



# Cancer Research Program

## Summer Student Research Day

August 26<sup>th</sup>, 2016

Centre universitaire  
de santé McGill  
Institut de recherche



McGill University  
Health Centre  
Research Institute

## Agenda

- 9:00-9:05** Welcome by Dr Peter Metrakos, CRP Program Leader
- 9:05 - 10:20** **Marissa Le Gallee**  
*Genomic alterations in prostate cancer metastases*
- Noémie Pothier**  
*Development of multiplex four-color fluorescence IN SITU hybridization (FISH) assay to detect MYC gain and PTEN deletion in prostate cancer tissues*
- Anthony Smith**  
*An immunohistochemical study in carcinoembryonic antigen cell adhesion molecule 1 in colorectal cancer liver metastases*
- Jack Mouhanna**  
*Circulating neutrophil extracellular traps as potential biomarkers for thoracic malignancies: a patient-specific therapeutic approach*
- Klaudiusz Stoklosa**  
*The immunoprofile as a prognostic tool in metastatic liver disease through specification of histological growth patterns*
- 10:20-10:45** **Coffee Break**
- 10:45-12:00** **Thomas Rosin**  
*Automated identification of growth patterns in liver metastases using dynamic contrast-enhanced MRI and shape analysis*
- Ambike Aarti Srivastava**  
*Correlation of clinical-pathological features with engraftment of pancreatic cancer in mice*
- Jérémie Richard**  
*A PIL (Purpose In Life) to live better with cancer: Results from a group psycho-educational intervention to improve emotional and existential wellbeing during treatment for cancer*

**Joseph DeCunha**

*Was dosimetry to blame? A retrospective analysis of beta emitting sources in intravascular brachytherapy*

**Julien Bancheri**

*Energy dependence of a graphite probe calorimeter for use in absolute clinical radiation dosimetry*

**12:00-1:30      Lunch**

**1:30              Presentation of awards**

**Acknowledgements**

The Cancer Research Program would like to thank volunteer judges Dr Nathalie Lamarche-Vane, Dr John Kildea and Dr Axel Thomson for evaluating the oral presentations.

## Genomic Alterations in Prostate Cancer Metastases

Marissa Le Gallee, Karl-Philippe Guérard, Eleonora Scarlata, Simone Chevalier, Jacques Lapointe

The Research Institute of the MUHC, McGill University

**Background:** Prostate cancer (PCa) is a disease that affects a large proportion of the male population in North America and better diagnostic tools are necessary to help identify early PCa with high risk of further progression. Our research team wishes to compare the genetic makeup of primary and metastatic tumour samples in the hopes of finding genetic similarities that could be detected early in diagnoses. Specific DNA copy number gains and deletions have been identified in metastatic PCa samples such as chromosome 16p13.3 gain and 16q23 deletion. Such genomic alterations have also been detected in primary tumors, but their respective contribution to the metastatic process remains unknown. The goal of this project is to survey PCa metastases and corresponding primary tumor samples for 16p13.3 gains and 16q23 deletions. Based on the results of the laboratory's previous studies, we hypothesize that these genomic alterations may be involved in disease progression and thus would be found in both the primary and the corresponding metastases.

**Methods:** Bacterial artificial chromosome (BAC) probes were fluorescently labelled along with control centromere probes to assess 16p13.3 gain and 16q23 deletion status by fluorescence in situ hybridization (FISH) in a small tissue microarray (TMA) including nine lymph node metastases and their corresponding primary tumors.

**Results:** The probes' specificity and their optimal fluorochrome combination for the assay were first tested on normal lymphocyte cells halted in metaphase. FISH is being performed on a TMA section and will be scored as previously reported by our laboratory.

**Conclusion:** Our set of FISH probes has shown sufficient specificity on metaphase spread to warrant its use in tissue sections. Whether the gain of 16p13.3 or the deletion of 16q23 can be retrieved in both primary and metastatic tissues will be evaluated once the TMA scoring is completed.

