (HA-PE) that efficiently penetrates the keratinocyte layer and forms coats around keratinocytes and dermal cells (Symonette et al., 2014). We assessed the effect of topical application of HA-PE to UVB-induced tumour initiation and progression in a patched mouse model of BCC and SCC susceptibility (Xu et al., 2014). HA-PE was applied topically to hairless (hr -/-) *ptch1* +/- mice five times weekly. Mice received 180 mJ/cm^2 dose of UVB twice weekly for 26 weeks prior to daily cream application. Cream vehicle alone and no cream treatment were used as negative controls for HA-PE treatment.BCC and SCC were examined by a mouse pathologist and identified by histology. Epidermal hyperplasia was quantified as epidermal thickness and by proliferation indices (ki67 positive). HA-PE treated mice show a significant increase in tumour latency compared to the no cream and vehicle cream control groups. HA-PE also decreased the tumour load (number of tumours per mouse) when compared to the control groups (HA-PE vs. no treatment p=0.036; HA-PE vs. base cream, p=0.063). These results show that increasing HA coat formation around basal keratinocytes significantly protects them from UV–induced transformation.

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56. PTCH53 AS A POTENTIAL SECONDARY MODIFIER IN LI-FRAUMENI SYNDROME

Anna JW. Pan[1,2], Benjamin Brew[2], Lauren Erdman[2,5], Anna Goldenberg[2,5], David Malkin[1, 2, 3, 4]

[1]*Institute of Medical Sciences, University of Toronto.*
[2]*Genetics and Genome Biology, SickKids Research Institute, Toronto.*
[3]*Division of Hematology/Oncology, The Hospital for Sick Children, Toronto.*
[4]*Department of Pediatrics, University of Toronto.*
[5]*Department of Computer Science, University of Toronto.*

**Presenting author is a graduate student (M.Sc. candidate).*

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Germline mutations in the *TP53* gene have been established as the underlying genetic event in Li-Fraumeni syndrome (LFS), predisposing affected families to a wide spectrum of early onset cancers. Despite rapid progress in elucidating the role of wild-type p53 in maintenance of genome stability, and mutant p53 in cellular transformation, much of the molecular basis underlying LFS remains unclear. Lack of predictability in age of onset, type(s) of cancers, and likelihood of subsequent malignancies prompts further research to examine secondary modifiers on the underlying *TP53* genotype. We have recently reported differential methylation and gene expression of the Hedgehog pathway associated *PTCH53 (Patched53)* locus, in *TP53* mutation carriers compared to a *TP53* wild-type cohort. The study herein examines the expression profile of *PTCH53* in the presence of different germline *TP53* mutations using primary patient-derived fibroblasts, and aims to elucidate the functional role of *PTCH53*. Preliminary results demonstrate that *PTCH53* expression indeed depends on *TP53* genotype. Through ddPCR analysis of *PTCH53* expression, different *TP53* mutations correspond with varied *PTCH53* mRNA levels. Nonetheless, mutant *TP53* fibroblasts have overall lower *PTCH53* transcripts compared to wild-type *TP53* fibroblasts. Fold-changes in *PTCH53* expression were detected upon Doxorubicin treatment and exposure to irradiation. These results suggest that induction of p53 via DNA damage activates a signalling cascade involving *PTCH53* up-regulation. Additional experiments are necessary to confirm these findings and to further examine the functional role of *PTCH53* in LFS patients via knockdown, knockout and overexpression in non-transformed fibroblast models.

57. CHARACTERIZING HETEROGENEITIES IN HYPOXIA TOLERANCE AND OXYGEN METABOLISM IN PANCREATIC ADENOCARCINOMA

M.Sc. Candidate **Ji Zhang[1],** Dan Cojocari[1], Bradly Wouters[1,2]

[1]Department of Medical Biophysics, University of Toronto, Ontario, Canada
[2]Department of Radiation Oncology, University of Toronto; Princess Margaret Cancer Centre, Ontario, Canada

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer related deaths, and has a 5-year survival rate of less than 5%. The hypoxic microenvironment of PDACs is extremely heterogeneous across patients, and the basis for this observation and its association to underlying genetic drivers of this disease are unknown. High levels of hypoxia are known to correlate with increased tumour aggressiveness and resistance to therapy. Although multiple hypoxia-targeted drugs have been developed in recent years, recent phase 3 trials such as in the case of evofosfamide were unsuccessful. This is likely due to the patient selection was not based on hypoxia, thus included large numbers of patients that could not benefit from such therapy. I hypothesize that genetic mutations in pancreatic cancer associated with two principal factors - oxygen metabolism and hypoxia tolerance - define the steady state levels of hypoxia in individual tumours. The consumption of oxygen by cells determines the levels and steepness of hypoxia gradients arising around perfused vessels. Tolerance to hypoxia determines the time cells can survive without oxygen and involves activation of stress response pathways such as HIF and UPR to enable the cells to adapt to low oxygen conditions.

We are generating 3D organoid models selected from 15 patients' tumours covering the known spectrum of tumour hypoxia levels from an ongoing clinical trial at the University Health Network (UHN). I will measure various aspects of organoid oxygen metabolism including basal oxygen consumption rate (OCR), maximum OCR, non-mitochondrial OCR, and mitochondrial leak in normoxic and hypoxic (<0.2% O_2) conditions. In addition, I will characterize the hypoxia tolerance in these organoids with a focus on the activation and importance of the UPR/PERK pathway in mediating survival. This will be done by measuring organoid growth, and secondary organoid growth under defined levels of oxygenation. UPR/PERK activation will be assessed via IHC of organoid sections staining for known makers of activation such as pEIF2a, ATF4, and GADD153. In addition, these same patient tumour samples have also been used to generate patient-derived-xenografts (PDXs) and were included as part of the ICGC providing whole genome and transcriptome sequencing. These organoid measurements will be simultaneous compared to estimates of oxygen metabolism, UPR activation, and overall levels of hypoxia as revealed by IHC staining of pimonidazole in the PDX and patient samples.

The long term goal of my project is to correlate the data generated from organoids with features of patient genetic characteristics to identify the underlying drivers of the hypoxic microenvironment. These genetic markers would be used to both select patients for alternative hypoxia-targeted therapies and develop new types of therapies to target hypoxic cells.

58. IDENTIFICATION OF NOVEL HIPPO PATHWAY REGULATORS IN CANCER

Mandeep Gill* and LILIANA ATTISANO

Department of Biochemistry and Donnelly Center, University of Toronto

**Trainee.*

The Hippo signaling pathway is an evolutionary conserved regulator of organ size and cell fate. It has emerged as a tumour suppressor pathway that acts to control the transcriptional coactivators, YAP and TAZ, which interact with transcription factors to regulate various biological processes. In response to diverse extrinsic and intrinsic signals, the Hippo pathway core kinases MST and LATS phosphorylate and inhibit YAP/TAZ activity. YAP and TAZ activity is key not only for normal organ growth and tissue regeneration but plays critical role in tumour initiation, epithelial-mesenchymal transition and stemness in multiple types of cancer. Thus restoring Hippo pathway activity or targeting YAP/TAZ represents a potential therapeutic approach to treat cancer. Thus, to uncover novel regulators of the Hippo pathway in cancer, I employed high-throughput RNAi and chemical inhibitor screening approaches. For this, the effect of siRNAs and inhibitors on Hippo pathway activity using luciferase TEAD-reporter and YAP/TAZ localization by immunofluorescence microscopy was examined in two breast cancer cell lines. Data from both the YAP/TAZ localization and the Hippo functional screen were compiled and analyzed to identify common hits. Selected top hits were validated by loss-of-functional studies using siRNA pool deconvolution and with distinct chemical inhibitors by examining their effects on YAP/TAZ localization, phosphorylation and regulation of target gene expression. Further studies on one of the top hits which acted as a strong negative regulator of the Hippo pathway are underway. Loss of expression of this kinase by siRNA or a chemical inhibitor that blocks activity promoted cytoplasmic accumulation and inhibited the transcriptional activity of YAP/TAZ. Analyzing the underlying molecular mechanism of this kinase in modulating Hippo pathway and its role in tumorigenesis is in progress.

This project is supported by the TFRI.

59. MONITORING AND PREDICTING CANCER TREATMENT OUTCOMES USING PHOTOACOUSTIC IMAGING

Eno Hysi[1,2,*], Lauren A. Wirtzfeld[1,2], Jonathan P. May[3], Elijus Undzys[4], Shyh-Dar Li[3] and Michael C. Kolios[1,2,§]

[1]Department of Physics, Faculty of Science, Ryerson University, Toronto, ON, Canada
[2]Institute for Biomedical Engineering, Science and Technology, Li Ka Shing Research Institute, Keenan Research Center, St. Michael's Hospital, Toronto, Toronto, Ontario, Canada
[3]Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, BC, Canada
[4]Drug Discovery and Formulation Group, Drug Discovery Program, Ontario Institute for Cancer Research, Toronto, ON, Canada

**Trainee: PhD student*

§Project partially funded by the TFRI New Frontiers PPG titled "Ultrasound and MRI for cancer therapy" (Project leader: Gregory Czarnota)

Cancer treatment monitoring typically relies on quantifying gross volume changes in tumour size, which often take several weeks to months to manifest. This is in contrast with tumour blood vessels which undergo more dynamic changes as a result of treatments. In this work, we propose that photoacoustic imaging can map the vascular changes (structural and functional) that occur early (2-5 hours) after the administration of cancer treatments and that these changes can be used to predict the therapeutic outcome.

