Hold'em for Life

Translating discoveries into breast cancer cures

Report to the Scientific Advisory Board
October 2017

Sinai Health System

Lunenfeld-Tanenbaum Research Institute
Sinai Health System

UHN Princess Margaret Cancer Centre

Executive Summary

With generous funding from the ***Hold'em for Life*** **Charity** we have built a successful integrated multidisciplinary breast cancer research program focused on the interface between the patient (host) and her cancer.

The dynamic team that we have assembled bridges clinical and basic researchers across Mount Sinai (MSH)/Lunenfeld Tanenbaum Research Institute (LTRI) and Princess Margaret Cancer Centre (PMH)/ Research Institute. We have leveraged the funds received from Hold'em (approximately $1.0 to 1.5 million per year) multiple times against institutional assets, peer-reviewed funding, and pooled funding from multi-center clinical trials.

Underpinning our success is our commitment to jointly aligning and conducting critical clinical and basic breast cancer research, deepening our understanding in the areas of our members' expertise and leveraging that knowledge to advance the central shared objectives of the Hold'em for Life program. The breadth and depth of the combined scientific and clinical expertise with which we approach our research is substantial and includes clinical medicine (medical oncology, radiation oncology and pathology), statistics and basic research (molecular biology and genomics) and is seldom achieved in multidisciplinary teams. Such a collaborative team, working interactively to investigate research questions from multiple perspectives is a major strength of our research program.

The Hold'em for Life funded program has four inter-related planks that reflect robust interaction between preclinical and clinical research focused on host hormonal factors (e.g. insulin, estrogen, progesterone) and tumor development and progression. We also provide support for clinical trials infrastructure and for trainees.

In 2017 we have added a new major plank – **Exploration of factors associated with late recurrence in hormone receptor positive breast cancer** – this work will involve initiation of a major prospective cohort study with rigorous and serial collection of blood, urine and patient-related factors in order to identify potential predictors of imminent risk of distant relapse. This project is described in detail later in the report.

PLANKS

Obesity & Metabolic Factors - We have been investigating the role of obesity and metabolic factors, by way of clinical trials of the diabetes drug metformin in breast cancer, and by looking at the molecular biology of the obesity-cancer relationship and mechanisms of action of metformin. Drs. Goodwin and Stambolic have led this area, along with Dr. Ryan Dowling. We have reported improved metabolism in women receiving metformin – important because abnormal metabolism (for example, high insulin levels) has been associated with an increased risk of breast cancer recurrence and death. We also have several ongoing translational research activities in CCTG MA.32 (A Phase III Adjuvant RCT of metformin vs placebo n early breast cancer that will provide definitive information regarding the potential therapeutic role of metformin in breast cancer) and we have conducted additional translational studies in this area (see below).

Liquid Biopsies - The study of metastatic breast cancer demands direct evaluation of tumor material, which is difficult to obtain because of the need for invasive biopsies of metastatic lesions. A promising alternative to tissue biopsies are "liquid biopsies", which sample tumor-derived cells or products present in the blood of affected individuals.

Initially, we examined patient metabolic host (patient-related) factor correlates of circulating tumour cells (CTCs) in metastatic breast cancer. There has been a shift in scientific thinking and we are now working with circulating tumour DNA ("liquid biopsy" or ctDNA). Dr. Scott Bratman, a scientific expert in this area, and Dr. Dave Cescon plan to examine ctDNA spikes post chemotherapy in the neoadjuvant and metastatic setting as potential early markers of tumor response to treatment.

Hormonal and Bone Related Factors - Drs. Rama Khokha (molecular biologist PMHRI) and David Cescon (clinician scientist, PMHRI) have explored hormonal and bone related factors such as estrogen, progesterone and Rank-ligand (RANK-L, a bone-related factor) in the development and treatment of breast cancer. As with our other core activities, this

work has contributed to hypotheses that will be tested in our planned new program of research focusing on late recurrence.

Dr. Khokha's research has provided an explanation for why certain types of hormone replacement lead to increased breast cancer risk while others do not, and her discoveries have contributed to the development of a multinational randomized trial of a RANK-L inhibitor as a potential means of preventing breast cancer in women at high genetic risk. Purna Joshi (Post-doctoral fellow) has expanded this work to investigate fat and stem cells in the breast, including examination of biologic properties of fat cells. Alison Casey's work (Post-doctoral fellow) has identified several drugs that target stem cells which can act as precursors of breast cancers. Dr. Daniel Schramek (LTRI scientist) has joined the Hold'em for Life team to investigate bone related trauma as a potential contributor to late recurrence in a mouse model.

Dr. Cescon has generated important results showing that a commonly used breast cancer drug – letrozole – effectively lowers estrogen levels in breast cancer patients, regardless of their degree of obesity (laying to rest a concern that the drug was not as effective in obese patients); he has also shown that there is no benefit to increasing the dose of the drug in obese women.

Translational Research Resource (BTRR) - The BTRR, an established research resource available to the Hold'em researchers and other University of Toronto investigators, currently includes tumor tissue, normal breast tissue, blood and clinical information. Almost 700 women with breast cancer have been enrolled into the BTRR with full clinical annotation; key patients (primary invasive cancer and followed clinically at MSH) are being followed annually for clinical outcomes.

This high quality resource will have its greatest value and impact in the next 3-5 years as some of the participants experience a recurrence or metastasis of their cancer. At that time it will become possible to conduct more detailed investigations into why some women develop metastases while others do not – an important complement to our planned late recurrence study. In the meantime, 5 researchers have proposed research utilizing these samples; two of those are underway and 1 project (investigating obesity associated blood and tumor markers in hormone receptor positive breast cancer) has been completed.

SUPPORTED ACTIVITIES

Clinical Trials Infrastructure Support - The program also supports clinical trials infrastructure that facilitates participation in multicenter clinical trials. A number of Hold'em funded studies have been completed with preliminary results already presented and manuscripts detailing the final results underway.

Much of the full complement of trials work (almost 30 studies) has taken advantage of funding from other sources and/or has been layered onto existing infrastructure for clinical trials – this has allowed the program to have an impact that is greater than would have been possible using only Hold'em funding. The enhanced level of clinical trial activity has recently facilitated membership of MSH in the Canadian Cancer Clinical Trials Network, which provides additional infrastructure support for oncology clinical trials.

Trainees - Hold'em has engaged 20 trainees and they are exposed to the full spectrum of research. Some of the trainees are now beginning to play a leadership role in their own studies and three trainees have accepted staff positions and continue to work with the group.

We are recognized internationally – having published 36 papers and 20 abstracts and presentations based on our Hold'em for Life funded research.

A New Program in 2017 – Investigation of Late Recurrences in Hormone Receptor Positive, HER2 Negative Breast Cancer

With committed expertise and the progress in our four planks, **we believe the Hold'em for Life research group is exceptionally well-positioned to initiate a bold new research program designed to identify women with imminent risk of recurrence and, ultimately, to determine how to best intervene to ensure that they do not recur**.

We are proposing an important new research initiative, which will tackle the problem of late recurrence of breast cancer. Late recurrences, which happen after completion of hormone therapy are currently a major leading cause of breast cancer death, underscoring the importance of this work. In Canada, almost 5000 Canadian women still die from breast cancer every year. Many of the life threatening/incurable breast cancer recurrences and deaths now take place more than 5 years after diagnosis, after completion of adjuvant therapy, particularly in hormone receptor positive breast cancer. It is a **major unmet need** to understand **who is at risk for these late recurrences** and to **identify interventions to prevent or delay** them.

To date, there has not been a reliable and validated way to identify which specific women will recur and when a recurrence is imminent (but still potentially avoidable). Overcoming this knowledge gap is important because the majority of women do not recur – treatment needs to be focused on those who are at greatest risk of recurrence in order to maximize benefit and minimize toxicity. This proposed research is novel, timely and impactful and we anticipate it will lead to international collaborations that we are well-placed to establish. **Hold'em funding would allow us to initiate this study in Toronto, cultivate international collaborations in the United States and Europe, and then leverage the program to obtain additional funding from granting agencies, including the Breast Cancer Research Foundation (New York).**

Questions to SAB Members

We look forward to any feedback that is provided by the SAB members relating to any aspect of the research that is presented. We would also ask SAB members to provide specific feedback in the following areas:

1. **How can we enrich the BTRR into an optimal research resource that will have maximum impact (e.g. continue to accrue, perform standardized genomic profiling, etc.)?**
2. **To what extent should more challenging and/or less developed assays be included in the Late Recurrence study – e.g. CTCs (requires immediate costly assay if CellSearch), metabolomics, proteomics, others?**
3. **How can we optimally translate our basic and translational findings into therapeutic and preventive interventions?**

Contents

Report of Activities February 2016-September 2017 and Description of Ongoing and Planned Core Activities

For the past 5 years, the generous support of ***Hold'em for Life*** has allowed us to accomplish much in building a multidisciplinary breast cancer research program. The dynamic team that we have assembled bridges clinical and basic research across Mount Sinai (MSH)/Lunenfeld Tanenbaum Research Institute (LTRI) and Princess Margaret Cancer Centre (PMH)/Research Institute. We have leveraged the funds received (approximately $1.0 to 1.5 million per year) multiple times against institutional assets, peer-reviewed grants, and pooled funding from multi-center clinical trials. The driving focus of our program is predominantly clinical with an emphasis on prevention and treatment of metastatic breast cancer; this focus also embraces the investigation of earlier stages of breast cancer to facilitate understanding of why some women develop metastases and how these recurrences can be prevented.

Our Hold'em for Life funded research program has focused on the interface between the woman and her cancer, exploring this interface in research spanning the molecular biology of the cancer to the physiology and lifestyle of the patient. Throughout, our focus has been on understanding why some patients develop metastases and how those metastases can be prevented and treated.

Underpinning our success has been the establishment of an integrated multidisciplinary breast cancer research team. This engaged research team has matured over the years to effectively conduct critical clinical and basic breast cancer research, deepening our understanding in the areas of our members' expertise and leveraging that knowledge to advance the central shared objectives of the Hold'em for Life program. The breadth and depth of the combined scientific and clinical expertise with which we approach our research is substantial and includes clinical medicine (medical oncology, radiation oncology, pathology), statistics and basic research (molecular biology and genomics) and is rarely achieved in multidisciplinary teams. Such a collaborative team, working interactively to investigate research questions from multiple perspectives, is both rare and highly prized; it is a major strength of our research program. Our Hold'em for Life program is cultivating the future scientists in this area through the support and mentoring of trainees, several of whom have moved into staff positions and are beginning to take a leadership role in their own breast cancer research programs. We are recognized internationally – having published 36 papers and 20 abstracts and presentations based on our Hold'em for Life funded research.

The Four Major Research Planks of the Hold'em Program

There are **four major planks** in our existing research program.

1. Obesity and Metabolic Factors, Including Metformin

We have been investigating the role of **obesity and metabolic factors,** by way of clinical trials of the diabetes drug metformin in breast cancer, and by investigating the molecular biology of the obesity-cancer relationship and mechanisms of action of metformin. This work has been led by Drs. Pam Goodwin (clinical scientist at MSH/LTRI) and Vuk Stambolic (molecular biologist at PMHRI); Hold'em scientists are recognized as international leaders in this area of research.

Our work in this area reflects our understanding of the obesity-cancer link described in the Figure below.

Impact of the Obesity Epidemic on Cancer

Pamela J. Goodwin and Vuk Stambolic (Annual Reviews in Medicine 2015)

The emerging understanding of the biologic nature of the association of obesity and cancer suggests a complex interplay of a range of factors at multiple levels: the whole patient, the adipose tissue, and the tumor cell and its fat-containing microenvironment (Figure 2).