Research Project was funded by: Dr. Clarke K. McLeod Memorial Scholarship

# **DEVELOPMENT OF A MULTIPLEX FOUR-COLOR FLUORESCENCE IN SITU HYBRIDIZATION (FISH) ASSAY TO DETECT MYC GAIN AND PTEN DELETION IN PROSTATE CANCER TISSUES**

Noémie Pothier, Karl-Philippe Guérard, Jacques Lapointe

The RI-MUHC – Glen Site, Department of Surgery – Division Urology, Faculty of Medicine, McGill University, Montreal, Qc, Canada

## **Background**

Prostate cancer (PCa) is the most common cancer and the third leading cause of cancer death in Canadian men. Distinguishing the indolent form from the aggressive form of PCa with high metastatic potential remains a major challenge in the clinical field. There is therefore a need for new prognostic tools to provide for a risk-adapted therapy. DNA copy number alterations (CNAs) have been identified and associated with progression of PCa including the gain of oncogene MYC (8q24) and the loss of tumor suppressor PTEN (10q23). Traditionally, fluorescence in situ hybridization (FISH) is performed on tissue sections to assess one CNA per assay, thus limiting the number of CNAs that can be analyzed with the small amount of tissue obtained from diagnostic biopsies and research specimens. The goal of this project is to develop a multiplex FISH assay to assess MYC gain and PTEN deletion that would perform as well as the single CNA assay and serve to further assess the molecular heterogeneity of PCa.

## **Method**

A four-color FISH assay was developed by combining fluorescently labelled bacterial artificial chromosome (BAC) probes for MYC and PTEN with commercial fluorescent control chromosome 8 and 10 centromere probes. FISH was performed on normal lymphocyte metaphase spreads and on formalin-fixed paraffin-embedded (FFPE) PCa tissue samples represented on a small tissue microarray (TMA), which included twelve radical prostatectomy specimens and four transurethral resections of the prostate (TURP) for advanced disease.

## **Results**

The four-color FISH showed similar signal specificity and intensity as the dual-color FISH for MYC or PTEN on normal metaphase spreads. The assay was optimized for FFPE tissue samples and sufficient signal intensity was obtained to assess both MYC and PTEN status. Results of the TMA FISH are currently under analysis.

## **Conclusion**

The new four-color FISH assay was able to detect MYC gain and PTEN deletion. This multiplex tissue biomarker assay is promising at reducing the amount of tissue required for the analysis of multiple CNAs and exploring tumor heterogeneity. The assay should be tested on more PCa samples, for which we have the clinical follow up, to evaluate its prognostic value.

Funded by Mach-Gaensslen Foundation of Canada

## **An immunohistochemical study of carcinoembryonic antigen cell adhesion molecule 1 in colorectal cancer liver metastases**

Anthony Smith, Zahir Fadel, Abdellatif Amri,, Stephanie Petrillo, Anthoula Lazaris, Nicole Beauchemin, Peter Metrakos

Colorectal cancer (CRC) is the third leading cause of cancer-related death in North America. Approximately 50% of patients will be diagnosed with CRC liver metastasis (CRCLM) during the course of their disease. Carcinoembryonic antigen (CEA) is currently used as the standard biomarker for monitoring response to therapy in metastatic disease, but a more reliable indicator must be found. CEA is a member of a large family of glycoproteins called CEA-related cell adhesion molecules (CEACAMs). CEACAM1, a member of the family, has been linked with the modulation of tumorigenesis. In CRC, loss of CEACAM1 demonstrated a tumour suppressing effect while over-expression in invasive CRC is correlated with clinical stage. CEACAM1 was also found to be an independent risk factor for lymph node involvement and shorter patient survival. Two main isoforms of CEACAM1 have been identified: CEACAM1-L with a long cytoplasmic domain, and CEACAM1-S with a short cytoplasmic domain. The two isoforms may have differing roles in tumorigenesis by virtue of their distinct signalling potentials.