The treatment consisted of a chemotherapy-loaded thermosensitive liposome (TSL) allowing for localized delivery of doxorubicin within subcutaneous EMT-6 breast cancer murine tumours. Mild hyperthermia (43°C) was used as the drug release trigger. Ultrasound and photoacoustic imaging was performed in 3D using the VevoLAZR preclinical imaging system (Fujifilm-VisualSonics, Toronto). All Balb/c mice were imaged pre-treatment, before imaging at early time points (30 minutes to 5 hours) post treatment and again at 24 hours and 7 days post-treatment. Heated saline injections were used as control. The photoacoustic data was analyzed to estimate the oxygen saturation (sO_2) of tumour blood vessels. Changes in the size of the blood vessels were monitored through spectral analysis of the photoacoustic signals and were then correlated with tumour growth measurements and histological findings.

After the TSL treatment, mice which exhibited a 22% drop in sO_2 2 hours post-treatment also experienced an average decrease of 75% in tumour size 30 days post-treatment. The drop in oxygenation persisted for the first 24 hours post-treatment. Heated saline control (100% of cases) and non-efficacious TSL (10% of cases) treatments did not show any significant changes in sO_2 at these time points. Photoacoustic spectral analysis showed that these changes were accompanied with a 73% decrease in the spectral slope, a parameter sensitive to the effective size of the optical absorbers (i.e. blood vessels). Histological findings revealed that the TSL treatment induced significant hemorrhaging within the tumour. This led the accumulation of red blood cells in the tumour interstitium eventually decreasing the tumour sO_2 and increasing the effective optical absorber size. These findings suggest that photoacoustic imaging could have clinical value in assessing therapeutic response of cancer treatments long before the manifestation of gross changes in tumour volume.

60. BUILDING AN ANALYSIS PIPELINE TO MEASURE DISTANCE RELATIONSHIPS BETWEEN PERFUSION VERSUS TUMOUR HYPOXIA AND PROLIFERATION

Mark Zaidi[1,2], Dan Cojocari[1], Trevor McKee[3], Brad Wouters[1]

[1]Princess Margaret Cancer Centre, University Health Network, Toronto,
[2]Ryerson University, Toronto,
[3]STTARR facility, University Health Network, Toronto.

Solid tumour's hypoxic environment (poorly oxygenated regions) is known to cause dramatic changes in tumour cell phenotypes, such as increased metastasis, radiation resistance, and overall poor survival of patients. Thus, understanding the root causes and molecular mechanisms of tumour hypoxia formation can help target these malignant phenotypes, improve the efficacy of hypoxia-targeted drugs, and extend patient survival.

The current study aims to develop an immunofluorescence image analysis pipeline to accurately quantify the oxygen and proliferation gradients as a function of distance from the nearest perfused blood vessel. To do this, mice bearing pancreatic (PANC1, KP4) or colorectal (HCT116) tumour cell line xenografts were injected with EF5 hypoxia probe and EdU (5-ethynyl-2'-deoxyuridine) proliferation marker. Vessel perfusion was detected by injecting mice with Hoechst one minute prior to tumour collection. The xenograft tumours were then serially sectioned and stained with fluorescently labeled anti-EF5 antibody and EdU Click-iT label. Blood vessels were detected using anti-CD31 immunostaining, while nuclei were stained with DAPI. Stained serial sections of the tumours were scanned and, using a MATLAB script, each image was aligned to one another such that all the stains can be viewed and analyzed against each other. This allowed for the comparison of blood vessel perfusion (Hoechst) to hypoxia (EF5) and cell proliferation (EdU). Following image alignment, regional and cellular analysis was performed in Definiens; an object-oriented, machine learning analysis software. Regions of hypoxia, perfusion, necrosis, and normal tumour areas were manually identified. Based on the morphology and stain intensities within these regions, Definiens classified the entire tumour into these regions of interest (ROIs). Within these regions, cellular analyses were conducted to identify useful properties of cells in each region including marker intensities, EF5 and EdU cell counts, cell location, and the calculated distance of each cell to the nearest perfused blood vessel (Hoechst ROIs). The resulting per-cell statistical data were then imported into our custom MATLAB programs to plot the relationship between mean cell EF5 intensity and the percent of EF5/EdU positive cells as a function of the distance to the nearest Hoechst stained ROI. Our results show that our method can accurately quantify the oxygen gradients within a tumour with reduced biological variability between tumour xenografts of a cell line. We observed unique slopes of oxygen gradients in xenografts established from different cell lines: KP4, PANC1, and HCT116. Furthermore, we could compare the percent viable hypoxic or proliferating cells as a function of distance from blood vessels. The nearly automated image analysis pipeline that we have developed can be used to quantify tumour hypoxia and proliferation as a function of distance to perfused blood vessels. This pipeline could be validated and used in various clinical scenarios, such as assessing the patient tumour hypoxic status, which ultimately could be used to improve the efficacy of hypoxia-targeting drugs or radiation treatment.

Mark Zaidi: Trainee (2nd year undergraduate student)

Conflict of interest: Nonet

61. ESTABLISHING A GENOME WIDE CRISPR SCREEN TO IDENTIFY NEW HIPPO PATHWAY REGULATORS

Sen, S.[1], Mis, M.[2], Angers, S.[2], Attisano, L.[1]

[1]Dept. of Biochemistry, Donnelly Center and 2Dept. of Pharmacy, Leslie Dan Faculty of Pharmacy, University of Toronto.

The Hippo pathway regulates tissue growth and organ size and its dysregulation/ inactivation leads to a host of cancers such as breast, colon and bladder cancer. YAP/ TAZ are transcriptional regulators which among other functions enhance cell growth. The core kinase cassette consisting of LATS1/2, MST1/2, SAV and Mob phosphorylate YAP and TAZ promoting their cytoplasmic localization and degradation. Currently much remains to be learned about the upstream regulators that modulate the pathway. The purpose of this study was to establish a method to carry out a genome wide positive selection CRISPR screen to uncover such upstream regulators of the Hippo pathway. For this, it was first necessary to identify cell lines with robust YAP/TAZ activity that would be appropriate for screening. Preliminary studies were carried out in representative colon and breast cancer cell lines using a YAP/TAZ responsive element located upstream of a luciferase transcriptional reporter. This indicated YAP/TAZ were highly active in colon cancer DLD1, SW480 and in breast cancer MDA-MB-231 cell lines.Knockdown studies of YAP/TAZ in these cell lines showed a marked decline in the mRNA expression of their target genes as seen by qPCR. For the screen it is necessary to generate stable cell lines expressing a fusion protein where the catalytic domain of Caspase-9 is fused to a modified FKBP (FK506 binding domain) under the transcriptional control of YAP/TAZ. This protein can be homodimerized by the addition of a chemical dimerizer AP20187 which will trigger an apoptotic pathway leading to cell death. When YAP/TAZ activity is disrupted, cells are expected to survive. Thus the cell lines are also designed to express Cas9 which will be used to knockout the genes in the human genome using a 180K guide RNA library. Using PCR to make a YAP/TAZ responsive FKBP-Caspase9 construct and by employing lentiviral transduction I generated cell lines of DLD1,SW480 (colorectal) and MDA-MB-231 (breast) cancer cells expressing FKBP-Caspase9 and Cas9. MDA-MB-231 cell lines showed very high activity of FKBP-Caspase9 and Cas9. I also generated a T24 bladder cancer cell line expressing highly active FKBP-Caspase9. Testing for the responsiveness of the FKBP-Caspase9 construct to the Hippo pathway in MDA-MB-231 and T24 is currently underway. This will enable us to select the most suitable system to carry out the CRISPR screen.

This project is supported by the TFRI.
Sen S is a graduate student.

62. MOLECULAR SUBTYPING AND CIRCULATING TUMOUR DNA ANALYSIS OF HEPATOCELLULAR CARCINOMA TO PREDICT RECURRENCE POST-SURGERY

Ting Ting Wang[1, 2], Anand Ghanekar [2], Sean Cleary[2], Scott Bratman[1, 2, 3], Trevor Pugh[1, 2, 4]

[1]Department of Medical Biophysics, University of Toronto, Toronto, ON;
[2] Princess Margaret Cancer Centre, University Health Network, Toronto, ON;
[3] Department of Radiation Oncology, University of Toronto, Toronto, ON;
[4] Ontario Institute for Cancer Research, Toronto, ON;

Background: Liver cancer is an etiologically diverse disease, with hepatocellular carcinoma (HCC) representing the predominant form of malignancy. Worldwide, HCC is the fifth most common cancer and the second leading cause of cancer-related deaths[1]. Curative therapies, including surgical resection and liver transplantation, are associated with high rates of recurrence. Despite 30-70% of patients having recurrent disease, no robust predictors of recurrence in HCC patients following surgery exist[2]. Current treatment decisions are made on crude tumour characteristics, such as size and number of lesions, which do not accurately reflect the underlying biology. Furthermore, our collaborators have shown that patients treated within surgical guidelines may still present recurrence[3]. These clinical predictors are inadequate and development of genomic predictors is complicated by regional variability in disease etiology and low mutation frequency in recurrently altered genes. A method more sensitive than imaging could allow for earlier detection of recurrence to further improve outcome of this disease.

Aims: Comprehensive molecular characterization of somatic mutations, methylation and gene expression to improve subtyping in HCC and to nominate clinically useful biomarkers predictive of recurrence. Additionally, these markers will be applied to ctDNA profiles collected serially post-surgery to monitor minimal residual disease.