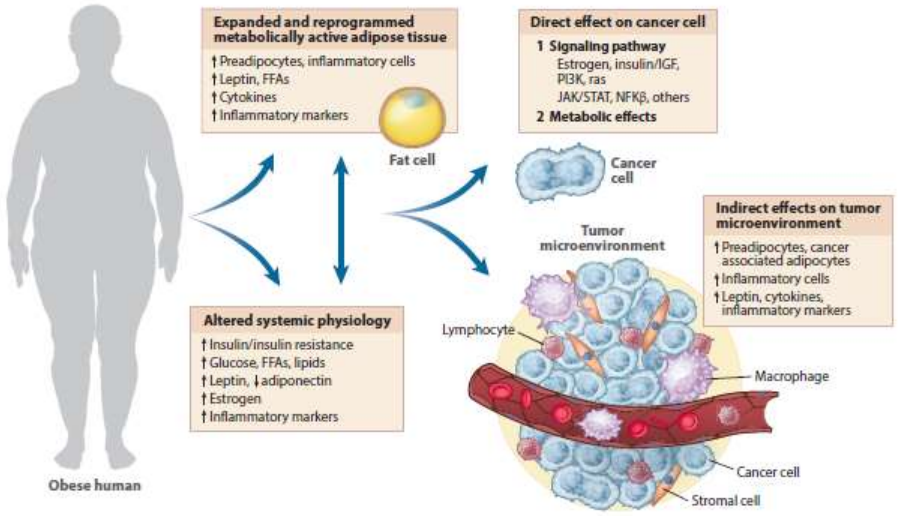


Figure 2

The complex association of obesity and cancer. Obesity is associated with expanded and reprogrammed adipose tissue that is metabolically active, leading to localized inflammation and altered cytokine/adipokine secretion; these local changes contribute to, and interact with, alterations in systemic physiology that reflect the insulin resistance/metabolic syndrome. Local adipose tissue and systemic obesity-associated alterations can impact cancer directly by (1) the activation of key signaling pathways or (2) an alteration in cellular metabolism, reflecting an abundance of glucose, free fatty acids (FFAs), and lipids. They may also act indirectly on the tumor microenvironment to promote proliferation, angiogenesis, invasion, and epithelial-mesenchymal transition.

A. OZM 027 – A Phase II Randomized Trial of the Effect of Metformin vs Placebo on Progression Free Survival (PFS) in Women with Metastatic Breast Cancer Receiving Standard Chemotherapy (anthracycline, taxane, platinum, capecitabine, vinorelbine) . (Detailed protocol attached.) Prepared by Drs. Pamela Goodwin and Marguerite Ennis

We have recently completed a Phase II trial of metformin (vs placebo) plus chemotherapy in the metastatic setting (OZM 027), and are currently finalizing the analysis of the data collected (see below). This trial was designed to evaluate, in a clinical population, the provocative observation that metformin enhanced the effect of chemotherapy by selectively targeting cancer stem cells in a mouse mammary cancer model (Hirsch H et al, Cancer Res 2009;19:7507-7511). The scientific rationale was supported by multiple observational studies in humans as well as both in vitro and in vivo preclinical work that provided evidence of a potential clinical benefit of metformin in breast cancer (see full protocol).

Study Design: We randomized 40 women starting a new chemotherapy (first, second, third or fourth line standard chemotherapy as selected by the treating physician) for progressing measurable or evaluable metastatic breast cancer at one of 6 participating Ontario centers to metformin (850 mg PO BID) or placebo . Randomization was stratified for line of chemotherapy (first versus second versus third and higher) and hormone status (ER and/or PgR positive versus both negative). Disease status was examined every 9 weeks and study treatment was stopped when disease progression was identified. One randomized woman withdrew from the study prior to the initiation of treatment but was included in our analyses, reflecting our intention to treat statistical analysis plan. Our sample size allowed detection of a HR of 0.58 with 80% power and a one-sided type one error of 0.20.

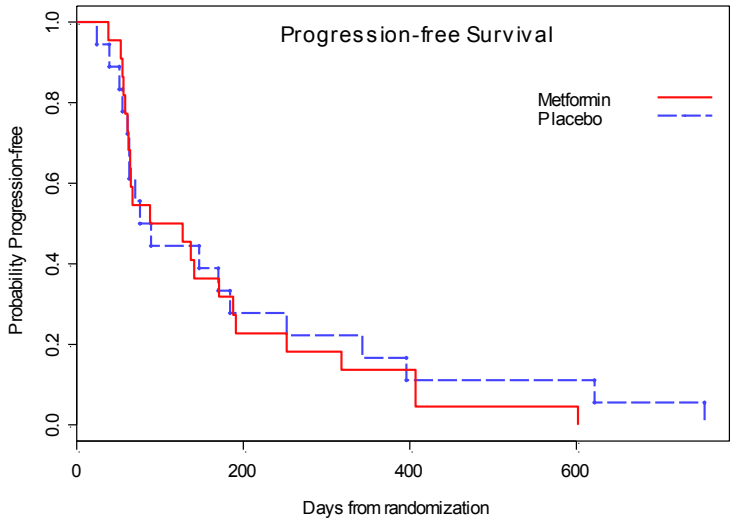
Patient Population: Mean age was 56.1 ±9.4 years (range 39-75). ER was positive in 33 (82.5%), PgR positive in 26 (65%) and HER2 was positive in 6 (15%) patients. Mean BMI was 26.5 kg/m^2. Twenty-seven (67.5%) were receiving first line chemotherapy, 7 (17.5%) second line and 6 (15%) third or fourth line chemotherapy. Baseline ECOG was 0 in 15 (37.5%), 1 in 21 (52.5%) and 2 in 4 (10%) patients. These factors did not differ between metformin and placebo patients. Sites of metastases included liver (77.3% metformin vs 55.6% placebo), lung (54.5% vs 33.3%), bone (77.3 vs 77.8%), lymph nodes (54.5% vs 44.4%) , pleural effusion (18.2 vs 11.1) or ascites (0 vs 11.1%); visceral disease (liver, lung) was present in 21 (95,5%) of metformin patients and in only 13 (72.2%) of placebo patients. No patients had brain metastases. Mean times from first histologic diagnosis to randomization were 6.5 and 4.0 years in metformin and placebo patients respectively (p = 0.18). Mean times from first distant metastasis to randomization were 1.1 and 0.8 years in placebo and metformin patients respectively (p = 0.87).

Treatment Administered: Type of concomitant chemo: single agent taxane (metformin arm 11=50% vs placebo arm 6=33.3%; capecitabine (metformin arm 7 = 31.2%, placebo arm 4 =22.2%, one of whom also received lapatanib); anthracycline with or without other agents (metformin arm 3 = 13.6%, placebo arm 5 = 27.8%); gemcitabine/cisplatin (metformin arm 1 = 4.5%, placebo arm 1 = 5.6%), vinorelbine (metformin arm none, placebo arm 1 = 5.6%).

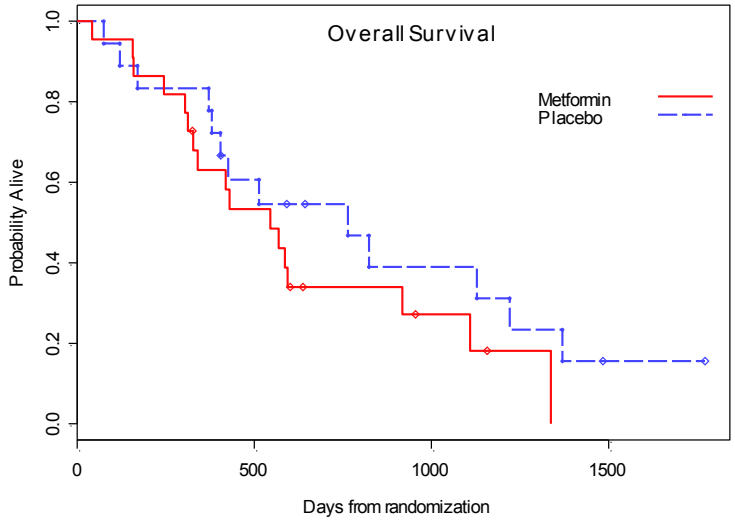
Best response (as reported by the treating physician): progressive disease (metformin 10 = 45.5%, placebo 9 = 50%), stable disease (metformin 7 = 31.8%, placebo 2 = 11.1%) partial response (metformin 4 = 18.2%, placebo 4 = 22.2%); response was not evaluable in 4 patients (metformin 1 = 4.5%, placebo 3 = 16.7%).

Toxicity: Only one grade 4 toxicity was reported (hyponatremia in a placebo patient). Grade 3 toxicities were reported 14 times in both the metformin and the placebo arms [hand foot syndrome, infection/febrile neutropenia/low neutrophils, diarrhea (1 on metformin and 2 on placebo), fatigue and transaminase elevations]. Grade 1 and 2 toxicities were more frequently reported in the metformin arm 193 events Grade 1 and 53 grade 2) than the placebo arm (136 events Grade 1 and 46 Grade 2) – in both arms GI toxicities were the most common Grade 1 and 2 toxicities.

Time to progression: Mean time to progression was 164 ± 149 days in the metformin arm and 192 ± 210 days in the placebo arm - univariable HR = 1.17 (95% confidence interval 0.62 – 2.24) , two-sided p = 0.63, one-sided p = 0.685 because the HR is in the opposite direction from that hypothesized); multivariable HR (adjusted for time from first distant recurrence to randomization and stratification variables) 1.14 (95% confidence interval 0.59 – 2.20), two-sided p = 0.7, one-sided p = 0.65. Further adjustment for visceral vs non-visceral metastases did not substantively change these results (multivariable HR 1.36, 95%CI 0.59-2.20).



Time to death: Mean time to death was 549 ± 349 days in the metformin arm and 703 ± 497 days in the placebo arm - univariable HR = 1.53 (95% confidence interval 0.72 – 3.25), two-sided p = 0.27, one-sided p = 0.87 because the HR is in the opposite direction from that hypothesized; multivariable HR 1.6 (95% confidence interval 0.72 – 3.54), two-sided p = 0.24, one-sided p = 0.88. Further adjustment for visceral vs non-visceral metastases did not substantively change these results (multivariable HR 1.31, 95%CI 0.55-3.13).



Conclusions: We found no evidence of a beneficial effect of metformin in this setting. The magnitude of the point estimates of HRs for PFS and OS suggest that the absence of demonstrable benefit of the addition of metformin was unlikely due to low power. Grade 1 an2 toxicities occurred more frequently on the metformin arm, but Grade 3 and 4 toxicities were comparable. Although these results do not suggest a beneficial effect of metformin in addition to chemotherapy in the metastatic setting, they do not preclude a potential benefit in earlier disease.

B. Correlative Research in CCTG MA.32, A Phase III Trial of the Effect of Metformin s Placebo on Invasive Disease Free Survival in Early Stage Breast Cancer – prepared by Dr. Ryan Dowling

Funding from the Hold'em For Life program has allowed us to significantly expand our correlative work on MA.32, a large phase III clinical trial (NCIC CTG MA.32) investigating the effects of metformin versus placebo on recurrence and survival in early stage breast cancer. This correlative research examines patient factors and physiology that underlie the obesity-cancer link, mechanisms of action of metformin in breast cancer, and predictors of metformin benefit (approximately $25 million in funding leveraged from other funders allowed us to perform this research in a cost-effective and timely fashion in order to definitively answer this important question). These outcomes are projected to be available in 2020 (the exact timing depends on when women experience recurrences). Dr. Martin Chang (a Mount Sinai based pathologist with the Hold'em group) is characterizing the breast cancers of the women participating in this trial to identify tissue markers of metformin benefit.