The goal of this study is to investigate whether CEACAM1 can be used as a prognostic tool to determine the aggressiveness of CRC and CRCLM. The study included FFPE tissue samples from 65 CRCLM lesions following liver resection. Samples of the matched primary were also obtained for 30 patients. All samples were cut and immunohistochemically (IHC) stained for CEACAM1 and CEA. Expression of CEACAM1 and CEA was compared between colon primaries and CRCLMs, as well as with clinical factors. Our results demonstrate that in the primary CRC, high expression of CEA correlated with disease staging and number of metastases while apical staining of CEACAM1 correlated with fewer metastases. In the CRCLMs, high expression of apical and cytoplasmic CEACAM1 correlated with a better response to chemotherapy. In the background liver, high expression of CEACAM1 inversely correlated with body mass index (BMI), number of metastases and metastatic differentiation. Analysis suggests that CEACAM1 has a tumour-suppressive role and could have potential use as a biomarker. Ongoing experiments pertaining to the role of CEACAM1 include more IHC staining, assaying matched patients' blood samples with a CEACAM-1 specific ELISA and using Western blots to determine the isoforms present.

# **Circulating neutrophil extracellular traps as a potential biomarker for thoracic malignancies: a patient-specific therapeutic approach**

Jack Mouhanna, Roni Rayes, Lorenzo Ferri and Jonathan Spicer

## **Introduction:**

Neutrophil extracellular traps (NETs) are neutrophil-derived decondensed chromatin fibres decorated with granule proteins. In addition to trapping invading pathogens, NETs may enhance circulating tumor cell adhesion and growth, promoting metastasis and comprising one mechanism through which neutrophils act as a key player in cancer biology. Furthermore, NET blood levels have been shown to be elevated in a variety of inflammatory conditions. Therefore, we sought to optimize the detection of circulating NETs in patients with thoracic malignancies and examine whether NETs can potentially be used as a biomarker for the presence and/or progression of cancer to identify patients who will likely benefit from a NET-based therapy.

## **Methods:**

NET levels in serum samples from 24 esophageal cancer patients and 11 healthy controls are measured using an enzyme-linked immunosorbent assay (ELISA), which detects myeloperoxidase (MPO)-DNA complexes characteristic of extruded NETs. Where applicable, phorbol myristate acetate (PMA) is used to stimulate NET formation, protein arginine deiminase 4 (PAD4) inhibitor or serine protease inhibitor serpinB1 (obtained through cloning) to inhibit NET formation, and DNase to degrade NETs.

## **Results:**

Sensitive detection of NETs in serum samples of esophageal cancer patients is achieved. The assay signal is specific for NETs and varies as a function of NET concentration. The assay indicates that serum NET levels are significantly higher in cancer patients compared to healthy controls, with more advanced cancer patients tending to have higher NET levels. Furthermore, circulating NET levels are rapidly and efficiently abrogated using DNase. Alternatively, PAD4 inhibitor and serpinB1 limit NET formation to maintain baseline levels.

## **Conclusion:**

We were able to optimize the performance and test the sensitivity of an ELISA to measure NET levels in serum samples from esophageal cancer patients. Our preliminary results suggest that circulating NET levels may be correlated with the presence and progression of cancer, advocating for a patient-specific therapeutic approach centered on degrading NETs and/or inhibiting their formation. Further investigation will show whether this assay may be used in the clinic as a practical prognostic tool predictive of oncologic outcomes.

Sources of funding: McGill Faculty of Medicine-- Canadian Institutes of Health Research

# The Immunoprofile as a Prognostic Tool in Metastatic Liver Disease Through Stratification of Histological Growth Patterns

Klaudiusz Stoklosa, Abdellatif Amri, Pablo Zoroquiain, Anthoula Lazaris, Peter Metrakos

Colorectal cancer (CRC) frequently metastasizes to the liver. Pathologists have noted histological heterogeneity within CRC liver metastases (CRCLMs). Three CRCLM histological growth patterns (HGPs) have been identified: desmoplastic HGP (DHGP), characterized by desmoplastic stroma separating liver parenchyma from the tumour; pushing HGP (PHGP), characterized by direct interaction between the tumour cells and hepatocytes with compressed liver cell plates running parallel to the tumour border; and, replacement HGP (RHGP), characterized by tumour cells replacing liver parenchymal cells in a random fashion.

Histopathological examination of resected tumour lesions is the predominant method of clinical and pathological risk prediction in cancer patients (AJCC/UICC-TNM classification). However, it provides limited information in estimating clinical post-operative outcome. Outcome may vary between patients within the same tumour stage due to the heterogeneity of the immune contexture in the tumour microenvironment. Consequently, a novel diagnostic involving CD3+ and CD8+ T cell densities, termed the Immunoscore, was developed. While effective at determining patient prognosis in primary cancers, it is ineffective in grading CRCLMs.