Results: To investigate the role of circulating tumour DNA (ctDNA) in HCC, we conducted a pilot study (n = 6) demonstrating detection of ctDNA containing clonal and subclonal mutations known from matched tumour tissue. Mutations were detected in all cases, although more mutations were found in plasma of patients with high tumour burden compared to low tumour burden. To improve the sensitivity of detection, we are adapting molecular barcoding methods for error suppression in ctDNA analysis. This method reduces background noise and identifies true variants through removal of polymerase and sequencing errors. Currently, we are doing RNA sequencing and methyl-seq on 87 tumour and paired normal liver samples. We are also in the process of recruiting 125 more patients.

63. HIGH-RESOLUTION CRISPR SCREENS REVEAL FITNESS GENES AND GENOTYPE-SPECIFIC CANCER LIABILITIES

Megha Chandrashekhar,*[1,2,12] , Traver Hart,[1,12] Michael Aregger,[1,12] Zachary Steinhart,[3] Kevin R. Brown,[1] Graham MacLeod,[3] Monika Mis,[3] Michal Zimmermann,[5] Amelie Fradet-Turcotte,[5,13] Song Sun,[1,2,7,9] Patricia Mero,[1] Peter Dirks,[2,8] Sachdev Sidhu,[1,2] Frederick P. Roth,[1,2,5,9,10,11] Olivia S. Rissland,[2,6] Daniel Durocher,[2,5] Stephane Angers,[3,4] and Jason Moffat[1,2,10]

[1]Donnelly Centre, 160 College Street, Toronto, ON M5S3E1, Canada
[2]Department of Molecular Genetics
[3]Department of Pharmaceutical Sciences and Leslie Dan Faculty of Pharmacy
[4]Department of Biochemistry University of Toronto, Toronto, ON M5S1A1, Canada
[5]The Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON M5G1X5, Canada 6Molecular Structure and Function Program, The Hospital for Sick Children Research Institute, 686 Bay Street, Toronto, ON M5G0A4, Canada
[7]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala SE-75123, Sweden 8Program in Developmental and Stem Cell Biology, Division of Neurosurgery, Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G1X8, Canada
[9]Department of Computer Science, University of Toronto, Toronto, ON M5G1X8, Canada
[10]Canadian Institute for Advanced Research, Toronto, ON M5G1Z8, Canada
[11]Center for Cancer Systems Biology, Dana-Farber Cancer Institute, Boston, MA 02215, USA 12Co-first author

**Graduate (Ph.D) student*

The advance that pooled CRISPR-Cas9 library technology brings to human genetics sets the stage for identifying cellular fitness genes which operate either globally or specifically within a particular genetic background or environmental context. To extend the catalogue of human core and context-dependent fitness genes, we have developed the TKO (Toronto KnockOut) library, a second-generation gRNA library of 176,500 guides targeting 17,661 human protein coding genes. We used the library to screen five human cell lines, representing a cross-section of wild type and cancer tissues, to identify genes whose knockouts induced significant fitness defects. We expand the known set of human core fitness genes more than fourfold to 1,580 genes, and identify dozens of essential protein complexes, both known and novel, whose heterozygous copy loss in chromosomally unstable cancers may induce a therapeutic window. We further characterize novel fitness genes of unknown function and find that they all likely exist in protein complexes with other essential genes. TKO screens accurately recapitulate pathway-specific genetic vulnerabilities induced by known oncogenes and reveal cell-type-specific dependencies for specific receptor tyrosine kinases, even in oncogenic KRAS backgrounds. We also identified a specific dependency on mitochondrial activity, which strongly supports the idea that oxidative phosphorylation dependency- a clear exception to the Warburg effect - is a targetable weakness of some tumours. Our findings demonstrate that the CRISPR-Cas9 system fundamentally alters the landscape for systematic genetics in human cells, affording a high-resolution view of the genetic vulnerabilities of a cell that may represent therapeutic opportunities in cancer.

64. DESIGNING THE NEXT-GENERATION ONCOLYTIC VACCINIA VIRUS

Tiffany Y. Ho*[1,2], Lili Li[2],David F. Stojdl[3], and J. Andrea McCart[1,2,4]

[1]*Insititute of Medical Science, University of Toronto;*
[2]*Toronto General Research Institute, University Health Network;*
[3]*Department of Biology, Microbiology, and Immunology, Children's Hospital of Eastern Ontario (CHEO) Research Institute;*
[4]*Division of General Surgery, Department of Surgery, Mount Sinai Hospital and University of Toronto*

**Presenting author is a trainee (graduate student)*

Background: Engineered gene deletions in the oncolytic vaccinia virus (VV), vvDD, enhance wildtype Western Reserve (WR) VV tumour-specificity based on cellular proliferation rates. While recent clinical studies have demonstrated tumour responses and remarkable safety, VV potency could be enhanced to increase clinical benefit by using alternate strategies of VV tumour-selectivity.

Hypothesis: Deletion of single VV immunomodulatory genes (N1L, K1L, K3L, A46R, or A52R) from WR VV will improve therapeutic efficacy compared to vvDD in *in vitro* and *in vivo* in terms of safety, tumour-selective viral replication, and anti-tumour responses.

Methods: N1L, K1L, K3L, A46R, and A52R VV deletion mutants were generated from WR VV and tested for replication ability, cytotoxicity, and ability to spread in vitro, in MC38 murine colon cancer, DLD1 human colon cancer and A2780 human ovarian cancer cell lines.Nude immunocompromised and C57BL/6 immunocompetent mice were injected with virus intraperitoneally (IP) to compare viral toxicity to vvDD. Twelve-day IP tumours of DLD1 or A2780 in nude mice or MC38 in C57BL/6 mice were treated with candidate viruses or vvDD and were followed for survival to determine tumour responses. Normal and tumour tissues were harvested for plaque assays to assess viral biodistribution.

Results: The K1L, A46R, and A52R VV deletion mutants demonstrated superior activity *in vitro* in viral replication, cytotoxicity, and spreading assays, respectively compared to vvDD, and were further investigated in vivo. Maximum tolerated doses (MTD) of the candidate viruses were at least 20 times lower than the MTD of vvDD (10e9 pfu in nude and C57BL/6 mice). All three viruses were as tumour-specific as vvDD in the biodistribution studies. Yet at MTD, K1L was the only VV to improve median MC38 tumour survival compared to mock-treated in C57BL/6 mice (35 vs 28.5 days, $p<0.01$). Similar results were seen in the A2780 model (53.5 vs 40 days, $p<0.01$). In the DLD1 tumour model, long term (>100 days) survivals of 25% and 37% were seen for the A46R and A52R-deleted viruses respectively compared to vvDD (12.5%).

Conclusions: Deletion of redundant immuno-modulatory genes from VV is a novel strategy to maintain tumour specificity while improving anti-tumour potency compared to vvDD. K1L, A46R, and A52R-deleted viruses are similarly tumour-selective and improve survival equally or better than vvDD in the models tested at significantly lower treatment doses.

65. RARE VARIANTS IN THE FAT1 GENE MAY PREDISPOSE TO FAMILIAL COLORECTAL CANCER

Ashton A. Connor[1,2], Caroline Badouel[1,2], Jordan Lerner-Ellis[1,2], Mohammad R. Akbari[1], Cezary Cybulski[3], Jan Lubinski[3], James G. Dowty[4], Mark Clendenning[4], Mark A. Jenkins[4], Daniel D. Buchanan[4], Helen McNeill[1,2] and Steven Gallinger[1,2]

[1]*Sinai Health System, Lunenfeld-Tanenbaum Research Institute*
[2]*University of Toronto, Canada*
[3]*Pomeranian Medical University, Szczecin, Poland*
[4]*University of Melbourne, Australia*

Introduction: Population- and family-based studies suggest hereditary predisposition contributes to as much as 35% of colorectal cancer (CRC) cases. Known genetic factors, including 3 high penetrant Mendelian cancer syndromes and approximately 70 low penetrant alleles, account for fewer than 15% of CRC cases. Identifying genes responsible for "missing" CRC predisposition will inform prevention and management.

Methods and Results: Our discovery cohort consisted of 127 CRC cases from 86 Canadian and Australian pedigrees that underwent whole exome sequencing of germline DNA. All pedigrees met the Familial Colorectal Cancer Type X definition (satisfy Amsterdam I Criteria and lack features of known cancer syndromes), likely enriching for heritable susceptibility. Using filter-based approaches, we discovered rare, non-silent variants in *FAT1* that co-segregated with CRC in Ontario (Canadian), Newfoundland (Canadian) and Australian pedigrees, including one stopgain variant that was found in all 4 affected of one Ontario pedigree and in 2 of 6 unaffected. Two replication cohorts were composed of pedigrees with multiple generation CRC cases. In one, an Australian cohort of 166 families, multiple affected and unaffected members were genotyped for rare (<0.01 MAF) germline likely deleterious variants in *FAT1*. Modified segregation analysis demonstrated an overall increased risk for CRC in *FAT1* carriers (HR 1.44, 95%CI 1.05-1.97), with greater hazard ratios (>2.5) for some individual *FAT1* variants identified in sufficient numbers of families. In the other, a Polish cohort, 496 probands underwent targeted deep sequencing of *FAT1*. Using the ExAC database as controls, variant-level association analyses revealed two strongly predisposing rare *FAT1* missense variants, namely c.9440T>G (OR 7.53, 95%CI 4.1-12.8, Fisher's exact test, $q < 0.05$ by false discovery rates) and c.3067G>A (OR 10.8, 95%CI 3.3-26.8, $q < 0.05$). Gene-level association testing was also significant for increased CRC risk when considering all rare, non-silent *FAT1* variants (OR 2.2, 95%CI 1.8-2.6, $q < 0.05$), including two rare truncating mutations. A *FAT1* hemizygous transgenic mouse model was developed on a CD1 background. 30 *FAT1* hemizygous and 28 wild type mice, balanced for sex and weight, were exposed to intra-peritoneal azoxymethane (AOM) to induce colonic dysplasia. Necropsies were performed when mice were 6 months of age, and the colons underwent gross and histologic inspection. We did not find significant differences in number or greatest size of aberrant crypts, adenoma or adenocarcinoma. Immunohistochemical analyses are pendng.