The clinical trial has now progressed sufficiently that we received DSMC approval in 2017 to proceed with the conduct and reporting of a broad range of statistical analyses of blood and tissue assays during the next 12-24 months, analyzing metabolic (blood) and tissue markers, diet, physical activity and quality of life. In order to predict which women will benefit from metformin, we are also examining genetic characteristics of individual patients (specifically a minor gene alteration called a SNP near the ATM gene that has been associated with metformin benefit in diabetes).

For example, we have completed a planned analysis of baseline and 6 month blood samples from the first 500 subjects enrolled on the trial and have demonstrated improved insulin metabolism and physiology in patients receiving metformin. Specifically, patients that received metformin exhibited decreases in circulating insulin, glucose, homeostatic model assessment (HOMA), leptin, highly sensitive C-reactive protein (hs-CRP), as well as reductions in body weight and body mass index after 6 months on trial (Goodwin et al. Journal of the National Cancer Institute, 2015). Analysis of these blood variables for the remaining MA.32 subjects (apart from approximately 150 UK subjects whose assays will be conducted in November 2017) has also been completed and the results are currently being analyzed with final reports expected shortly – an abstract submission to ASCO 2018 is planned. All baseline and 6 month blood samples have also been evaluated for levels of CA15-3, a previously characterized tumour marker that has demonstrated prognostic value in breast cancer patients. In addition to assessment of these blood variables, we have recently completed an evaluation of a single nucleotide polymorphism (SNP; namely rs1121617, which is located at a locus near the ataxia telangiectasia mutated (ATM) gene) in the germline DNA of all trial subjects. The minor allele of this SNP (C/C) has been associated with metformin benefit in patients with diabetes, but the significance of this SNP in non-diabetics remains uncharacterized. Within the MA.32 cohort, 30.3% patients exhibited the major allele (A/A), 48.7% were heterozygous (A/C) and 21% exhibited the minor allele (C/C). A detailed analysis of associations of these genotypes with metabolic and tumour related variables (including change in glucose, insulin and HOMA) is currently underway in an attempt to explore the previously reported association of the minor allele with metformin benefit in this non-diabetic cohort and to uncover potentially novel cancer-related associations in patients within MA.32. These data are expected to be reported in 2018.

In addition to these blood based assays, significant progress has been made in the organization and analysis of MA.32 tissue samples. Dr. Martin Chang has completed assessment and marking of all tissue slides associated with the trial (over 3500, including both tumour and normal tissue) and TMA construction is well underway, with more than 50% of TMAs having been completed by September 2017. In fact, the tissue samples from the first 500 patients enrolled on MA.32 have been evaluated by immunohistochemistry (IHC) for a number of proteins of interest. Specifically, IHC staining has been completed for the insulin receptor (IR), the tumour suppressor PTEN,

and the phosphorylation of downstream effectors of IR, including Akt (S473) and ERK1/2 (T202/Y204). Dr. Chang is currently scoring (Allred and H-Score) these slides with results expected in early 2018.

These studies build on our long standing interest in evaluating the use of metformin in breast cancer and characterizing the impact of obesity and hyperinsulinemia on breast cancer, which represents a major theme of our Hold'em research. The results of these analyses have the potential to elucidate the effects of insulin on breast cancer development and progression and to identify new targets for the treatment of cancers that are sensitive to the growth promoting effects of insulin. As the proportion of obese individuals in Canada increases, characterizing the impact of insulin on breast and other cancers will be critical to the identification of patients at risk of developing the disease and the establishment of personalized treatment strategies targeting IR positive tumors and/or those in patients exhibiting obesity and hyperinsulinemia.

C. Other Clinical Studies (Hepcidin, ER/PgR Obesity, CLS-B)

(i) Hepcidin - prepared by Dr. Katarzyna. J. Jerzak (Clinical Fellow and MSc student, now on staff at Sunnybrook Odette Cancer Centre)

Intra-tumor RNA expression of hepcidin (a marker of iron metabolism/flux out of cells and a potential marker of inflammation and predictor of breast cancer recurrence) has been linked to adverse metastasis-free survival in women with early breast cancer (BC), but the prognostic implications of this inflammatory marker and iron-regulating plasma peptide in the blood are unknown.

Using an ELISA assay, hepcidin was measured in the banked blood of 518 women who were recruited from 1989-1996 for a prospective cohort study regarding diet and lifestyle factors in BC. Blood was obtained 4-12 weeks post-operatively, prior to treatment with chemotherapy or tamoxifen.

Hepcidin was not associated with time to distant BC recurrence (primary outcome) nor time to death due to any cause. However, a pre-planned interaction test of BMI was statistically significant ($p<0.01$); among obese women (BMI>30 kg/m^2), higher hepcidin was associated with a shorter time to distant BC recurrence in univariable (HR 1.81; 95%CI 1.06–3.10) and multivariable (HR 1.84; 95%CI 1.04–3.25) models adjusted for age, T stage, tumor grade, N stage and ER/PR expression.

This work formed the basis of a Master's thesis by Dr. Jerzak; Dr. Goodwin was her thesis supervisor. It has been accepted for Poster presentation at SABCS 2017.

(ii) ER/PgR Obesity - Prepared by Dr. Martin Chang

Using BTRR resources, we have undertaken a study of blood and tissue factors related to metabolic and inflammatory signaling in 129 patients with obesity and non-obesity-associated estrogen-receptor-positive breast cancer. Data from this study have been presented at SABCS 2016 and USCAP 2017 meetings, with a manuscript under preparation.

We found that, as expected, BMI was associated with fasting circulating metabolic factors (glucose Spearman R = 0.42, insulin R = 0.65, HOMA R = 0.63, leptin R = 0.79) as well as markers of inflammation (hs-CRP R = 0.51, IL-6 R = 0.30, IL-8 R = 0.20), estrogen related factors (estradiol R = 0.39, SHBG R = -0.47), VEGF R = 0.22 and PAI-1 R = 0.37 (all $P < 0.026$). Obesity was not associated with circulating EGF R = -0.05, IL-1B R = 0.0, IL-2 R = -0.07 or other estrogens (estrone R = -0.04, estriol R = 0.005), all $P >0.15$.

However, in these ER positive breast cancers (89.9% also PgR positive), BMI was not associated with any of the tissue markers we examined, scored using the Allred scoring system; OB-R Spearman R = 0.0, p = 1; Insulin receptor R = -0.02, p = 0.87; ER R = 0.15, p = 0.10; PgR R = 0.0, p = l; pAKT R = -0.17 p = 0.063, pERK R = -0.11, p = 0.14.

One novel aspect of this work was the demonstration that OB-R was ubiquitously expressed in these ER+ cancers. OB-R expression was associated with the presence of LVI and extensive DCIS. It was not associated with other tumour characteristics (grade, focality, stage ki67, ER/PgR score Allred, growth patterns, borders, stromal/peritumoral features, cytoplasmic appearance, mitotic score and presence of LCIS). OB-R expression was significantly associated with insulin receptor expression (R = 0.26, P = 0.005), ER (R = 0.27, p = 0.004), PgR (R = 0.29, p = 0.002) and pAKT (R = 0.24, p = 0.01) but not p ERK (R = 0.08, p = 0.36). OB-R expression was not significantly associated with any of the blood markers listed above (all R < +/- 0.21, all P > 0.11). Notably, OB-R expression was not down-regulated by systemic leptin levels.

Based on these observations, further investigation of OB-R and JAK-STAT signaling in breast cancer (including hormone receptor negative cancer) is warranted.

(iii) CLS-B – Prepared by Dr. Martin Chang

Dr. Martin Chang has explored the association of crown-like structures of the breast (CLS-B) and altered patient metabolism/obesity in women with early breast cancer, with initial results reported in a poster presentation at SABCS 2015. This dataset has been expanded in 2017 to include additional cases and to investigate prognostic associations of CLS-Bs; manuscript preparation is underway.

In a group of 162 pre- and postmenopausal breast cancer patients with available tissue (of 221 enrolled onto a prospective cohort study at the time of breast cancer diagnosis) and followed prospectively for recurrence and death, CLS-B were identified in adjacent normal tissue in 59 (36%) of patients. As expected, CLS-B were more commonly identified in women with BMI > 30 (59%) vs <25 (27%), p = 0.0083 and with greater waist circumference (a marker of central obesity), p = 0.0037. The presence (vs absence) of CLS-B was not associated with age, menopausal status, type of surgery, tumor stage or grade. ER, PgR or the presence of LVI. The presence of CLS-B was associated blood levels of hs-CRP (p = 0.049) and 25-OH Vitamin D (p = 0.04) but not with fasting glucose, insulin, HOMA, leptin, adiponectin, lipids, IGF1/2 or IGFBP1/3 (all P > 0.014). When the Dannenberg CLS-B index (#slides with CLS-B present/total number of slides examined) was used, these associations did not differ significantly.

Importantly, the presence of CLS-B was not associated with either distant recurrence, p = 0.56, or death (p = 0.55).

These findings refute prior reports in which the presence of CLS-B, or the CLS-B index, was associated with circulating metabolic factors, and with disease outcomes. They do not support the hypothesis that local breast inflammation in breast cancer patients mediates the obesity-cancer link.

D. Impact of Insulin on Breast Cancer – prepared by Dr. Vuk Stambolic

Obesity is a key risk factor in the etiology of type 2 diabetes and cardiovascular diseases. Emerging evidence indicates that obesity and the associated increase in circulating insulin levels are major adverse factors in the development and severity of breast cancer (BC). Moreover, many BCs ectopically express the insulin receptor (IR) and feature activation of signaling pathways downstream of the IR, including the PI3K signaling pathway, which is deregulated in as many as half of all BCs.

The overarching hypothesis of our program is that insulin, in part, mediates the adverse effect of obesity in BC. Deregulation of PI3K signaling, both by insulin-dependent hyperactivation of the insulin signaling pathway and the oncogenic mutations of various pathway components may sensitize BCs to the action of insulin and impact their potential dependence on this hormone. The objective of our work is a detailed molecular characterization of IR signaling in BC and the development of therapeutic strategies countering insulin/IR-associated BC.

Working with Dr. Martin Chang, we are evaluating IR and PI3K signaling throughput in tumor material from various cohorts using our optimized IHC assays for the insulin receptor (IR), insulin-like growth factor 1 receptor (IGF1R), activated/total PKB/Akt, leptin receptor, activated Erk and ribosomal protein S6, as well as the negative regulator of the pathway, the tumor suppressor PTEN.

We are in the scoring phase of the assessment of the archival breast cancer patient material from 949 patients from the NCIC CTG MA.21, a randomized adjuvant BC phase III clinical trial comparing CEF vs CMF chemotherapy that was completed in 2005. In addition to the remarkable insights this analysis is providing, it also represents a valuable training run for the ongoing characterization of patient material from MA.32, a phase III randomized trial of metformin vs placebo in early stage breast cancer, where Dr. Stambolic serves as a correlative science chair. To this end (as noted above), we have completed the analysis of matched (0 and 6 months) blood specimens from over 3000 MA.32 patients. Further, germline genomic DNA has been extracted for over 2500 MA.32 patients and we have completed the scoring for rs11212617, a SNP associated with metformin response in patients with type 2 diabetes (GoDARTS, 2011, Nature Genetics).

On the basic research front, we are in the final stages of completing the assessment of the impact of IR loss in a well-characterized BC mouse model system, which has shown that deletion of IR in mammary tissue of mice considerably reduces mammary tumor burden and the metastatic potential. A manuscript describing these findings is in preparation and will be submitted in the coming months. We have also completed a study establishing the pharmacokinetics of metformin in mouse models, effectively defining clinically relevant doses of this drug for experimentation in mice (Dowling et al. Cell Metabolism, 2016).