This study aims to add a new parameter, the CD4+:CD8+ T cell ratio, termed the Immunoprofile, as a liver metastasis biomarker. We hypothesize: the Immunoprofile has a high efficacy as a prognostic tool for patient outcome and stratification for treatment options, such as administering chemotherapy with or without Avastin (angiogenesis inhibitor anti-VEGF-A antibody); and, DHGP tumours have greater overall cytotoxic immune activity than RHGP tumours, characterized by quantifiably stronger CD4+ and CD8+ T cell staining. We analyzed 30 resected DHGP and RHGP tumour immune contextures using CD4/CD8 double staining immunohistochemistry and scoring. Our preliminary analysis demonstrates a greater concentration of CD4+ T cell staining the periphery of DHGP, whereas RHGP have distinct islands of CD4+ T cell staining. We are now in the process of analyzing samples from chemo-naïve, chemo only and chemo + Avastin CRCLM lesions



## Automated identification of growth patterns in liver metastases using dynamic contrast-enhanced MRI and shape analysis

Thomas Rosin, Zaki Ahmed, Martin Vallières, Ives R. Levesque

It has been proposed that liver metastases follow one of three growth patterns: desmoplastic, replacement, or pushing. These can be identified following surgery using a histological analysis. We hypothesize that these patterns can be identified based on their perfusion characteristics as measured by MRI. Our group has developed technique to identify high and low perfusion regions on dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) with a shape analysis method. In breast cancer, this technique was able to predict which patients would respond to chemotherapy before treatment. The goal of this work was to extend the method to applications in liver. We obtained a set of 21 patients from which we have 24 lesions and their type, classified as desmoplastic (14) and non-desmoplastic (10, combining replacement and pushing patterns). Four volumes of T1-weighted MRI data of the patient were acquired during a single exam, one before injection of the contrast agent and three after venous injection. The contrast agent was gadobutrol, a common gadolinium-based contrast agent. After registration of each dataset, we applied our analysis, which identifies shapes in the time-course data based on the Tofts model, to the measured concentration curve at each voxel. The shapes, or “sources” we use are normalized. In this analysis, one source was defined for low perfusion and a second for high perfusion, based on the  $k_{ep}$  parameter used to compute the shape. The analysis returns maps of “weights” throughout the tumour, *i.e.* the combination of shapes that explains the data, for all shapes simultaneously. We believe these weights reflect the tumour metabolism. We then calculated the mean of low or high perfusion weights inside the tumour. We compared the distribution of mean weight to the outcome (desmoplastic or non-desmoplastic). Using the area under receiver operating characteristic (AUROC), we evaluated if an optimal test to separate the outcome is possible. We sought the  $k_{ep}$  values that give rise to an AUROC closest to 1. With our dataset we obtained optimal AUROCs of 0.65 to 0.75 depending on the model we applied.

### References:

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## **Correlation of Clinical-Pathological Features with Engraftment of Pancreatic Cancer in Mice**

Ambike Aarti Srivastava, George Zogopoulos

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Pancreatic adenocarcinoma (PC) is a lethal malignancy due to its late presentation and chemoresistance. Patient-derived xenograft (PDX) programs facilitate research studies in models that more closely resemble human disease, and provide a platform for personalized medicine (Andrei et al. *Cancer Lett* 2015). The Quebec Pancreas Cancer Study (QPCS, Smith et al *Curr Oncol* 2015) is a pancreas cancer patient research registry with an integrated (subcutaneous) PDX program. My summer research project focused on reviewing the QPCS PDX experience with the specific aims of 1) identifying clinical and histological tumour features associated with tumour engraftment in mice and 2) evaluating if tumour engraftment in mice correlates with patient disease-free (DFS) and overall (OS) survivals.