Conclusions: We have shown that rare germline variants in *FAT1* may predispose to CRC. Discovery of such causal genes and associated pathways will improve our understanding of early onset CRC for both familial and sporadic cases. Stratifying CRC risk by familial susceptibility genes should foster tailored, cost-effective primary and secondary prevention strategies. This work was supported by grant from the Terry Fox Research Institute (#1063).

66. TARGETED INACTIVATION OF Rb AND p53 VIA WAP-CRE INDUCES PINEOBLASTOMA

Philip E.D. Chung[1,2], Deena Gendoo[3], Ronak Ghanbari[1,2] , Adrian Dubuc[4], Marc Remke[1,4], David Shih[1,4], Jennifer Tsui[1], Zhe Jiang[2], Livia Garzia[4], Sidney Croul[1,5], Benjamin Haibe-Kains[3], Michael D. Taylor[1,3], Eldad Zacksenhaus[1,2]

[1]*Dept. of laboratory medicine and pathobiology, University of Toronto, Toronto;*
[2]*Division 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*

**Presenting author is a graduate student*

Introduction: Pineoblastoma (PB) is the most aggressive WHO IV tumor 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 tumors with a median latency of 133 days (100% penetrance; 149 out of 149). The tumors 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 (IC_{50}=~30nM). Using our mouse model, we demonstrated strong inhibition of PB tumor 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* (IC_{50}=~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.

*The project is supported by TFRI

67. TMPRSS2:ERG FUSION CO-OPTS MASTER TRANSCRIPTION FACTORS TO DEPLOY A UNIQUE CIS-REGULATORY LANDSCAPE AND ACTIVATE NOTCH SIGNALLING IN PRIMARY PROSTATE CANCER

Ken Kron[1,*], Alexander Murison[1,*], Vincent Huang[2], Takafumi N. Yamaguchi[2], Yu-Jia Shiah[2], Stanley Zhou[1,3], Michael Fraser[1], Theodorus van der Kwast[4], Paul C. Boutros[2,3,5], Robert G. Bristow[1,3,6], Mathieu Lupien[1,2,3]

[1]Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada, M5G 1L7
[2]Ontario Institute for Cancer Research, Toronto, Ontario, Canada, M5G 0A3
[3]Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada, M5G 1L7
[4]Department of Pathology and Laboratory Medicine, Toronto General Hospital, University Health Network, Toronto, Ontario, Canada, M5G 2C4
[5]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada, M5S 1A8
[6]Department of Radiation Oncology, University of Toronto, Toronto, Ontario, Canada, M5T 1P5

Structural rearrangements leading to the TMPRSS2:ERG (T2E) fusion typify ~50% of prostate tumours and result in overexpression of the ERG transcription factor. Using T2E and non-T2E primary prostate tumours, we assessed the impact of ERG overexpression on chromatin by integrating ChIP-seq against H3K27ac, a chromatin modification found at active cis-regulatory elements, with paired genomic and expression data. We show that T2E tumours have a consistent and distinct cis-regulatory landscape to non-T2E tumours which drives their unique transcriptional profile. The T2E-specific cis-regulatory landscape is driven by ERG-mediated co-option of prostate master transcription factors HOXB13 and FOXA1 and is typified by Cluster Of Regulatory Elements (COREs), including one spreading into the ERG locus on the structurally rearranged allele. This gives rise to a cis-regulatory element within the rearranged *ERG* gene necessary for ERG expression. The unique cis-regulatory landscape in T2E primary prostate tumours also reveals the activation of the NOTCH signalling pathway. Chemical NOTCH pathway inhibition limited the invasive nature of T2E prostate cancer cells, revealing a novel therapeutic opportunity against T2E prostate tumours. Taken together, our work delineates the role of ERG over-expression in co-opting master transcription factors to deploy a unique cis-regulatory landscape inducing a dependency on NOTCH signaling in T2E prostate tumours.

68. THE DESIGN AND VALIDATION OF THE LYNCH SUPER PANEL IN ENDOMETRIAL CANCER PATIENTS: A PILOT STUDY

Leslie Oldfield[1], Tiantian Li[2], Arnavaz Danesh[2], Mark Mansour[2], Jessica Liu[2], Blaise Clarke[3], Alicia Tone[4], Sarah Ferguson[4], Trevor Pugh[1,2]

[1]MSc. Candidate, Department of Medical Biophysics, University of Toronto, Toronto, ON
[2]University Health Network, Toronto, ON
[3]Cancer Clinical Research Centre, Princess Margaret Cancer Centre, Toronto, ON
[4]Division of Gynecologic Oncology, Princess Margaret Cancer Centre, Toronto, ON

Endometrial cancer is one of the most common and lethal gynecologic cancers affecting Canadian women. Of these cases 1-3% arise from inherited cancer syndromes, including Lynch Syndrome (LS), a disease with a lifetime risk of 33-61% for endometrial cancer (EC). In Ontario, EC patients with a family history of these cancers often undergo testing for LS through analysis of mismatch repair (MMR) function by either microsatellite instability (MSI), or immunohistochemistry (IHC). Based on the results, patients are referred to genetic counselling followed by germline mutation testing for MMR-deficient or MSI-positive (MSI-H) tumours. The serial nature of current testing results is timely and limits the potential discovery of new genetic causes of LS and Lynch-associated syndromes.

To address this we have created a targeted panel for clinical and research use, termed the Lynch Super Panel. This panel simultaneously queries LS genes for germline and somatic mutation, copy number alteration, structural rearrangement, and methylation as well as infer MSI status from analysis of microsatellites. To validate the accuracy of this panel, we have started a pilot study with the overall goal of testing this panel on 170 EC and OC patients. To date, the Lynch Super Panel has been used to characterize 12 patient's disease. This has allowed us to detect somatic methylation of MLH1 in 6 patients, thus ruling out LS, detect LS in one patient, and detect LS-like syndrome in another patient.

This pilot study has begun to show the utility of the Lynch Super Panel to inexpensively and accurately diagnose LS in women. Optimizing screening methods and exploring cases with unknown etiology is crucial to preventing future cancers and to better understanding LS in women.

69. PHARMACOPx: AN R PACKAGE FOR PATIENT DERIVED XENOGRAFT DATA MANAGEMENT AND ANALYSIS

Arvind Singh Mer[1,2], Ben Brew[3,4], Wonjune Tai[3,4], Janosch Ortmann[5], Venkata Manem[1,2] Anna Goldenberg[3,4], Benjamin Haibe-Kains[1,2]

[1]Princess Margaret Cancer Centre, University Health Network, Toronto, Canada
[2]Department of Medical Biophysics, University of Toronto, Toronto, Canada
[3]Hospital for Sick Children, Toronto, Canada
[4]Department of Computer Science, University of Toronto, Toronto, Canada
[5]Centre de recherches mathématiques (CRM),University of Montreal, Canada

Finding robust biomarkers for drug response prediction is a key challenge in precision medicine for cancer. The cell line-based preclinical models of cancer have shown limited success due to their modest ability to recapitulate tumour heterogeneity and their adaptation to in-vitro growth conditions. In recent years an increasing amount of evidence has shown that patient-derived tumour xenografts (PDXs) are reliable preclinical models, as they better recapitulate tumour biology and can predict drug response with higher accuracy. This led to several efforts to create PDXs for different cancer types and conduct drug screening. However, the published molecular and pharmacological profiles of PDX lack standards and is scattered throughout. Efficient storage, access and analysis is the key to the realization of the full potential of PDX pharmacogenomic data. To address these issues, we are implementing PharmacoPx, an open-source package for integrative analysis of multiple PDX-based pharmacogenomic datasets, providing versatile functions for efficient processing, visualization and analysis of these complex data-. We demonstrate the utility of our package using recently published PDX-based pharmacogenomic data such as the Novartis PDX Encyclopedia (PDXE) and the biobank of breast cancer explants (PDTX). With increasing amount of PDX-based pharmacogenomic data, our package will open new avenues of research for integrative and meta-analysis of pharmacogenomic data.

70. ASSESSING RESPONSE TO RADIOTHERAPY IN HNSCC THROUGH CIRCULATING TUMOUR DNA DETECTION

Ariana Rostami (Trainee)[1], Caberry Yu[1], Marco DiGrappa[2], Scott Bratman[1,2,3]

[1]Department of Medical Biophysics, University of Toronto, Princess Margaret Cancer Center, Toronto, ON
[2]University Health Network, Princess Margaret Cancer Center, Toronto, ON
[3]Department of Radiation Oncology, University of Toronto, Toronto, ON

Purpose: HPV-negative and HPV-positive head and neck squamous cell carcinoma (HNSCC) have different prognosis, with the latter displaying increased survival rates and better response to treatment, which is thought to be due in part to increased radiosensitivity. Despite these differences, the same treatment regimes are applied to all HNSCC patients, leading to prolonged courses of RT for patients whom lower doses might be sufficient. In this study, we are evaluating the use of circulating-tumour DNA (ctDNA) as a real-time biomarker of radiosensitivity and response to treatment.