In pursuit of the molecular mechanism(s) of IR action in BC, we have mapped the IR interactome in mammary epithelial cells. Emerging from this data set is the notion that ectopic IR in breast cancer cells utilizes components of the epidermal growth factor receptor (EGFR) family to transmit proliferative signals downstream. Specifically, IR was found to interact with ErbB2, ErbB3 and ErbB4, but not EGFR, in insulin-dependent manner, considerably changing our understanding of the biology of breast cancers expressing IR. This newly identified tyrosine kinase (RTK) crosstalk, where IR works in concert with certain members of the EGFR family to transmit insulin signals downstream, may have far-reaching implications for management of IR positive breast cancers, especially in obese patients. In the coming year, the detailed molecular aspects of IR interactions will be dissected biochemically, genetically and pharmacologically, towards mechanistic understanding of the relationships between these critical cellular receptors.

Completion of the planned work could lead to an extension of these observations into the development of new breast cancer therapeutic strategies (including repurposing of existing, approved therapies targeting these pathways, particularly in obese individuals. Taken together, our research progress is integral to several facets of the Hold'em program. As we continue to build the knowledge and expand on the characterization of the specimens within the Hold'em biorepository, a number of research directions are developing on the foundation of our previous work.

In 2017-2018 we plan to continue with the obesity-associated research we have outlined above; many of our observations will impact the design and analysis of our new late recurrence research.

2. Liquid Biopsies

The study of metastatic breast cancer demands direct evaluation of tumor material, which is difficult to obtain because of the need for invasive biopsies of metastatic lesions. A promising alternative to tissue biopsies are "liquid biopsies", which sample tumor-derived cells or products present in the blood of affected individuals. The use of ctDNA as an early marker of

tumour cell death "**liquid biopsy**" could provide clinicians with a tool to rapidly measure treatment efficacy and adjust treatment course accordingly.

Our work initially focused on circulating tumor cells (CTCs) in metastatic breast cancer – Dr. Martin Chang established the first clinical measurement facility in Canada at MSH and we participated in an international study investigating whether the characteristics of the CTCs could be used to predict response to hormonal therapy in metastatic breast cancer. We also completed a study involving 100 metastatic breast cancer patients described below.

i. **CTC Host Factor - Association of Metabolic, Inflammatory and Tumor Markers with Circulating Tumor Cells in Metastatic Breast Cancer – prepared by Dr. Ana Lohmann, PhD student and Clinical Fellow**

We evaluated the association of metabolic/ inflammatory/tumor markers and Circulating tumor cells (CTCs) in women with progressing metastatic BC prior to commencing a new line of systemic therapy. Ninety-six (96) patients with metastatic BC without current diabetes or use of anti-inflammatory agents were recruited from four Ontario cancer hospitals. Women provided fasting blood for measurement of metabolic/inflammatory/tumor markers and CTCs; CTCs were assayed within 72 hours of collection using *CellSearch*.

Results: Median patient age was 60.5 years; 87/96 (90.6%) were post-menopausal. The cohort included hormone-receptor positive (84/96, 87.5%), HER2-positive regardless of hormone receptor status (15/96, 15.6%), and triple-negative (10/96, 10.4%) BCs. Patients were starting first (35.4%), second (26%), or third-or-more line therapy (38.5%). CTC counts measured using EPCAM-based CellSearch technology (per 7.5cc) ranged from 0 to 1238 (median 2); none were detected in 29 (30.2%) patients, 1 to 4 in 25 (26%) and 5 or more in 42 (43.8%) patients. Median CTCs were higher in the presence of bone (3.5 vs.0; p=0.019) and liver metastases (8 vs 1; p=0.006). CTCs were significantly associated with higher hs-CRP (Spearman R =0.22, p=0.028), IL-6 (R=0.25, p=0.01), IL8 (R=0.38; p=0.0001), PAI-1 (R=0.31, p=0.001), CEA (R=0.31, p=0.002) and Ca15-3 (R=0.40, p=<0.0001) and inversely associated with BMI (R= -0.23, p=0.02), leptin (-0.26, p=0.01) and leptin/adiponectin ratio (-0.26, p=0.01).

Conclusion: CTCs were positively associated with bone and liver metastases and inflammatory/tumor markers, and inversely associated with metabolic markers; potentially reflecting tumor burden and cachexia.

ii. **Circulating Tumor DNA (ctDNA) – prepared by Dr. Scott Bratman**

In the recent past, there has been a scientific shift to novel forms of liquid biopsies, particularly those focusing on circulating tumor DNA (ctDNA) which can be detected in minute quantities thanks to rapid evolution of genomic sequencing technologies and computational algorithms. Our change in focus has paralleled this shift and has strengthened our proposed work relating to late recurrences.

Circulating tumour-derived DNA (ctDNA) has emerged as a highly promising cancer biomarker because it provides non-invasive access to cancer-specific genomic/epigenomic changes (Wan et al., 2017). Distinct from circulating tumour cells, ctDNA is cell-free and can be collected from peripheral blood plasma, urine, or other bodily fluids. In most cases, the vast majority of the cell-free DNA (cfDNA) found in circulation is from non-cancerous tissues (e.g., leukocytes), so highly sensitive techniques are necessary for reliable detection and quantification of the tumour-derived fraction.

Recently, next-generation sequencing (NGS) methods have been developed that allow for ultrasensitive detection of ctDNA and for inferring tissue-of-origin of cfDNA. Dr. Bratman is an internationally recognized expert in ctDNA detection technologies using NGS who has pioneered two different and potentially complementary methods to address limitations of earlier NGS-based ctDNA detection methods (e.g., modest sensitivity, high cost, and insufficient patient coverage); these methods differ in their focus on detecting either cancer-specific somatic mutations (Cancer Personalized Profiling by deep Sequencing – CAPP-Seq (Bratman et al., 2015)) or epigenetic changes (cell-free DNA Methylation Immuno-Precipitation and Sequencing – cfMeDIP-Seq (Shen et al., 2017)). The Bratman Lab will apply these and related methods to

cohorts of breast cancer patients in distinct clinical settings in order to evaluate the feasibility and clinical utility of ctDNA analysis for improving personalized medicine.

Project 1: Design and validation of ctDNA assays to predict relapse and guide systemic therapy in early breast cancer

The neoadjuvant setting has increasingly become the focus for the study of residual disease biomarkers, since it permits dynamic assessments across the treatment arc (from locoregionally-restricted at baseline, through to no clinically evident disease following surgery) as well as correlation with standard clinical, radiographic and pathologic measures of response and prognosis. In this multi-centre project, for which we have obtained independent external funding from Canadian Cancer Society (PI: David Cescon), we will develop an assay to monitor response to neoadjuvant chemotherapy and accurately predict subsequent breast cancer recurrence, enabling more selective development of future salvage adjuvant strategies in those at highest risk. To do so, we will employ novel approaches to quantify ctDNA to detect and track residual disease in patients before, during, and after completion of treatment for their high risk early breast cancer. We will employ digital PCR, CAPP-Seq, and cfMeDIP-Seq and compare their performance characteristics in women with high risk early breast cancer (n=30; 10 each of ER+/HER2-, HER2+, TNBC) and 1:1 age-matched healthy controls. Next, we will prospectively describe the dynamics of the optimized ctDNA assay in women undergoing neoadjuvant chemotherapy and surgery for high risk early breast cancer (n=90; 30 each of ER+/HER2-, HER2+, TNBC) and correlate with clinical, radiographic, and pathologic endpoints. The technical validation performed in this project will inform future studies such as those described below.

Project 2: Characterization of rapid release of ctDNA as a Biomarker of Chemotherapy Response in Advanced and Locally Advanced Breast Carcinoma

Rapid release and turnover of ctDNA has been observed in a number of contexts. Following surgery for colorectal cancer or nasopharyngeal cancer, levels of ctDNA follow an exponential decay pattern with a half-life of ~2 hours (Diehl et al., 2008; To et al., 2003). This signifies a continual steady-state release of ctDNA from dying cancer cells within intact tumours. Release of ctDNA can also be accelerated by treatments such as chemotherapy or radiotherapy (Cao et al., 2012; Lo et al., 2000; Rago et al., 2007). A ctDNA spike within the 1st week of treatment may therefore reflect inherent sensitivity of the tumour to treatment and could predict for favorable response. In this project, our primary objective is to establish the feasibility of detecting cfDNA/ctDNA spikes during the first week of treatment for locally advanced and metastatic (stage III and IV) breast carcinoma in women who are undergoing neoadjuvant or first line palliative chemotherapy. Women with locally advanced (n=20) or metastatic (n=20) breast cancer who are set to receive neoadjuvant or first line chemotherapy, respectively, will provide blood for ctDNA analysis at serial time points within the first week of initiating chemotherapy. The primary endpoint of this study is the feasibility of analyzing ctDNA spikes during the first week of chemotherapy.

Project 3: Exploration of Factors Associated with Imminent Risk of Late Recurrence in Hormone Receptor Positive Breast Cancer (Full project described in detail below)

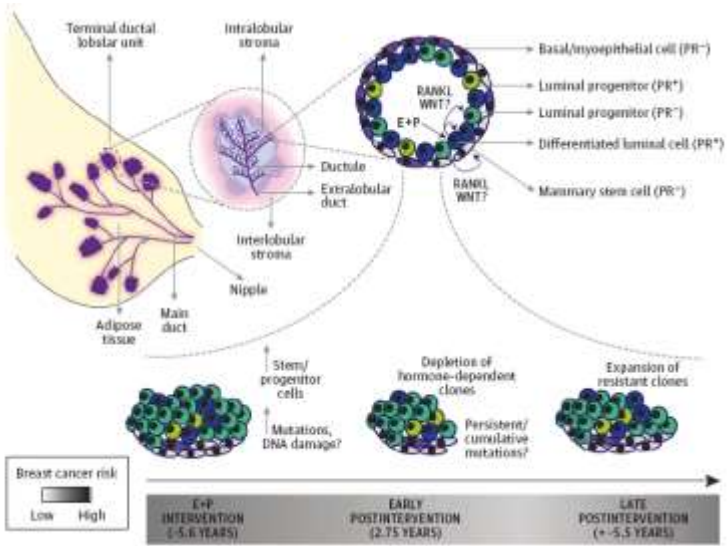
The majority of the life threatening breast cancer recurrences and deaths in women with hormone receptor positive (HR+) HER2- breast cancer take place more than 5 years post-diagnosis, after completion of adjuvant hormonal therapy. *Hold'em for Life* plans to conduct a prospective observational study of patient and tumour-related factors in high risk women with HR+/HER2- breast cancer who have completed adjuvant hormonal therapy, in order to identify a parsimonious group of factors that will identify those who are at imminent risk of recurrence. We hypothesize that such late recurrences will be anticipated by detectable increases in tumour-derived blood markers including ctDNA. We will recruit 2500 high risk patients with HR+ HER2- BC who have stopped adjuvant hormone therapy within the past two years. Plasma will be collected at baseline and annually until a distant recurrence or new invasive primary cancer is identified. We will then conduct nested case-control analyses to test whether ctDNA can differentiate women who develop late recurrences from

those who do not. Multiple proof-of-concept studies suggest that the presence of ctDNA in this setting is highly predictive of metastatic recurrence (Garcia-Murillas et al., 2015; Olsson et al., 2015). Acknowledging the fast pace of ctDNA technical and commercial development, the ctDNA analysis platform(s) selected for this study will depend upon perceived accuracy, cost, and plasma requirements at the time of analysis. Results from Projects 1 and 2 described above will also inform the selection of the analysis platform.