From July 19th 2012 to May 3rd 2016, 89 patients enrolled in the QPCS had their primary tumours, which were resected with curative intent, implanted in mice with a median of 18.1 months follow-up time. An engraftment rate of 65.2% (n=30) was observed, with node positivity ( $p=0.0202$ ) and poor differentiation ( $p=0.0112$ ) being predictive of engraftment. The median of patient DFS and OS were 14.6 and 18.2 months, respectively. The relationship of DFS and OS with age at diagnosis, sex, resection type, clinical stage at diagnosis, nodal status, resection margin positivity, tumour differentiation, lymphovascular invasion, perineural invasion, neoadjuvant therapy, adjuvant therapy, and PDX engraftment was evaluated. A correlation between perineural invasion ( $p=0.031$ ) and DFS was observed

These data suggest that tumours from patients with more advanced disease (node positive and less differentiated histology) are more likely to implant. This observation is likely due to the higher proportion of tumour initiating cells in these cases, supporting the utility of PDX models to study aggressive subtypes of PC. The absence of significance between all clinical and pathological features known to impact DFS and OS is likely due to our small sample size. Consequently, with a larger sample size, an association of tumour engraftment in mice with patient outcome may be evident as we would expect if engraftment is associated with more aggressive disease.

Support

This study was supported by funding from the MICRTP Summer Studentship.

# **A PIL (Purpose In Life) to live better with cancer: Results from a group psycho-educational intervention to improve emotional and existential wellbeing during treatment for cancer**

Jérémie Richard, Maude Paradis, Virginia Lee

Research-Institute of the McGill University Health Centre (RI-MUHC), McGill University Ingram School of Nursing, McGill University Department of Education and Counselling Psychology

## **Background**

As fundamental research continues to search for a cure for cancer, psychosocial oncology research continues to search for ways to help patients endure the physical and psychological effects of cancer treatment. One-on-one interventions are clinically effective approaches that help patients cognitively process and emotionally adjust to the effects of cancer. However, considering long waiting lists and the high cost of specialized psychological services, distress is prevalent amongst cancer patients. The purpose of this study was to examine the efficacy of a group-based Meaning-Making Intervention (MMi) that addresses meaning and purpose in life while living under the threat of cancer.

## **Methods**

A single group, pretest–posttest design explored the effect of a 3 hour group psychoeducational workshop for cancer patients at the MUHC Cancer Centre. Between November 2015 - June 2016, six groups of 3-8 participants attended a standardized workshop and completed a self-administered patient workbook over a recommended 1 month period. Generalized well-being and self-efficacy were measured at baseline (T0), immediately after (T1), and 1 month (T2) after the workshop. Repeated measures paired sample t-tests were conducted to compare the means within subjects and across time.

## **Results**

Thirty-five patients completed the questionnaires at T0 and T1 and twenty participants completed these questionnaires at T2. Immediately after the workshop, at T1, patients reported significantly greater emotional wellbeing (increased hope, less sadness and decreased worry about dying) which was maintained one month after the intervention. One month later, at T2, patients reported significantly improved spiritual wellbeing (being at peace), greater self-efficacy to accomplish goals and problem solve, and were sleeping better.

## **Conclusions**

Our results demonstrate the MMi delivered as a brief, group workshop is a clinically accessible and cost-effective approach to assist cancer patients in dealing with various emotional and existential issues. A group workshop offered within the routine care of a busy cancer centre reduces many barriers patients encounter when seeking psychological and social support and can foster personal growth in a secure and structured setting.

## **Sources of Support/Funding**

Fonds de la recherche du Québec - Santé (FRQ-S), Canadian Association of Nurses in Oncology (CANO), MGH Foundation, Newton Foundation, Cedars CanSupport

# Was Dosimetry to Blame? A Retrospective Analysis of Beta Emitting Sources in Intravascular Brachytherapy

Joseph DeCunha, Shirin Enger

Coronary artery disease involves the deposition of plaque along the walls of a coronary artery leading to narrowed or blocked vessels (stenosis) and is one of the main causes of death in developed countries. Percutaneous Transluminal Coronary Angioplasty (PTCA) is used to reverse stenosis. Restenosis of the treated vessel is a major complication of PTCA and occurs after as many as half of PTCA procedures. A metal mesh tube (stent) can be placed inside the vessel to prevent restenosis. Tissue stress incurred during PTCA and stenting can start cell proliferation leading to in stent restenosis (ISR). Intravascular brachytherapy (IVBT), a form of internal radiotherapy, is used to treat ISR. Successful irradiation of the proliferating tissue requires precise treatment planning (TP) to enable delivery of an optimal dose to the lesion and spare healthy tissues. However, TP of IVBT is limited and based on simplistic dose calculations, where human tissue is approximated by water. The interactions of arterial tissue, plaque, the treatment catheter and guidewire, and the stent with radiation delivered to the artery are ignored.