Methods: Four cell lines, two HPV-negative (Cal33, FaDu) and two HPV-positive (HMS-001, 93-Vu147T) were irradiated (IR) at 2, 8, and 20 Gy doses or treated with 0.5 μM staurosporine (positive control). At time-points between 6-144h post-treatment, cell lines were evaluated for caspase 3/7 activity, cell viability and ctDNA release using quantitative polymerase chain reaction. *In vitro* radiosensitivity was measured using clonogenic assays.

Results: All cell lines underwent increased caspase 3/7 activity following treatment with staurosporine. Both HPV-positive and HPV-negative cell lines displayed an increase in ctDNA release, with the latter exhibiting a more pronounced increase. Radiation response at high doses (8-20 Gy) demonstrated increased caspase activity in all cell lines as early as 72 hours post-IR. However, ctDNA release and HPV-status showed no associations, with variations in magnitude of release observed across all cell lines. Completed radiosensitivities of HMS-001 and FaDu resulted in SF2 values of 0.358 and 0.524 respectively.

Conclusions: Despite differences in HPV-status, instrinsic radiosensitivities, and overall radiation-induced apoptosis and subsequent ctDNA release, possible associations between these parameters has yet to be fully elucidated. On-going studies will attempt to determine whether other mechanisms of cell death play a more primary role. TP53 status may be a major factor in radiation-induced apoptosis that could explain the observed differences in ctDNA release.

71. PRE-CLINICAL VALIDATION OF PHARMACEUTICAL SELECTION TO ENHANCE SYNERGY WITH RADIATION

Meghan Lambie[1,*] (Trainee), Ryan Elliot[2,3], Venkata Manem[4], David Jaffrey[1,3,4], Bradley Wouters[1,4], Benjamin Haibe-Kains[1,4], Scott V. Bratman[1,4,5]

[1]Dept. of Medical Biophysics, University of Toronto, Toronto ON;
[2]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto ON;
[3]Techna Institute, Toronto ON;
[4]Princess Margaret Cancer Centre, Toronto ON;
[5]Dept. Radiation Oncology, University of Toronto ON
**Strategic Training in Transdisciplinary Radiation Science for the 21st Century (STARS21) training program scholar*

Purpose: Radiotherapy is a widely used curative modality for many cancer types, including head and neck squamous cell carcinoma (HNSCC). However, some HNSCC tumours are inherently resistant to killing by radiotherapy yet treatment causes significant side effects. Concurrent administration of radiosensitizing drugs can improve upon the efficacy of radiotherapy, but known radiosensitizing drugs provide only modest benefits and have significant side effects of their own. There is a pressing clinical need for additional novel radiosensitizers that cause less toxicity in normal tissues as a result of their use. Our goal is to identify novel radiosensitizing drugs and to ascertain genomic features within HNSCC tumours that render them sensitive to particular drug-radiotherapy combinations.

Methods: In collaboration with Venkata Kumar, a postdoctoral fellow in the Haibe-Kains Lab, we analyzed published cell line radiosensitivity data (*from Yard et al, Nature Communications, 2016*) and gene expression data (from Broad Institute) to create a gene expression signature for intrinsic radiosensitivity, which can then be applied to create a list of putative radiosensitizing drugs. For *in vitro* studies I am utilizing a novel robotic-assisted, imaging-based clonogenic assay platform developed by our collaborators, Drs. David Jaffray and Brad Wouters, in order to confirm synergistic effects and probe the molecular biology of this change. This clonogenic assay platform is designed to overcome shortcomings of the classical clonogenic assay, which is resource intensive, time consuming, and subject to biased interpretation.

Results: Meta-signatures were elucidated to determine significant pathways involved with radiosensitivity responses to radiotherapy in HNSCC. Among these pathways included the DNA damage response pathway, immune related pathways, and pathways related to stem-cell response, all previously implicated in radiation response.

Conclusions: Our signature is a promising method of predicting HNSCC *in vitro* response to radiotherapy. Ongoing work will determine whether it is capable of selecting drugs that successfully enhance synergy, by measuring changes in radiation response *in vitro*.

72. IDENTIFYING THE ROLE OF NONCODING SINGLE NUCLEOTIDE VARIANTS (SNVS) ON TRANSCRIPTION FACTORS ACTIVITY IN LIVER CANCER

Parisa Mazrooei, Tahmid Mehdi, Anna Goldenberg, Mathieu Lupien

95% of single nucleotide variants (SNVs) including somatic point mutations or single nucleotide polymorphisms (SNPs) occur in noncoding DNA is outside gene coding sequences. Non-coding SNVs directly contribute to cancer development [1], by, for example, affecting the binding intensity of proteins called transcription factors (TFs) to the DNA, thus changing the expression of genes. Previous works have revealed such a disruption for a handful of SNVs and TFs in various cancer types [2,3]; however, cancer is not likely to arise from one or two SNVs. Thousands of SNVs exist in each tumour sample the functional effects of which are unknown. Therefore, there is a growing need to identify and analyze the combinatorial effect of SNVs that affect complexes of TFs binding intensity, which we refer to as signatures.

To do so, we first infer the effect of any SNV on a TF's binding intensity to the DNA using ChIP-seq data [2]. Then, we utilize our new ensemble method for bi-clustering based on bipartite network analysis to extract the TFs-SNVs signatures. We used this approach to characterize the impact of 2,233 somatic mutations in liver cancer on the binding of 76 transcription factors to the chromatin and utilized our method to identify TFs-SNVs signatures. Our preliminary results reveal a TF-combination consisting of CTCF, Rad21 and SMC3 affected by a collection of SNVs. This combination agrees with their co-localization across the genome and their complimentary function in regulating chromatin interactions.

This work provides a framework to identify the functional contribution of noncoding SNVs in cancer based on their impact on transcription factor binding to the chromatin. It allows to identify the SNVs-transcription factor combinations most significantly affected in cancer, pinpointing the transcriptional machinery to target for therapeutic action.

References:

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73. HIGH-COMPLEXITY NEUTRAL GENOMIC BARCODING TECHNOLOGY REVEALS EXTENSIVE CLONAL DYNAMICS IN MULTIPLE CANCER MODEL SYSTEMS

Nixon AML, Brown KR, Haynes J, Donovan L, Taylor MD, O'Brien CW and Moffat J.

Increasing evidence of extensive intratumoral heterogeneity, along with advances in high-throughput in vivo functional genetic screening technologies, have together highlighted the need to observe growth in cancer models at the clonal level. To address this, we have designed, constructed and validated multiple high-diversity lentivirally delivered barcode libraries, which utilize next-gen sequencing technology to read out millions of clones in heterogeneous cancer populations. These libraries can be used to address a multitude of biological questions in many cancer model systems. To date, we have completed, sequenced and analyzed three different types of barcoding applications to explore clonal dynamics in a variety of human cancer models. First, we tested the serial limiting dilution analysis (LDA) assay for tumor initiating cells (TICs) in PDX colon tumor cells. In the LDA assay, a range of cell dilution, down to a single cell, are transplanted into immunocompromised mice. The TIC frequency is calculated using a single-hit model from the proportion of tumors established at each cell dose including the limiting dose. However, quantifying minimum cell numbers required for tumor formation does not reveal the actual diversity of clonal contribution during tumour engraftment and progression. In fact, recent research suggests that the single-hit model, which assumes a static hierarchy of initiating cells, may not be biologically relevant as many cells may have the potential to form or contribute to a tumor given a specific environment, or in cooperation with another clone. Our barcoded, serial LDAs show interesting and informative patterns of clonal dynamics. Second, we have performed clonal lineage tracing on established human cancer cell lines in vitro compared to in vivo. Our findings demonstrate the presence of TICs in standard cell lines and serve as precursors to future in vivo CRISPR-Cas9 screens by defining upper limits of library size. Finally, we used a patient-derived xenograft (PDX) model of brain tumor metastasis to investigate the presence of pre-existing metastatic clones with site-specific homing abilities. Overall, given the degree of heterogeneity present in cancer models, we propose the use of barcode technology to validate novel cancer targets, including TIC markers in the LDA assay. Furthermore, we suggest the use of barcodes for careful optimization of the system before large functional genomic screens in animal models.

74. LIPID-STABILIZED NANOEMULSIONS AS VASCULAR CT CONTRAST AGENTS FOR IN VIVO IMAGING

Ana Maria Jaimes Castillo[1], Elizabeth Huynh[5], Juan Chen[4], Gang Zheng[1,2,3]

[1]Leslie Dan Faculty of Pharmacy, University of Toronto, Ontario, CA
[2]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Ontario, CA
[3]Department of Medical Biophysics, University of Toronto, Ontario, CA
[4]Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, CA
[5]Department of Radiation Oncology, Harvard Medical School, Boston, MA, U.S.A

Purpose: X-ray computed tomography (CT) enables rapid, high-resolution acquisition of anatomical images. Clinically, exogenous contrast agents are administered that provide enhanced X-ray attenuation and therefore, visualization of blood vessels and organs. Currently, vascular CT contrast agents are iodine-containing small molecules, which are effective in enhancing X-ray attenuation. However these agents are rapidly cleared from the body and thus, they must be administered in high doses for effective imaging to take place, which often raise chemotoxicity concerns. This study designs a nanoparticulate CT contrast agent that may overcome these limitations to improve contrast-enhanced CT imaging in cancer applications.

Methods: A lipid-stabilized nanoemulsion with specific contrast properties for vascular CT imaging was developed. The key building block of this formulation is Lipiodol®, an FDA approved CT contrast agent composed of iodinated poppy seed oil. Using microfluidic processing and phospholipid surfactants, Lipiodol® was dispersed in aqueous media, forming nanodroplets, termed Lipiodol-LNE. The formulation was then optimized and its stability and morphology characterized. The X-ray attenuating properties were assessed in vitro as well as in vivo.