3. Hormonal and bone related factors – prepared by Dr. Rama Khokha

Drs. Rama Khokha (molecular biologist PMHRI) has explored **hormonal factors** such as estrogen, progesterone and Rank-ligand (RANK-L, a bone-related factor) in the development and treatment of breast cancer. As with our other core activities, this work has contributed to hypotheses that will be tested in our proposed late recurrence study.

Dr. Khokha's group is unravelling the role of hormones in the development of breast cancer, with a focus on progesterone and RANK-L (a bone-related molecule) and their impact on breast cancer stem cells. Her work has provided an explanation for why certain types of hormone replacement lead to increased breast cancer risk while others do not, and her discoveries have contributed to the development of a multinational randomized trial of a RANK-L inhibitor as a potential means of preventing breast cancer.



From an editorial in JAMA Oncology (2015) written by Hold'em scientists Drs. Purna Joshi (Post-doc), Pamela Goodwin and Rama Khokha.

Purna Joshi (Post-doctoral fellow) has expanded this work to investigate fat and stem cells in the breast, including examination of biologic properties of fat cells, and their potential contributions to breast cancer development. Specifically, she is investigating adipose tissue progenitor cell dynamics in the mammary gland to gain an understanding of their potential contribution to the link between adiposity and breast cancer. She is using a combination of experimental tools including lineage tracing in mouse models, confocal imaging, flow cytometry, clonogenic and transplantation assays, and RNA-seq analysis to characterize adipocyte progenitors in the mammary fat pad and determine their interactions with the epithelium.

Dr. Alison Casey (Post-doctoral fellow) is using a systems biology approach to identify key epigenetic regulatory proteins that are distinctly abundant in specific breast cell fractions. We matched these epigenetic targets with drugs (from the Structural Genomics Consortium) to test their cytostatic effects in selected in vitro and in vivo assays to determine which of our top drugs inhibit adult mammary stem cell expansion. We have also integrated our mouse mammary datasets at multiple levels (proteome, methylome, transcriptome, ATAC-seq) to create mammary molecular maps. Further, we have

extended our proteomic analyses to include high-risk patient samples, producing two separate proteomic datasets on primary human breast cells FACS-purified from prophylactic mastectomy samples (collaborations with T. Kislinger & H Berman at PMH & C. Eaves at UBC). Our bioinformatics analyses are focused on identification of i) specific biological features of the different HR+ and HR- breast cell types and ii) differences in the breast cell proteomes of young vs old women.

Dr. Daniel Schramek (LTRI scientist) has joined the Hold'em for Life team to investigate bone related trauma as a potential contributor to late recurrence in a mouse model. Previously he identified the essential osteoclast-differentiation factors RANKL/RANK as the major drivers of hormone-driven breast cancer (Schramek et al. Nature 2010). To further re-fine this finding his lab has generated a RANK reporter mouse, which expresses EGFP as well as CreERT2 under the control of the endogenous RANK promoter. EGFP allows us to characterize RANK expression in various cell types and mammary epithelial linages during tumor initiation, progression and metastasis (dormant micro- as well as macro-metastasis) while the inducible CreERT2 recombinase will enable us to perform linage tracing studies as well as genetically ablate and test any genes of interest. Together, this mouse will allow us to elucidate the role of hormones and RANKL in late recurrence of breast cancer.

In earlier work, Dr. Cescon has generated important results showing that a commonly used breast cancer drug – letrozole – effectively lowers estrogen levels in breast cancer patients, regardless of their degree of obesity (laying to rest a concern that the drug was not as effective in obese patients); he has also shown that there is no additional benefit to increasing the dose of the drug in obese women.

In 2017-18 we will continue to explore contributions of hormones to breast cancer development and metastasis and plan to contribute to both translational aspects of a multinational trial of denosumab (a RANK-L inhibitor, also a common osteoporosis treatment) in breast cancer prevention, with Drs. Goodwin and Elser. Dr. Schramek's mouse models of bone-related trauma and Dr. Khokha's work on RANK-L will contribute to assays in our planned late recurrence study.

4. Breast Translational Research Resource (BTRR) – prepared by Dr. Martin Chang

With Hold 'em funding we have established a **Translational Research Biorepository** that now includes tumor tissue, normal breast tissue, blood and clinical information (including follow-up) on almost 700 women with early/non-metastatic breast cancer – this biorepository is a rich research resource that will have its greatest value and impact in the next 3-5 years as some of the participants experience a recurrence or metastasis of their cancer. At that time it will become possible to conduct more detailed investigations into why some women develop metastases while others do not – an important complement to our planned late recurrence study. Recognizing the importance of fully annotated tissue samples, we have designated all BTRR tissues representing a first diagnosis from a patient fully consented for clinical annotation as "Key Patients". Tissues from Key Patients are flagged within the BTRR database, allowing additional oversight over tissue use, and protection against tissue depletion.

In the meantime, this resource has already been proven valuable to several groups of investigators at Mount Sinai's Lunenfeld Tanenbaum Research Institute, Princess Margaret and University of Toronto to conduct targeted research into, for example, differences in the characteristics of breast cancer in obese vs. non-obese breast cancer patients.

Starting with a cohort of 132 ER-positive/HER2-negative patients, we have constructed formalin-fixed-paraffin-embedded tissue microarrays of tumour tissue, to facilitate future high-throughput phenotypic and molecular characterization. Our plan is to periodically review and select tissues for further tissue microarray construction, organizing the collection both by year and by ER/PgR/HER2 phenotype.

Key Results

As of September 2017, a total of **684** breast cancer patients have been enrolled into the BTRR (653 have completed surgery), from which **499** have provided blood samples, 286 fasting blood samples, and 297 fresh frozen tissues collected. Full clinical annotations have been registered for 543 patients, including 359 ER+/HER2-, 75 HER2 + (both ER+ and ER-negative), and 45 triple-negative carcinomas. The BTRR includes annual chart reviews for primary invasive breast cancer participants. Of the 362 invasive cases, 349 have reached their one-year follow-up, 279 have reached year 2, 100 have reached year 3 and 7 have reached the fourth year follow up time point. In addition, 55 patients have had benign tissues registered with the BTRR in response to investigators' need to examine markers of breast physiology.

The following studies have been approved by the BTRR for access to samples:

- TRR Obesity: A study of estrogen signaling, insulin signaling, and inflammatory pathways in women with ER+/HER2- breast cancer (Dr. P. Goodwin, PI - described above)
- qTAP in breast cancer tissue: A component of a proteomics study that assess the signaling state of tumours, in particular receptor tyrosine kinase networks in HER2+ cancers and the Hippo pathway in triple negative disease. (Prof. J. Wrana, PI) – this team has just finished parallel testing on human colorectal cancer tissue in IP/mass spec experiments, with good results"' – work involving breast tissue will begin in the next few months.
- Targeting precursor cell determinants to personalize cancer therapy (Dr. R. Khokha)

Additional studies are pending approval, including a collaborative integration of BTRR data with an international study on Reducing the Burden of Breast Cancer in Young Women ("RUBY"), led by Dr. Steven Narod (Toronto) and Dr. May Lynn Quan (Calgary).

Future Focus

We are committed to ensuring access, to BTRR samples, is collaborative and free of prohibitive administrative barriers. The qTAP in breast cancer study (see above) was successful in obtaining funding from the CQDM/CIHR Collaborative Funding Program in Personalized Medicine to Accelerate Drug Discovery based on the availability and support of the BTRR. We will continue to emphasize availability of the BTRR to support a wide range of investigations. Information on available holdings can be found in Appendix 7.

This is an important resource for the entire University of Toronto community, developed, curated and maintained using Hold'em for Life funds.

BTRR Holdings

- blood (fasting & non-fasting; aliquots of whole blood, serum, and plasma)
- tissue (tumour & benign)
- clinical annotation (demographics, risk factors, pathology and staging, treatment, recurrence, death)

Sample Access & Utilization

- HIGH QUALITY, COLLABORATIVE & FREE OF PROHIBITIVE ADMINISTRATIVE BARRIERS
- TRR Obesity: A study of estrogen signaling, insulin signaling, and inflammatory pathways in women with ER+/HER2- breast cancer (Dr. P. Goodwin, PI)
- qTAP, A Novel Platform for Personalized Medicine in Cancer: a Study Nested within the BTRR (Prof. J. Wrana, PI)

Biorepository facilities (Lunenfeld-Tanenbaum Research Institute) where BTRR samples are processed and stored.

▲ Targeting precursor cell determinants to personalize cancer therapy (Dr. R. Khokha, PI)

In 2017-18, work related to the BTRR will continue as in previous years, allowing this resource to mature. We anticipate we will continue to enroll 150-200 women annually (with an ultimate goal of 1250-1500 breast cancer patients, with 7-8 years of follow-up in another 5 years). This number will be sufficient to conduct a broad range of detailed prognostic and biologic investigations. In the interim, we will continue to support novel research questions as they arise, ensuring that we do not deplete our resources for the critically important prognostic studies that will be possible once this resource matures. All key subjects have been flagged in order to preserve these specimens; non-key samples are dispensed whenever possible.

Clinical Trials Infrastructure Support

In addition to these four planks, we have also supported clinical trials infrastructure that facilitates participation in multicenter Phase 2 and 3 clinical trials. To date, this clinical research infrastructure support has been allocated primarily to MSH – a listing of studies/accrual is appended (Appendices 4 & 5). This support has enhanced accrual and will continue to be allocated. We are now expanding our support to three PMH based studies, two of which are led by Dr. Dave Cescon (medical oncologist and clinician scientist, PMH); (i) REFLECT – a prospective study incorporating the generation and pharmacologic testing of patient-derived xenografts (PDX) in the context of a drug development and clinical cancer genomics program for triple negative breast cancer patients with residual disease after neoadjuvant therapy or with metastatic disease and (ii) GENIUS – an exceptional responders (or non-responders) protocol incorporating genomic characterization of clinical tumour material similar to the Exceptional Responders program based at the NIH (US). The third study, led by Dr. Bratman, is the Characterization of Circulating Cell-Free DNA (cfDNA) as a Biomarker of Chemotherapy Response in Advanced and Locally Advanced Breast Carcinoma (described above). These studies are specifically aligned with "treatment of metastatic disease" and will recruit patients from PMH and MSH.

Much of our work has leveraged funding from other sources and/or has been layered onto existing infrastructure or clinical trials – this has allowed us to have an impact that is greater that would have been possible using only Hold'em funding. The enhanced level of clinical trial activity (Appendix 5) has recently facilitated membership of MSH-Oncology in the Canadian Cancer Clinical Trials Network (3CTN) which provides additional infrastructure support for clinical trials.

Trainees

Throughout our Hold'em work we have been dedicated to involving trainees (both basic and clinical) in our research activities. These trainees have been embedded into all of our clinical and basic research and have been given the opportunity to be part of a truly multidisciplinary research team. Support has involved one or more of: salary, access to research subjects and databases, material support for laboratory supplies and other direct research costs, travel to scientific meetings to present results of Hold'em funded research. Some trainees have leveraged this Hold'em support to obtain additional peer-review fellowship/studentship support, freeing up Hold'em funds for additional trainees. The majority of our 20 trainees have been enrolled into Graduate programs and/or post-doctoral positions. Several have gone on to hold academic positions at the University of Toronto and elsewhere.

A list of trainees is provided in the Appendix 1.