A retrospective analysis of commercially available IVBT systems was performed by utilizing the Monte Carlo method, which is the most accurate method for calculating absorbed dose of radiation in inhomogeneous systems such as the human body. Absorbed dose to the lesion site was calculated using a model of a human coronary artery with a calcified plaque and stent. Dose delivered in water was also calculated in order to evaluate the accuracy of a water approximation.

Our findings show that the water approximation used in clinical practice to calculate dose is inaccurate when inhomogeneities are present. For the Novoste Beta Cath device, delivered dose was reduced by 20% in regions behind the stent struts and by as much as 60% in a region occluded by the guidewire, plaque, and stent. For the Guidant Galileo, dose was also reduced by 20% behind stent struts and as much as 72% in the same region.

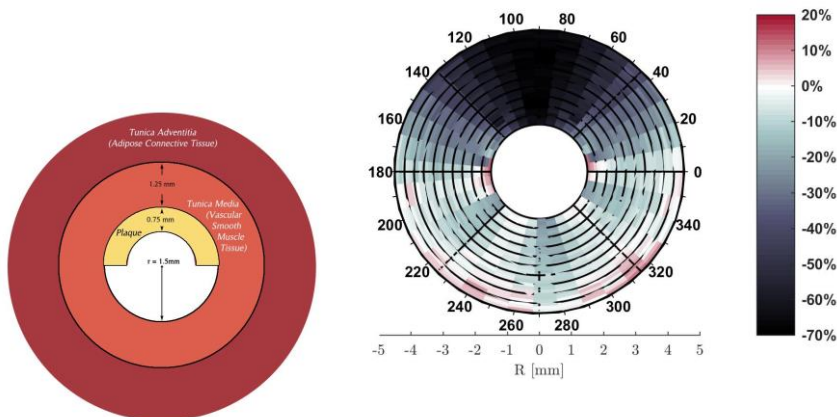


Fig 1: (Left) Cross sectional diagram of the artery phantom used for dosimetric calculations. (Right) Percent difference of dose delivered between artery and water used calculation models for the Guidant Galileo Device. Plaque subtends the region from 0 to 180° and guidewire subtends the region about 90°.

# Energy dependence of a graphite probe calorimeter for use in absolute clinical radiation dosimetry

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McGill University Medical Physics Unit

## Purpose

The aim of this work is to evaluate the energy dependence of a previously introduced graphite probe calorimeter (GPC) for use in absolute clinical radiation dosimetry through Monte Carlo simulations.

## Methods

Three models of the GPC were required. These were an ideal model, a realistic model and a water model. The realistic model was one where the core contained impurities such as thermistors and nickel leads, whereas the ideal model contained no impurities. The water model was one where all the component materials were assigned to water. Each model was embedded into a water phantom as well. The egs-chamber user code from the EGSnrc toolkit, a radiation transport software package, was used. Dose was scored at the core of the GPC for both photon and electron beams, ranging from Cobalt-60-24MV and 6-20MeV, respectively. Inhouse measured electron beam spectra and standard photon beam spectra were used for the beam sources. A 10x10cm<sup>2</sup> field size was used throughout the simulations.

## Results

For each photon and electron beam, the ratio of dose to graphite and dose to water was recorded. For photon beams, in the ideal GPC case, these ranged from 0.990 to 1, normalized to Cobalt-60. The relative uncertainties were approximately 0.1%. These ratios decreased as the photon beam quality increased. This is consistent with experimental results for the ratio of the total mass stopping power of graphite and water. For a 6MV photon beam, the percent difference between the real and ideal case was 2.5%. For the electron beam, the ratios varied from 0.851 to 0.872, increasing with beam energy. Relative uncertainties were between 0.1-0.2%.

## Conclusions

The GPC is weakly energy dependent for both photon and electron beams. As a rough, primary approximation, the dose delivered to graphite can be considered the dose delivered to water.



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