Results: Lipiodol-LNE particles were monodisperse, ~80nm in size and stable for over 100 days under 4°C storage conditions. This was validated through transmission electron microscopy. The formulation was also found to be stable in serum. The effective iodine concentration (mg/ml) in Lipiodol-LNEs was compared with a clinically approved iodine-based agent in a μCT phantom, and was significant enough for contrast to be achieved. In a pilot μCT mice imaging study, differentiation of soft organs was possible post Lipiodol-LNE delivery.

Conclusion: Lipiodol-LNEs were fabricated and characterized as a unimodal nanoparticulate CT contrast agent. Incorporating poorly soluble drugs or imaging markers for other modalities into the nanoemulsion droplets' hydrophobic core opens up the possibility of Lipiodol-LNEs being a platform to eventually develop a multimodal/theranostic vascular CT contrast agent library.

75. FOLATE RECEPTOR-TARGETED PHOTODYNAMIC PRE-TREATMENT TO ENHANCE TUMOUR NANOPARTICLE ACCUMULATION

Marta Overchuk[1], Kara Harmatys[2], Juan Chen[2], Gang Zheng[2,3]

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

Presenting author is a trainee (PhD candidate). This work is supported by the TFRI.

Rapid development of nanotechnology over the past two decades has led to the introduction of several cancer nano-therapeutics into clinical practice. While encapsulation into a nanostructure alleviates some drug-associated off-target toxicities, therapeutic efficacy of the currently available nanomedicines remains suboptimal. Multiple studies demonstrate that only a small percentage of the nanoparticle injected dose is able to reach the tumour. High interstitial fluid pressure, poor blood perfusion and high cell packing density collectively impede nanoparticle extravasation and interstitial diffusion, significantly decreasing their therapeutic potential. Therefore, a novel strategy that can locally alter tumour microenvironment and enhance nanoparticle delivery to the tumour is in high demand.

A variety of studies have investigated the ability of radiation therapy, hyperthermia and chemotherapy pre-treatment to facilitate nanoparticle accumulation in tumours and achieve better therapeutic results. Photodynamic therapy is a versatile cancer treatment strategy, exploiting the combination of a photoreactive molecule (photosensitizer) and an external light source in order to generate reactive oxygen species and induce cell death. Recent studies demonstrate the potential of photodynamic therapy, enabled by a targeted antibody-photosensitizer conjugate, to enhance nanoparticle delivery in subcutaneous tumour mouse models.

Dr. Gang Zheng's group previously reported the development of a highly selective photodynamic therapy agent, consisting of a porphyrin-derived near-infrared photosensitizer conjugated via a peptide linker to a folate-targeting ligand. In the current study we explored the potential of this photosensitizer for the photodynamic pre-treatment of tumours to enable subsequent fast and efficient nanoparticle delivery. Such an approach is expected to decrease tumour cell density and alleviate solid tissue pressure, resulting in the improved nanoparticle extravasation. We have investigated various treatment parameters in order to achieve a balance between efficient cell death induction and preservation of vascular structures, crucial for systemic nanoparticle delivery.

Overall, state-of-the-art light delivery technologies enable access to a variety of folate receptor-positive solid tumours, while simple chemistry of the developed agent makes it an appealing candidate for clinical translation. Folate-targeted photodynamic pre-treatment holds the potential to become a relatively simple tool for the enhancement of cancer nanochemotherapy.

76. MEASUREMENT OF DYNAMIC HYPOXIA AT SINGLE CELL RESOLUTION USING SEQUENTIAL LABELING OF ISOTOPOLOGOUS PROBES (SLIP)-MASS CYTOMETRY TECHNIQUE

Ravi N. Vellanki[1], Landon J. Edgar[2], Trevor D. McKee[1], David Hedley[1], Mark Nitz[2], Bradly G. Wouters[1]

[1]Departments of Radiation Oncology, Medical Biophysics, and the STTARR Innovation Centre, Princess Margaret Cancer Centre, University Health Network, Toronto, Canada
[2]Department of Chemistry, University of Toronto, Toronto, Canada

Hypoxia is a common and heterogeneous feature of tumors caused by an imbalance between oxygen supply and consumption. Hypoxic regions have been found in the majority of solid tumors, and high levels of hypoxia are associated with metastasis and poor response to chemotherapy and radiotherapy. There have been extensive efforts made to develop tools to measure hypoxia in patients' tumors as a means to select patients to evaluate new therapeutic approaches. However, none of these methods are currently capable of assessing changes in tumor oxygenation over time. Tumor sub-regions may experience repeated cycles of hypoxia/reoxygenation due to intermittent changes in blood flow (cyclical hypoxia) leading to the formation of a highly dynamic microenvironment, with important implications on both cell phenotype and tumor progression. Changes in tumor oxygenation also occur during therapy, with unknown consequences on response. Consequently, there is a need to develop robust and reproducible methods of detecting and quantifying dynamic changes in oxygenation.

We report the development of a series of tellurium containing isotopologous probes for measurement of cellular hypoxia that are compatible with imaging mass cytometry (IMC) technology. The tellurium isotopes are used to quantify bound hypoxia probes through the use of either flow or imaging based atomic mass spectrometry. Using isotopes of tellurium, analytically distinguishable but pharmacologically identical versions of this hypoxia probe were used for measurement of dynamic changes in cellular hypoxia in tumors in vivo. Sequential labeling with isotopologous probes (SLIP) injected in mice bearing pancreatic tumor xenografts, revealed dynamic changes in cellular oxygenation state that correlated with the distance from vasculature, the proliferative potential of cell populations, and proximity to necrosis. These probes were also able to demonstrate dynamic changes in oxygenation introduced following application of therapeutic agents or changes to the oxygen concentration of inhaled gas. These new TELOX probes set the stage for quantitative analysis of cellular oxygen dynamics in tumors.

77. IFN-γ SIGNALLING RENDERS CBL-B DEFICIENT CD8+ T CELLS UNRESPONSIVE TO REGULATORY T CELL IMMUNOSUPPRESSION

Douglas C. Chung[1*], SeongJun Han[1,2*], Pamela Ohashi[1,2,3].

[1]Princess Margaret Cancer Center, University Health Network, Toronto ON, Canada, M5G 2M9
[2]Department of Immunology, Faculty of Medicine, University of Toronto, Toronto, ON, Canada, M5S
[3]Ontario Institute for Cancer Research, Toronto, ON, Canada, M5G 1L7

** Research trainees*

Adoptive cell therapy (ACT) utilizes patient-derived $CD8^+$ T cells to mount anti-tumour response, leading to tumour regression and increased survival in cancer patients. Despite recent advances, ACT is limited by the presence of immunosuppressive regulatory T cells (Treg) within solid tumours. Previous attempts to overcome immunosuppression by systemic depletion of Tregs have led to severe autoimmune toxicities. As an alternative approach, we are investigating the adoptive transfer of genetically modified $CD8^+$ T cells to enhance anti-tumour immune response. Cbl-b is an E3 ubiquitin ligase that negatively regulates TCR signalling. Ablation of Cbl-b in $CD8^+$ T cells have been shown to enhance proliferation and induce an inflammatory phenotype, however it's role in evading immunosuppression has not been extensively explored. The primary objective of this study was to investigate the mechanism of Cbl-b deficient $CD8^+$ T cell resistance against Tregs. First, Cbl-$b^{-/-}$ CD8 T cells were characterized by surface marker expression and cytokine secretion profile. Furthermore, Treg suppression assays (TSA) were conducted with $CD8^+$ T cells from either C57BL/6 or Cbl-$b^{-/-}$ mice to determine the role of Cbl-b dependent resistance against Tregs. We demonstrated that Cbl-$b^{-/-}$ CD8+ T cells had an increased expression of activation markers (CD25 and ICOS), hyper-secretion of pro-inflammatory cytokines (IFN- γ, TNF-α and IL-2), and were unresponsive to Treg immunosuppression. Interestingly, exogenous introduction of IFN-γ was sufficient in inducing Treg resistance specifically in WT $CD8^+$ T cells. Accordingly, this effect was lost when IFN-γ signalling was abrogated by use of neutralizing antibodies. Finally, TSA performed using $CD8^+$ T cells or Tregs from IFN-γ$R1^{-/-}$ mice demonstrated that IFN-γ confers Treg resistance in an autocrine-dependent manner. We report that ablation of Cbl-b results in IFN-γ dependent $CD8^+$ T cell resistance against Treg cells. While IFN-γ has previously been shown to enhance anti-tumour immunity by enhancing T cell activation, this study is the first to demonstrate a role of IFN-γ in rendering $CD8^+$ T cells unresponsive to immunosuppression by Tregs.

78. IMPROVING IMMUNOTHERAPY THROUGH EPIGENETIC MODULATION IN TRIPLE-NEGATIVE BREAST CANCER

Ingrid Kao [1,2], Ali Madani [1,2], Pam Ohashi [1,2,3], Benjamin Haibe-Kains [1,2,3], Mathieu Lupien [1,2,3*]

[1]The Princess Margaret Cancer Centre-University Health Network, Toronto, Canada M5G 1L7.
[2]Department of Medical Biophysics, University of Toronto, Canada M5G 1L7.
[3]Ontario Institute for Cancer Research, Toronto, Canada M5G 1L7.