Major New Focus 2017-2025: Investigation of Late Recurrences in Hormone Receptor Positive, HER2 Negative Breast Cancer (Draft Protocol appended)

We are proposing an important new research initiative, which will investigate the problem of late recurrence of HR+, HER2- breast cancer. The evolution of our scientists into a highly functioning inter-institutional multidisciplinary research team has placed us in a unique position to conduct this novel, impactful research. All of the activities described above will take place in parallel to this new initiative. Although we have described these activities separately, the ongoing and new work is interconnected. This new initiative arises from advances in the understanding of late recurrence risk in breast cancer patients and from the discoveries we have made in our ongoing and completed Hold'em for Life funded research and it reflects the strong (and effective) interdisciplinary collaborations our team has developed with Hold'em funding. The late recurrence study will utilize the advances we have made in all 4 planks described above.

The goal of our new research focus is to identify predictors of late recurrence of HR+, HER2- breast cancer - this would allow us to know which individual women are most likely to develop a recurrence in the near future (the next one to two years – called "imminent risk"). Our ultimate goal is to develop treatments targeting these women at imminent risk that will prevent late recurrences and death. Successful completion of this work will likely require international collaboration. We plan to initiate this study in Toronto with Hold'em for Life funding and to develop international collaborations in the United States and Europe, and obtain funding from granting agencies (including the Breast Cancer Research Foundation) to leverage Hold'em for Life funding.

As with much of our Hold'em for Life funded research, this research will focus on the interface between the patient and her cancer; we hypothesize that changes in the patient may contribute to re-activation of dormant breast cancer cells, leading to late recurrence and that late recurrences will be heralded by increase in circulating tumor DNA (ctDNA), circulating cancer cells (CTCs) and other markers including tumor markers such as CA15.3 and CEA. Our work will be multi-disciplinary (involving all of our current scientists) and inter-institutional involving Mount Sinai and Princess Margaret Hospitals. For the first time we will expand to include Sunnybrook Odette Cancer Center (notably, Dr. Katarzyna Jerzak, who has recently joined the Hold'em for Life research group after conducting her Master of Science research work with our team). Several Hold'em for Life funded medical fellows, graduate students and post-docs (Drs. David Cescon, Ryan Dowling, Katarzyna Jerzak who now have staff positions at our participating hospitals as well as Ana Lohmann who is just finishing her PhD) have contributed actively to the development of this research and will play leadership roles in this work going forward. We plan to involve additional trainees in this new work, continuing to foster the next generation of scientists.

This work is in progress – here we summarize a draft protocol reflecting current activities; we expect to continue to modify and finalize this protocol during the upcoming months, and to establish international collaborations. We anticipate we will initiate work to demonstrate feasibility of recruitment early in 2018.

Background

Earlier diagnosis of breast cancer as a result of screening, as well as more effective adjuvant drug therapies (chemotherapy, hormonal therapy, trastuzumab) administered around the time of surgery have led to improved breast cancer outcomes by preventing the development of lethal metastatic recurrences. In Canada, breast cancer mortality rates are at the lowest levels since the 1950s, when statistics were first collected. However, almost 5000 Canadian women still die from breast cancer every year. Many of the life threatening/incurable breast cancer recurrences and deaths now take place more than 5 years following diagnosis, after completion of adjuvant therapy.

Overall Study Goal - To identify a parsimonious group of factors that will differentiate women with HR+ HER2- BC at high risk of late recurrence following completion of adjuvant hormone therapy (defined by baseline tumor characteristics) who will develop a late recurrence during the next 1-2 years from those who will not develop a late recurrence during that time.

Specific Goals:

1. To recruit 2500 patients with HR+ HER2- BC (regardless of menopausal status) who have completed adjuvant hormone therapy (including extended hormonal therapy) within the past two years and have not experienced a distant breast cancer recurrence.
2. To obtain information on characteristics of breast cancer at diagnosis (including T and N stage, ER, PgR, HER2, grade, lymphovascular invasion, genomic test results if available), systemic therapy administered and any local/regional breast cancer recurrences. We plan to collect original tumor tissue when possible.
3. To prospectively collect (i) information on patient-related factors using standardized questionnaires, (ii) biospecimens (blood and urine) annually for up to 10 years or until a distant recurrence or new primary cancer is identified.
4. To follow patients prospectively to ascertain the occurrence of distant metastases.
5. To conduct (i) prospective cohort statistical analyses of patient-related factors, and CBC/differential counts (which will be available in real time at all measurement points) and (ii) nested case-control analyses of ctDNA, CTCs, leptin, inflammatory and other blood factors. In these case-control analyses, women who experience distant recurrences (cases) will be compared to 4 matched women per case who do experience recurrences (controls).
6. To perform statistical modelling to identify factors that differentiate women who develop late recurrences from those who do not, with a particular focus on factors predicting risk within the next 1-2 years. In these analyses we will consider a range of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) as different test characteristics may be optimal for different clinical scenarios (e.g. high sensitivity for well tolerated treatments, high specificity for more toxic treatments. We will also consider that some factors (e.g. trauma) may contribute to the underlying biology of late recurrence while others (e.g. ctDNA) may herald an imminent recurrence.

Scientific Background

By the time a breast cancer is surgically removed, microscopic cancer cells have often spread to other parts of the body; many of these cells are killed by post-operative adjuvant drug treatments (chemotherapy, trastuzumab, hormone therapy), leading to lower risk of future recurrence. Unfortunately some cells are not killed – they remain in a dormant state (in essence, they are inactive, or under control) for years. At some point, in some patients, often many years after diagnosis, and particularly in women who have had hormone receptor positive breast cancer, these dormant (sleeping) cells grow to become incurable metastases.

Metastatic dormancy is regulated by complex interactions between disseminated tumour cells, their microenvironment, and systemic host factors. Three major processes have been implicated:

(i) Cellular dormancy: A major contributor to metastatic dormancy is the failure of disseminated tumour cells to proliferate within a foreign environment. Cells enter into a quiescent-like state, which is characterized by activation of pathways that reduce cell proliferation and maintain cell cycle arrest. Escape from dormancy is believed to be induced by changes in the microenvironment surrounding the disseminated tumour cells. These changes may represent a co-evolution of the dormant cells and their microenvironment, which ultimately contributes to disease progression.

(ii) Angiogenic dormancy: Tumour dormancy can be induced by the absence of sufficient nutrients and oxygen to support the level of disseminated cell proliferation necessary for tumour growth; cells can proliferate but lack the blood supply required for full metastatic outgrowth. Exit from angiogenic dormancy, or the angiogenic switch, can be stimulated either locally within the microenvironment (by stroma and cancer cells) or systemically via alterations in circulating factors such as VEGF and placenta growth factor (PlGF). Surgery or physical trauma and the subsequent healing process can stimulate

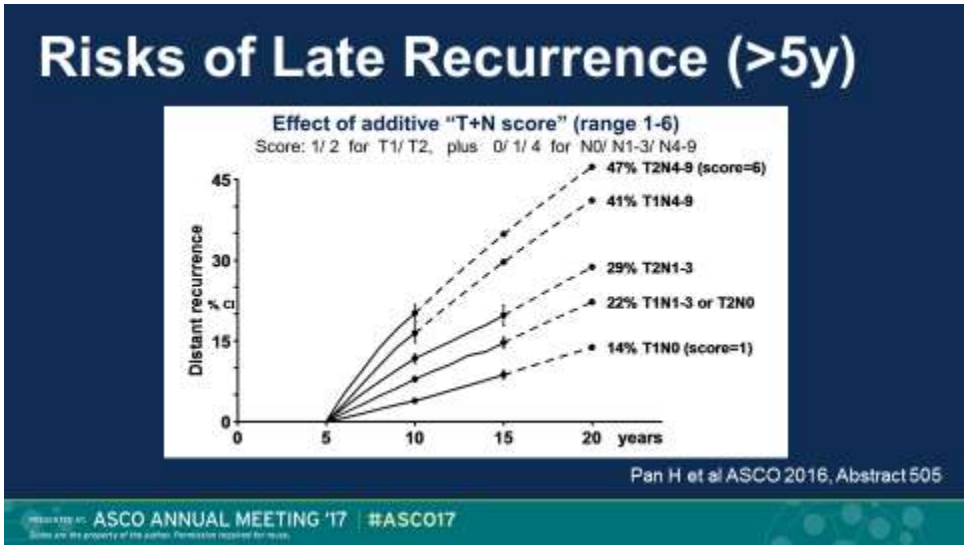
systemic increases in angiogenic factors; changes in diet, alterations in use of medications (such as those for cardiovascular disease, or NSAIDS) may also affect systemic levels of angiogenic factors and inflammatory cytokines.

(iii) Immune system induced dormancy: Both the innate and adaptive immune systems detect and eliminate cancer cells; some cancer cells may emerge as resistant to elimination or masked from detection. Equilibrium (maintained by the adaptive immune system) will be reached between the immune system's ability to eliminate cancer cells and proliferation of the resistant subclone(s). Escape occurs when a subclone emerges that proliferates at a rate exceeding elimination due to immune resistance or immune system suppression.

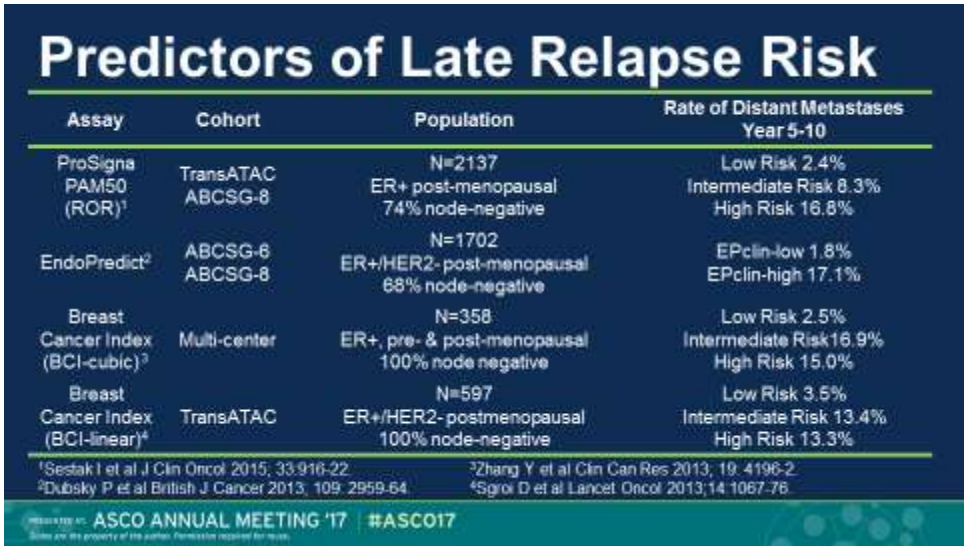
The specific contribution of these (and other) mechanisms to late recurrence, which remains a major clinical problem in hormone receptor positive breast cancer, is unclear.

Clinical Factors Associated with Late Recurrence in HR+, HER2- BC:

1. Tumor Characteristics: Research to date has focused largely on tumor characteristics – more advanced stage (larger tumor size, greater nodal involvement), lobular (vs. ductal) histology and biologic characteristics of the tumors, potentially including grade, have been identified as predictors. Recently reported data from the Oxford Overview, showing risk of recurrence out to 20 years post-diagnosis in women with ER+ breast cancer who are free of recurrence at completion of their adjuvant hormone therapy at 5 years, have guided patient selection using clinical factors for this study. While these prognostic factors permit the identification of populations of women at risk), our focus will be on identification of factors associated with imminent risk of distant recurrence at an individual level.



Several tests, performed on primary tumor tissue, often developed as predictors of early relapse have also proven useful as predictors of late relapse – these include the PAM 50 Risk of Recurrence (ROR), Endopredict (EP) and the Breast Cancer Index (BCI - HOXB13/IL17BR but not the Molecular Grade Index proliferation module). High risk scores on these indices are associated with 15-17% risk of recurrence over 5 years (at least 3% annual risk) after completion of adjuvant hormonal therapy (slide prepared by Phil Bedard ASCO 2017).



2. Patient-related factors (including medications)

(i) From a patient perspective, drugs that lower estrogen levels or block estrogen action (e.g. aromatase inhibitors or tamoxifen) lower risk of both early (within 5 years) and late (5 to 10 years and beyond) recurrence. In contrast, administration of a synthetic estrogen (tibolone) increases risk of late recurrence. These observations provide evidence that **estrogen** is one factor that is linked to late recurrence.

(ii) **With Hold'em funding, our group has shown that obesity and higher leptin (a growth factor associated with obesity) are associated with late recurrence**, providing evidence that **obesity-related** factors are also important. Obesity may lead to changes in the microenvironment around dormant cancer cells (e.g. inflammation) or to higher levels of growth factors that more directly stimulate cells. Physical activity has also been associated with lower risk of both early and late recurrence via mechanisms that may be similar to those associated with obesity but have not been well delineated.

(iii) There are anecdotal reports that trauma and surgery can lead to late recurrences, possible because wound-healing leads to new blood vessel growth, microenvironment changes and secretion of growth factors. Women who undergo breast reconstruction after they have completed their hormone therapy have been reported to have higher rates of recurrence than those who have breast reconstruction while they are still receiving hormone therapy, consistent with an impact of surgery and wound healing on activation of dormant cells. **Dr. Daniel Schramek (scientist at LTRI), who has recently joined our group is investigating mouse models of trauma (bone fracture) as a potential contributor to late recurrence – this work will inform our planned research. Dr. Angela Shellenberg (Surgical Breast Fellow) is conducting a critical appraisal of the clinical literature in this area.**

(iv) There are also reports that aspirin (an anti-inflammatory agent), beta-blockers and bone targeting drugs (bisphosphonates, including zoledronic acid) may reduce risk of recurrence, potentially reflecting contributions of inflammation or changes in the bone micro-environment in the development of late recurrences. **With Hold'em for Life funding Dr. Rama Khokha has been conducting research in this area**.

(v) Diabetes has been associated with increased risk of recurrence in some (but not all) studies, while commonly used drugs such as ASA, metformin and beta-blockers may be associated with lower risk of recurrence.

3. Circulating Factors that may Herald a Late Recurrence

   We hypothesize that re-activation of dormant cancer cells will be reflected in rises in tumor markers and/or release of ctDNA and/or CTCs into the circulation, before clinically overt metastases develop. **Dr. Scott Bratman, a Hold'em for Life Scientist with expertise in measuring this DNA, will investigate this hypothesis as part of our proposed research.**

To date, there has not been a way to identify which specific women will recur and when a recurrence is imminent (but still potentially avoidable). It is expected that our research will ultimately lead to the development of interventions that target individual women who have an imminent risk of recurrence in order to prevent incurable recurrence and subsequent death.

Research Design

Brief Summary of Study Design (A draft protocol is appended)

Using a prospective cohort design, 2500 women with prior HR+ HER2- BC who have not experienced a distant recurrence will be enrolled during the first 2 years after adjuvant/extended adjuvant hormone therapy; host and circulating factors will be measured annually (blood will be stored for future analyses) until distant recurrence or final study analysis, whichever occurs first. We plan to collect data remotely (via telephone, with blood draws and urine collections handled by commercial laboratories) to maximize access to the study by potential eligible women and we will request tumor tissue from the institution where participants had their primary surgery. Statistical analyses will use a cohort design for questionnaire based factors and CBC/differential data (which will be available on all subjects at all measurement points) and a matched case control design (matching for time since completion of adjuvant hormone therapy, baseline T, N and grade) for factors requiring additional costly analysis (e.g. blood assays including ctDNA, CTCs, diet questionnaires). With 3-4 years of enrolment and a projected annual event rate of 2.5% and a gradual ramp-up in recruitment, we project 147 distant recurrences after four years, 271 after six years and 300 mid way through the seventh year. Analyses will compare measurements over the 1-2 years prior to relapse (matched time in controls) in those who recur to those who do not. Statistical modelling will be undertaken to identify a parsimonious group of factors that optimally identify women at imminent risk of recurrence, first in a training set (200 recurrences) and subsequently in a test set (100 recurrences). We will explore sensitivity, specificity, PPV and NPV, potentially developing different models that have different test characteristics that would be relevant in different clinical scenarios. For example, when considering future endocrine treatment associated with low toxicity, we may want to maximize sensitivity (i.e.: ensuring that we miss few women who will recur) while accepting modest specificity (i.e.: we will accept that not all women labelled as being at imminent risk will actually recur within the subsequent 1- 2 years- that is, we will accept a low positive predictive value). In contrast, we may want to maximize specificity (and accept a lower sensitivity) when more toxic treatments are being considered in order to focus treatment on those most likely to benefit.

Key design features include:

Inclusion Criteria:

1. Diagnosis of ER and/or PgR positive (at least 10% positivity of at least one of these receptors), HER2 negative (ASCO/CAP guidelines) invasive breast cancer. In the case of multiple/bilateral breast cancers, all cancers must be HR+ and HER2 negative and hormone therapy for the most recent cancer must have lasted at least 2 years and been stopped within the last 2 years; all other entry criteria must be met.
2. At least 2% annual risk of recurrence - based on the EBCTC analysis presented by Pan et al ASCO 2016 this will include those with breast cancer that was staged as T2, T3 or T4 with any N+;T1 at least 4 nodes +. Those with T2N0 or T1 N1 (1-3 nodes+) cancers will be enrolled if their tumor was grade 3 or high risk based on tissue scores (PAM50 – high risk, Endopredict – High Risk, BCI Intermediate or High Risk), Oncotype - high.
3. Receipt of adjuvant hormone therapy for at least 2 years, with stopping of hormone therapy within the past 2 years. Receipt of adjuvant chemotherapy, biologic therapy including HER-2 and bone targeted therapies is acceptable provided they have also been completed.
4. Willingness to comply with study procedures, including follow-up for up for 10 years.
5. Life expectancy of at least 10 years.

Exclusion Criteria:

1. Previous invasive cancer (non-breast) that has not been treated with curative intent at any time; cancers diagnosed more than 5 years ago and treated with curative intent without recurrence will not lead to exclusion nor will non-melanoma skin cancers or in-situ cancers at any site diagnosed at any time.
2. Metastases outside of the ipsilateral breast and axilla at any time.
3. Inability to speak, read and write English.
4. Unwillingness to provide informed consent or to comply with study procedures.
5. Current receipt of any systemic therapy for breast cancer.

Blood, Urine and Data Collection

i. Clinical data (obtained from medical record) – date of breast cancer diagnosis, T,N, grade, ER PgR HER2, histologic subtype (ductal, lobular, mixed ductal/lobular, other to be specified), PAM50/EndoPredict/BCI score/Oncotype Dx (if performed), treatment (medical, surgical, radiation) including dates and doses, adjuvant hormone therapy including duration and date of completion, breast cancer events since diagnosis (locoregional recurrences, new primary cancers). Tumor tissue obtained at primary surgery will be requested from the institution at which the surgery was performed.
ii. Host factor questionnaire – breast cancer risk factors including family history, overall health, alcohol intake, smoking, trauma including surgery/accidents, medical conditions, medications/alternative therapies, life stressors, social support, physical activity, and diet.
iii. Anthropometrics - height and weight (self-report).
iv. Blood - (approximate volume 75 cc = 5 tbsp.) – whole blood (1 tube), plasma (3 tubes), serum (1 tubes), Streck tube (3 tubes), Cell Search tube for CTCs (1 tube), purple top (for immediate CBC/differential assay). Immediate measurement of CBC with differential. Serum and plasma will be aliquoted into 1cc tubes and those aliquots, as well as Streck tubes, will be stored locally at -80C then shipped to the central biorepository at Mount Sinai. Participants will be given the option of having blood drawn at Mount Sinai (Toronto), another participating hospital or at a designated commercial blood drawing facility (patients will be provided labels that include only their study numbers). Specimens not drawn at Mount Sinai will be handled as described above and then shipped

on dry ice to a monitored biorepository based at Mount Sinai Hospital, Toronto until future analysis. Details of specimen handling are outlined below.

v. Random urine – 10 cc will be collected and aliquoted into 1cc vials and frozen at -80C.

Follow-up will stop when patients develop a distant recurrence (outside of the breast and ipsilateral axilla) or the final study analysis is undertaken. Participants with a new invasive cancer (breast or other) or an ipsilateral locoregional recurrence of breast cancer will continue to participate in study measurements and be followed; their data will be analyzed separately.

Blood and urine assays

i. Complete blood count with differential will be assayed in the local blood drawing facility using CAP/CLIO certified procedures when blood is drawn as storage impacts these measurements.
ii. Other biochemical assays will be finalized by an expert group based on scientific knowledge at the time of analysis (likely to include leptin, estrogens, and inflammatory markers including interleukins, VEGF, PlGF, CA 15-3, CEA and other factors). These will be assayed using standard validated laboratory kits.
iii. Circulating tumor DNA – methylation, mutation analysis, using validated assays selected based on information available at the time the assays will be performed.
iv. CTCs – to be measured using standard methodology at a central laboratory
v. Metabolomics/Proteomics – to be explored
vi. Urine will be assayed for estrogens and other factors

When assays are performed, aliquots (identified using study ID numbers only) will be pulled, batched and shipped to the laboratory that will perform the assay. When relevant, samples from the same participant will be performed in the same batch. Results of all blood assays will be integrated into the main study database for statistical analysis.

Outcomes:

Outcomes will be ascertained by regular self-report (via annual telephone calls), and confirmed by medical record review. Participants will be asked to provide the name(s) of the physicians involved in their breast cancer care, as well as their family doctor. The physician designated by the patient as currently primarily responsible for their breast cancer follow-(usually their family doctor) will be asked to complete a form annually that reports whether or not a recurrence or new primary cancer has occurred.

Primary Outcome – distant BC recurrence (outside of the breast and ipsilateral axilla), excluding new primary cancers (breast or other).

Secondary Outcome – any BC event, including loco-regional recurrence,

Statistical Analysis:

Our aim is to develop models that can identify subjects at high risk of developing a late recurrence during the next 1-2 years, using a parsimonious group of factors. We also require that the models be interpretable in order to increase understanding of the processes and generate hypotheses. All model development will take place using a Training data set and all final model assessments will be done using a separate Test data set to obtain unbiased assessments of the predictive ability of the models.

We will classify study variables as follows:

Regular and special factors: Regular factors are data collected regularly for every subject at every time point (e.g. trauma, surgeries, global health, diet, exercise). They are available for analysis in a cohort design but can also be used in a case-control design. Special factors (e.g. ctDNA, CTCs, leptin, inflammatory factors) are – due to their cost – measured only in subjects in the case-control study and only at selected time points (initially only at index and antecedent follow-ups). Special factors can only be analyzed in a case-control design; they are in fact the reason for having a nested case-control study in the first place. We will also consider factors that may reflect biologic processes associated with recurrence (i.e.: many of our patient-related factors) separately from those that may herald a developing distant recurrence (e.g. ctDNA, CTCs, tumor markers) in some analyses.

Matching of controls to cases in case-control study: Matching of controls to cases for the case-control study will be performed at the end of the study when follow-up has stopped. For each evaluable case, four evaluable controls will be randomly selected among all available evaluable controls that have the same baseline T, N and grade as the case and for whom the time since completion of adjuvant hormone therapy to the index follow-up is within 12 months of that of the case. Matching will not be done on the type or duration of adjuvant therapy which will be controlled for in the multivariable models.