Purpose: Patients with triple negative breast cancer (TNBC) have the lowest survival rate among all breast cancer subtypes. Therefore, new curative therapeutic approaches are urgently required. Recent studies suggest that patients expressing more mutant peptides respond better to immunotherapy. However, mutations in silent or lowly expressed genes remain undetected by the immune system. Given the fact that gene silencing involves epigenetic processes, new chemical reagents have been invented to modulate DNA or histone methylation. In this study, we aim to increase recognition and elimination of tumour cells by the immune system, by using epigenetic therapy to re-express lowly expressed mutated genes in TNBC, thus improving the outcome of immunotherapy.

Method: 1. Delineating the mutational burden of lowly expressed genes and immune related pathways in TNBC using RNA and exon sequencing data from The Cancer Genome Atlas (TCGA).
2. Induce and assess mutation expression in lowly expressed regions by treating TNBC cell lines with epigenetic chemical probes (EZH2/G9a/DNMT inhibitors).
3. Perform *in silico* immunogenicity scoring on target mutated peptides to identify potential immunogens.
4. Assessing the efficacy of epigenetic chemical probes to increase the immune response against breast cancer using a TNBC mouse model.

Results: Patient data from TCGA shows high mutational burden within silent and poorly expressed genes in TNBC patients. In addition, we found that immune related pathways are lowly mutated and highly expressed. Furthermore, TNBC cells treated with EZH2 inhibitors show decrease in H3K27 trimethylation, possibly leading to re-expression of genes regulated by these marks.

Conclusions: In conclusion, we reported that TNBC harbors lowly expressed genes with high mutation rates, therefore, is a suitable target for epigenetic therapy. We are continuing to test chemical probes and to analyze the effect of epigenetic therapy on the efficiency of immunotherapy.

79. UTILIZATION OF METHYLATED CIRCULATING TUMOUR DNA IN ORAL SQUAMOUS CELL CARCINOMA FOR RISK STRATIFICATION AND DETECTION OF RECURRENCE

JUSTIN BURGENER[1,2], Daniel DeCarvalho[2], Scott Bratman[2]

[1]*Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada*
[2]*Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada*

Background: Gene-specific DNA methylation occurs early in the development of oral squamous cell carcinoma (OSCC); thus, methylation is a promising diagnostic and prognostic biomarker for OSCC. PCR-based methods have been used with limited success to interrogate individual methylation marks within circulating tumour DNA (ctDNA) from OSCC patients. Although cost prohibitive, genome-wide methylation mapping of ctDNA by whole genome bisulfite sequencing improves sensitivity by simultaneously identifying many cancer-specific methylation marks. Hence, there is a need for inexpensive ctDNA detection methods capable of analyzing genome-wide methylation in OSCC

Methodology: cfMeDIP-seq is a novel method for genome-wide DNA methylation analysis from low-input specimens, such as cell-free DNA. This method has been used to detect tumour-specific differentially methylated regions (DMRs) within cell-free DNA. The clinical utility of cfMeDIP-seq to predict diagnosis, treatment response, and prognosis has yet to be assessed in clinical studies.

Hypothesis and Aims: Genome-wide methylation mapping of OSCC ctDNA by cfMeDIP-seq will be highly accurate, predict recurrence, and identify methylation-based prognostic signatures.

Aim 1: Determine sensitivity and specificity of cfMeDIP-seq for ctDNA detection in OSCC patients. Cell-free DNA has been isolated from pre-treatment plasma samples of OSCC patients and risk-factor matched healthy donors. For each OSCC patient, primary tumour and adjacent normal epithelium has been isolated from banked surgical specimens. Methylation profiles for plasma and tissue samples were generated by cfMeDIP-seq and WGBS respectively. DMRs have been identified between OSCC vs. healthy donor cfDNA, and primary tumour vs. normal epithelium.

Aim 2: Determine the prognostic significance of ctDNA detection by cfMeDIP-seq in OSCC patients following curative treatment. DMRs within post-treatment cell-free DNA have been analyzed by cfMeDIP-seq. Plasma samples taken 1 month, 3 months, and 12 months after surgery will be analyzed. The presence of residual detectable ctDNA will be compared with clinical outcomes (i.e., recurrence and overall survival). The strength of prognostic association will be estimated by Cox proportional hazards. Lead-time for detection of clinical recurrence by ctDNA will also be determined.

Aim 3: Build a signature of OSCC-specific DMRs detectable within cell-free DNA with the greatest prognostic association. Detectable DMRs from pre- and post-treatment plasma will be ranked, and DMR signatures with improved prognostic power will be determined. Pre-treatment plasma from additional OSCC patients will be used for validation of these signatures.

Conclusion: Survival of OSCC patients has improved relatively little over the last 25 years. Our work demonstrates the clinical use of cfMeDIP-seq with potential applications in earlier OSCC detection, treatment guidance, and post-treatment disease monitoring.

80. A ROLE FOR NEK10 IN THE DNA DAMAGE RESPONSE

Nasir Haider[1,2,3], Previn Dutt[2,3], Vuk Stambolic[1,2,3]

[1]Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada.
[2]Princess Margaret Cancer Center, Toronto, ON, Canada.
[3]University Health Network, Toronto, ON, Canada.

Presenting Author is a Graduate Student

The DNA Damage Response (DDR) is a cellular process responsible for the maintenance of genomic stability in response to a host of endogenous and environmental insults. Although the roles of many major players in DDR have been elucidated, recent work has uncovered key functions for the members of the NIMA Related Kinase (Nek) family, such as Nek1, Nek11 and Nek10. Nek10 is a largely uncharacterized member of the Nek kinase family that has been implicated in the response to UV irradiation. Moreover, lowered expression of Nek10 has been associated with poor breast and lung cancer prognosis and higher tumour grade.

However, Nek10's function in response to other clinically relevant DNA damaging agents remains uncharacterized.

To directly probe the function of Nek10 in the DDR, using CRISPR/Cas9 methodology, we have developed Nek10-deficient human cancer cell lines (*Nek10* Δ/Δ). These cells display an increase in proliferation, accompanied by a decrease in p21 protein and mRNA levels. Moreover, Nek10 Δ/Δ cell lines have increased sensitivity to cisplatin and olaparib, both DNA damaging agents. In response to ionizing radiation, Nek10 Δ/Δ cells displayed less sustained Chk2 and p53 activation, and a simultaneous delayed increase in p21 protein and mRNA levels. Curiously, although both Nek10 +/+ and *Nek10* Δ/Δ cells present with similar levels of □H2AX foci upon irradiation, indicative of comparable levels of sustained DNA damage, *Nek10* Δ/Δ cells display fewer Rad51 and BRCA1 foci. Collectively, these data point towards a potential defect in p53 and p21 mediated homologous recombination repair in the context of Nek10 loss. Given that Nek10 has been shown to be mutated in multiple cancers, understanding its role in the DDR, may aid in the development of novel treatment strategies for tumours exhibiting Nek10 mutation.

81. EXAMINING THE ROLE OF PROTEIN KINASE C IOTA IN BREAST CANCER GROWTH AND METASTASIS

Jennifer L. Gorman[1], Jami E. Kronick[1], Emma M. Jones[1], Rhea Jangra[3], Jessica R. Adams[3], Sean E. Egan[3,4] and James R. Woodgett[1,2]

[1]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada
[2]Medical Biophysics, University of Toronto, Toronto, Ontario, Canada
[3]Hospital for Sick Children Research Institute, Toronto, Ontario, Canada
[4]Lab Medicine & Pathobiology, University of Toronto, Toronto, Ontario, Canada

Protein Kinase C Iota (PKCι) is a serine/threonine kinase, activated downstream of PI3K, that plays an important role in cell survival, apico-basal polarity, and invasion. Examination of human breast tumours has revealed that PKCι expression is elevated early in tumour development and remains over-expressed in metastatic lesions, making it a potential shared maintenance gene. Since the products of such genes represent excellent therapeutic targets, affecting all stages of tumour growth, we examined the affect of PKCι knockout on primary and metastatic tumour growth, with 4 possible outcomes; importance solely for primary tumour development, requirement for metastatic spread but not primary growth, impact on all tumour growth or no overall effect.

CRISPR/Cas9 technology was used to generate PKCι knockout MDA-MB-231 cell lines, with gRNAs designed to target exon 2 or exon 5 of the gene. To assess the affect of PKCι knockout on tumour growth and metastasis, control and knockout cell lines were injected into the #4 mammary gland of immune-compromised mice, with growth tracked for up to 6 months.

Xenograft experiments have revealed stark differences in the development of primary tumours across various PKCι knockout cell lines. Of the 4 knockout cell lines examined, 2 (F22 and F40) develop primary tumours at a similar rate to controls, while the other 2 (F33 and F39) fail to develop palpable tumours. Histological examination of the primary tumours revealed no differences between control and F22/F40 tumours, with a low rate of metastasis observed in these mice.At 6 months post injection, only 1 mouse injected with F33/F39 cells showed a small developing mass, with all other mice displaying normal gland histology.Further studies *in vitro* are being undertaken to ascertain the differences between the knockout cells lines, in order to understand the differences in tumour development observed *in vivo*.

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82. FLUORESCENT MONITORING OF LIPID-BASED NANOMEDICINES USING STABLE PORPHYRIN J-AGGREGATES

Danielle M Charron [a,b,†], Richard Liu [a,c,‡], Gang Zheng[a,b,d,*]

[a]*Princess Margaret Cancer Research Centre, University Health Network, Toronto ON*
[b]*Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto ON*
[c]*Faculty of Science, McGill University, Montreal QC*
[d]*Department of Medical Biophysics, University of Toronto, Toronto ON*

†PhD student
‡Undergraduate summer student mentored by DMC
**Corresponding author: gang.zheng@uhnres.utoronto.ca*

Nanomedicine delivery vehicles have widely demonstrated heterogeneous tumour accumulation in animal studies and clinical trials. New imaging techniques that can dynamically monitor nanomedicines during target nano-bio interactions (e.g., tumour cell uptake) have the potential for significant positive impact on preclinical nanomedicine development and personalized treatment planning. We propose the use of porphyrins as labelling tools for monitoring lipid-based nanomedicine stability, by taking advantage of their nanostructure-dependent ratiometric fluorescence. Conjugating porphyrins to lipids enables their self-assembly into lipid-based nanomedicines such as liposomes, lipoproteins, and microbubbles. Nanoscale porphyrin supramolecular interactions within the lipid layer red-shift porphyrin fluorescence through an exciton coupling interaction called J-aggregation. As a result, the intact and disrupted nanomedicines are encoded with unique emission spectra. Maintaining stable J-aggregation in vivo has so far been difficult due to the inherent sensitivity of porphyrin-lipid packing to complex biological environments. Previous approaches to enhancing J-aggregate stability have focused on introducing or strengthening specific interactions between the porphyrins, such as hydrogen bonding or metal-ligand coordination. Using a liposome system, we systematically substituted the host lipid to reveal differences in J-aggregate formation by two different porphyrin-lipids: pyropheophorbide-lipid and bacteriochlorophyll-lipid. Here, we show that the formation and stability of porphyrin-lipid J-aggregates is modulated by the host lipid environment, but the nature and extent of this dependence is dictated by the specific porphyrin. Pyropheophorbide-lipid forms J-aggregates having positive chirality in a concentration dependent manner, with maximal conversion to aggregate optical properties when host and porphyrin-lipid have matching carbon chain lengths. In contrast, bacteriochlorophyll-lipid forms concentration independent J-aggregates having negative chirality in all host lipid environments studied, but has a stronger negative impact on liposome physical stability. We postulate that these differences result from the porphyrin nanodomain size required for efficient exciton coupling. This discovery opens the opportunity for inducing stable J-aggregate formation of poorly-aggregating porphyrins by tuning the lipid environment. Our approach involving the substitution of host lipids in liposomes is applicable to other lipid-based drug delivery vehicles as well as lipid-coated inorganic nanoparticles, which will lead to further insights concerning porphyrin-lipid J-aggregate stability for optical imaging and theranostic applications.

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83. OPTIMIZING THE VACCINIA BACKBONE FOR USE AS AN ONCOLYTIC VIRUS USING INSERTIONAL MUTAGENESIS§

Brian A. Keller[1,2,*], Russell Barkley[2], Adrian Pelin[1,2], Jiahu Wang[2], Fabrice LeBoeuf[2], Carolyn Nessim[3], Carolina Ilkow[2], Harry L. Atkins[1,2], John C. Bell[1,2]

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

**Graduate student;*
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Oncolytic viruses (OVs) are an emerging class of multi-mechanistic biologic cancer therapeutics designed to: (1) directly lyse cancer cells, (2) destroy tumour vasculature, and (3) induce anti-tumour immunity. This latter effect has been demonstrated to have therapeutic efficacy on existing sites of disease, prevent further metastases, and provide immune-mediated surveillance. Poxviruses are being tested clinically in a variety of settings, however a systematic analysis of the types of virus gene mutations/deletions that favour both oncolytic activity and immune stimulation has not been previously completed. We have identified Copenhagen as the fittest clinical candidate strain of Vaccinia virus, and utilized a transposable element to randomly mutagenize the Copenhagen genome. With next-generation sequencing (NGS), we confirmed that we have generated a library of 89 unique Vaccinia virus clones and inserted into 52 unique genes, from which we have developed individual clonal stocks. We have demonstrated a loss-of-function that is caused by insertion of our transposable element into Vaccinia virus and have begun characterizing our library of novel clones. To date, we have identified several clones of interest as oncolytic candidates based on novel phenotypes in primary patient-derived and immortalized cancer cell lines (replication, cytotoxicity, spread, specificity). We are in the process of investigating the potential of our novel clones to initiate or augment existing anti-tumour immunity. Currently, our lead candidates are being more completely characterized and we have begun generating individual backbones with combined mutations of interest. We hypothesize that this may provide additive phenotypic effects and lead to the optimal oncolytic Vaccinia virus backbone.

Conclusions: We have generated a unique library of viral clones that have allowed us to probe the Vaccinia genome more comprehensively than previously possible. Using this tool, we have gained fundamental insights into the eradication and prevention of metastatic disease and recurrent cancers. We have identified several candidate clones that demonstrate more desirable oncolytic characteristics than the current clinical standard Vaccinia-based OV. We have also identified novel gene functions of previously described and undescribed Vaccinia virus genes. This tool has the potential to impact the fields of oncology, virology, and vaccine development by providing novel fundamental insights into the biology of human cancers and Vaccinia virus.

84. TARGETING LACTATE TRANSPORTERS MCT-1 AND MCT-4 INHIBITS HNSCC CELL GROWTH IN VITRO

Pedro Boasquevisque [1,2], Marianne Koritzinsky [1,3,4], and Bradly G. Wouters[1,2,3].

[1]*Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada*
[2]*Department of Medical Biophysics, University of Toronto, Toronto, Ontario Canada*
[3]*Department of Radiation Oncology, University of Toronto, Toronto, Ontario, Canada*
[4]*Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada*

(Graduate Student)

Purpose: Tumours display an aberrant metabolism when compared to healthy tissues, one prominent aspect of this being increased glucose consumption and concomitant lactate production. High tumour lactate levels have been associated with aggressive, treatment-resistant disease, particularly in head and neck squamous cell carcinoma (HNSCC). In order to sustain this typical metabolic profile, tumour cells must export the lactate generated through the action of the integral membrane proteins monocarboxylate transporters 1 and 4 (MCT-1 and MCT-4). We hypothesize that inhibition MCT-1 and MCT-4 will selectively target the growth of tumours displaying such altered metabolism.

Methods: 74B (HNSCC cell line) cells had MCT-1 and MCT-4 loss-of-function achieved through CRISPR-Cas9 mediated genetic ablation. Cells were grown for 5 days under conditions of 21% O_2 (normoxia) in a regular CO_2 incubator or 0.2% O_2 (hypoxia) in a hypoxia chamber. Cell proliferation was measured under normoxia and hypoxia through the use of IncuCyte automated imaging. The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of cells was measured using a Seahorse® XF analyzer.

Results: The use of CRISPR-Cas9 to knockout MCT-1 and MCT-4 allowed us to isolate individual clones where these membrane transporters could no longer be detected through immunoblotting. Validation of MCT-1 functional knockout was achieved by verifying that clones were completely resistant to a lethal dose of 3-bromopyruvate (a toxic molecule transported inside cells by MCT-1). Measurements of ECAR showed a decreased ability to export lactate in all MCT-1 and MCT-4 knockouts. Additionally, the loss of MCT-1 and MCT-4 did not significantly alter cell proliferation under normoxic conditions, but MCT-4 loss under hypoxia produced a significant growth-impairment in the loss-of-function clones.

Conclusion: The inhibition of MCT-4 is capable of functionally impairing cancer cell lactate export and has a significant effect on the ability of HNSCC cells to grow *in vitro* under hypoxic conditions. Our findings warrant further analysis in vivo of MCT targeting in addition to verifying a synergistic effect of inhibiting both MCT-1 and MCT-4 by a double knockout mechanism.

85. EVALUATING THE FEASIBILITY AND SPECIFICITY OF PIMONIDAZOLE AS A SPECIFIC MARKER OF HYPOXIA IN MALIGNANT GLIOMAS

Sheila Mansouri, Julie Metcalf, Takyee Tung, Mark R. Wilson, Farshad Nassiri, Alireza Mansouri, Kenneth Aldape, Gelareh Zadeh

MacFeeters Hamilton Center for Neuro-Oncology Research and Princess Margaret Cancer Center, Toronto, Ontario, Canada

Malignant gliomas are the most frequent primary brain tumour in adults and are also the most aggressive. Despite recent therapeutic advances, malignant gliomas are notoriously resistant to treatment and the survival time of patients is between 3-8 years for low-grade and anaplastic gliomas, and 6-12 month for glioblastoma. Increasing malignancy of gliomas correlates with an increase in cellularity and a poorly organized tumour vasculature leading to insufficient blood supply, hypoxic areas and ultimately to the formation of necrosis, which is a characteristic of glioblastoma. Hypoxia is a predominant feature of malignant glioma microenvironment, and it is associated with the tumour growth, progression, and resistance to conventional chemotherapy and radiation. Hypoxia induces direct or indirect changes in the biology of a tumour and its microenvironment through the activation of specific transcription factors, leading to increased aggressiveness and tumour resistance to chemotherapy and radiation. Current clinic-pathological markers are insufficient to identify patients at risk of treatment failure. Therefore, a hypoxia biomarker would be of value for clinical decision-making. The exogenous hypoxia marker pimonidazole is a 2-nitroimidazole compound, which forms covalent bonds with cellular macromolecules at oxygen levels below 1.3% and demonstrates poorly oxygenated regions in histological sections from tumours. In the present study, we have investigated the feasibility and specificity of pimonidazole as a specific marker of hypoxia in higher-grade glioma patients treated with pimonidazole preoperatively.We have quantified the extent of pimonidazole staining in tumour sections and determined its correlation with presence of other hypoxia markers such as carbonyl anhydrase IX.We have also determined the characteristics of tumour vasculature and its association with extent of pimonidazole staining. Our ultimate goal is to establish the association between pimonidazole uptake and pattern of staining in malignant glioma tumours with patient clinical outcome in order to establish pimonidazole as a prognostic marker for higher grade gliomas.