Multivariable models to identify predictors of imminent recurrence

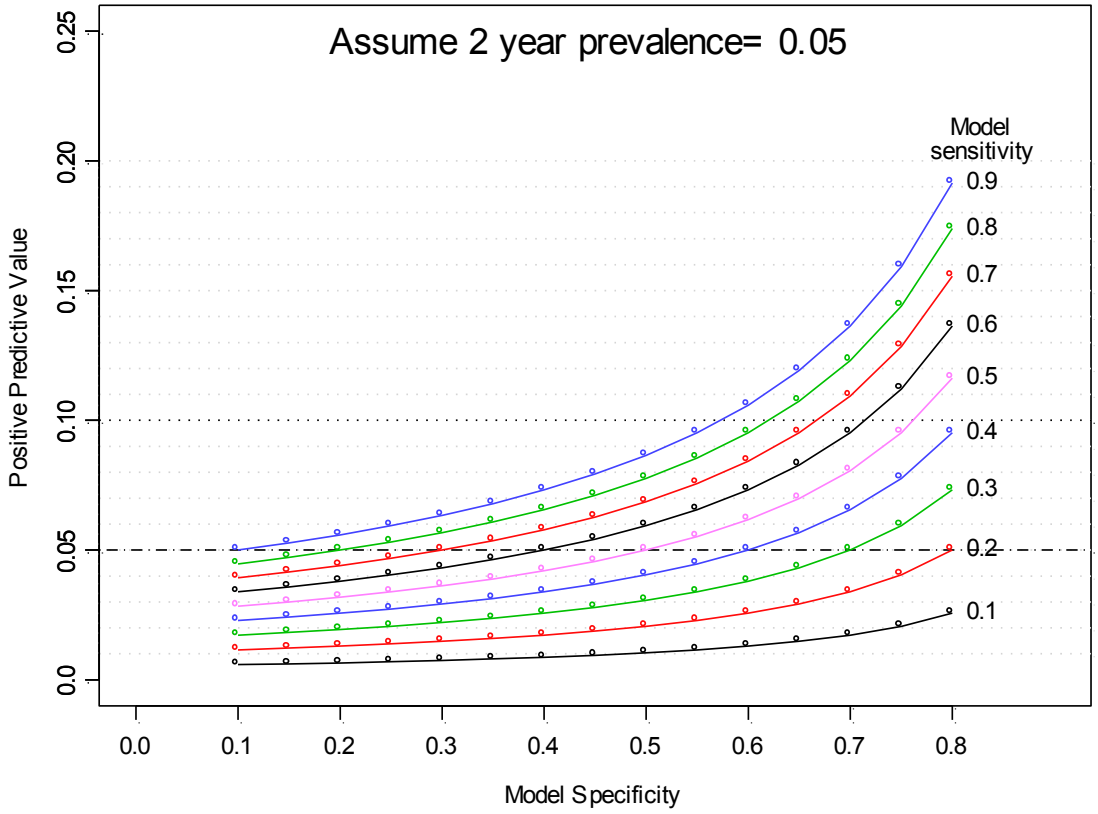
With 3-4 years of enrolment and a projected annual event rate of 2.5% and a gradual ramp-up in recruitment, we project 147 distant recurrences after four years, 271 after six years and 300 mid way through the seventh year.

The cohort Training set will be used to develop a small number of models using the regular factors to predict risk of distant recurrence within the next 2 years. The case-control Training set will be used to a similar aim using the special factors plus selected regular factors. Factors will be come mainly from the index follow-up and the change from the index versus antecedent follow-ups. Baseline (enrollment) variables may also be included. Logistic regression will be used to fit univariable and multivariable models, although other methods may like regression and classification trees may also be explored. Final models will be chosen based on performance on the Training set and scientific considerations.

Model performance

The performance of the model developed on the cohort Training set will be assessed in the cohort Test set and the model developed in the case-control Training set will be assessed in the case-control Test set. The primary assessment criterion will be the area under the receiver operating characteristic (ROC) curve (AUC). The ROC curve is obtained by plotting sensitivity versus 1-specificity for different thresholds. These sensitivities and specificities will be obtained by thresholding on the log odds calculated for each Test subject using the model developed in the Training set and comparing actual versus predicted status for cases and controls at this threshold. Secondary outcomes will be sensitivity and specificity of the model for specific thresholds. In general the performance of a model depends on the model's sensitivity, specificity and the prevalence of the disease. We expect a "usual" prevalence of 5% of subjects to recur with a distant metastasis over two years. The following graph shows how this probability is changed by a positive prediction by the model, depending on the

sensitivity and specificity of the model at a specific threshold.



For example, a model that achieves 80% sensitivity and 65% specificity at a specific threshold will have a positive predictive value (PPV=probability of a recurrence happening in the subsequent two years given the model predicted it) of 10% which is double the baseline prevalence of 5%. As noted above, differing test characteristics will be explored in relation to different clinical scenarios.

Timeline (Note: some activities overlap in time)

July 2017-December 2017	• protocol development, ethics approval, development of recruitment strategies
January 2018 – December 2021 (4 years)	• recruitment and baseline measures
January 2018 – December 2024	• ongoing follow-up (to 300 distant recurrence events in women who have provided blood and data annually)
July 2019 – June 2021	• analyses of selected baseline factors
January 2025 - December 2025	• Biochemical and ctDNA assays
July 2025 – December 2026	• statistical analysis of factors associated with late recurrence

Anticipated Impact

Successful completion of this research, allowing us to identify breast cancer survivors at imminent risk of late recurrence, will open up new possibilities for interventional research, which could leverage an increasing number of relevant anti-cancer drugs or non-pharmacologic interventions which could be curative in this setting. Rationally-designed clinical trials targeted to these women at imminent risk will permit efficient evaluation of treatments that could be effective in preventing many (possibly a majority) of these recurrences, dramatically lowering breast cancer mortality rates for hormone receptor positive breast cancer, the most common form of breast cancer in Canada and around the world. Our research is also expected to contribute to enhanced understanding of the biologic factors associated with late recurrence. We will require funding for at least 7-8 years to successfully implement and enroll women onto this study. We have already received approximately $650,000 CAD from the Breast Cancer Research Foundation to implement this study. Hold'em has allocated an additional $600,000 CAD during the current year of funding. We anticipate ongoing funding from these two sources and expect to further leverage this support through other funders.

SUMMARY AND CONCLUSIONS

Over the past five years, the Hold'em team has evolved into a successful multidisciplinary team that has effectively conducted research in four inter-related planks (Obesity, Hormonal Factors, Liquid Biopsies and Biorepository), with plans for ongoing activities in each of these planks. We have also provided support for clinical trials infrastructure and 20 trainees and we have published 36 manuscripts and presented 20 abstracts.

In 2017, we have embarked on a new, timely and potentially impactful area of research – identification of individual patient factors associated with imminent risk of distant recurrence after hormone treatment for hormone receptor positive breast cancer. This work will involve international collaborations and will require funding beyond Hold'em (currently the Breast Cancer Research Foundation has matched Hold'em funding). We anticipate this new project will require 7-8 years for completion and that we will obtain funding from multiple sources to conduct this research.

Appendix 1: List of Hold'em Scientists, Trainees and Staff

	Mt. Sinai Hospital (MSH) Lunenfeld-Tanenbaum Research Institute (LTRI)	Princess Margaret Hospital (PMH-University Health Network) Princess Margaret Hospital Research Institute (PMHRI)
Medical Oncology/ Surgical Oncology	**Pamela Goodwin** *Trainees* • **Ana Lohmann, MD** (2013-present) - (1) Prognostic associations of vitamin D in NCIC MA.21, a phase III adjuvant randomized trial of three chemotherapy regimens in high risk breast cancer, (2) Pilot study of the association of obesity associated factors with circulating tumour cells in metastatic breast cancer, (3) Anthropometric measurements, metabolic factors, diet and physical activity in long-term breast cancer survivors: change from diagnosis and comparison to non-breast cancer controls. (4) Effects of metformin versus placebo on Vitamin B12 metabolism in non-diabetic breast cancer patients in CCTG MA.32 (5) Association of obesity with breast cancer outcome in relation to breast cancer subtype (5) Association of metabolic, inflammatory and tumor markers with circulating tumor cells in metastatic breast cancer • **Katarzyna Jerzak** (MSc student, 2016-present; MSH & Sunnybrook) Hepcidin in Breast Cancer (Note – Primary Supervisor, MSc) • **April Rose** (2013) - Vitamin D and early stage breast cancer prognosis (Note – Medical Student, McGill University, Montreal) • **Ariadna Tibau, MD** (2012-2014) - (1) Pilot study of the association of obesity associated factors with circulating tumour cells in metastatic breast cancer, (2) Post-surgical highly sensitive C-reactive protein and prognosis in early-stage breast cancer, (3) Non-estrogenic obesity-related variables and breast cancer prognosis – A systematic review and critical appraisal (Note – Primary Supervisor, Clinical Research Fellowship)	**David Cescon**

	Mt. Sinai Hospital (MSH) Lunenfeld-Tanenbaum Research Institute (LTRI)	Princess Margaret Hospital (PMH-University Health Network) Princess Margaret Hospital Research Institute (PMHRI)
	• **David Cescon, MD** – (2012-2014) - Vitamin D in breast cancer. (Note – Medical Oncology Fellowship Secondary Supervisor) • **Saroj Niraula, MD, MSc** (2009-2012) – Clinical and biologic effects of metformin in early stage breast cancer. (Note – Medical Oncology Fellowship Co-Supervisor 2009-2012; MSc Supervisor 2010-2012; completed MSc in 2012 but is still involved in related publications) • **Ryan Dowling, PhD** (2009-2014) - Biological characterization of patient samples, relationship between obesity, insulin and breast cancer, CTCs (*Note – Research Fellow, Co-Supervisor, Dr. Vuk Stambolic Princess Margaret Cancer Centre Primary Supervisor*) • **Sara Soldera** (2016-2017) – Clinical research fellow – investigation of sexual functioning in breast cancer survivors • **Angela Shellenberg** (2017-2018) – Surgical fellow – The impact of surgery and trauma on risk of late recurrence in breast cancer patients	
Pathology	**Martin Chang** *Trainees*: • **Dr. Zohreh Eslami**- CLSB and Obesity • **Dr. Mahdi Rahimi** - MA.21/MA.32 correlatives (to June 30, 2017) • **Dr. Aysegul Sari** (July 1, 2017- current)	
Radiology	**Pavel Crystal (2012-2016)**	**(Pavel Crystal; 2012-2016)** **Scott Bratman**
Statistics	**Marguerite Ennis, PhD**	

	Mt. Sinai Hospital (MSH) Lunenfeld-Tanenbaum Research Institute (LTRI)	Princess Margaret Hospital (PMH-University Health Network) Princess Margaret Hospital Research Institute (PMHRI)
Laboratory	**Jim Woodgett** *Trainee*: • **Jennifer Gorman,** PhD (2011 - present) - Evaluation of genes implicated in metastatic spread and circulating tumour cell populations. **Daniel Schramek** *Trainee*: Ellen Langille (PhD student, 2016-present) - breast cancer models	**Vuk Stambolic** **Ryan Dowling** *Trainees*: • **Ryan Dowling, PhD** (2009-2016) - Biological characterization of patient samples, relationship between obesity, insulin and breast cancer, CTCs • **Yekaterina Poloz, PhD** (2014-present) - The role of insulin receptor in breast cancer. • **YingJu Chang, PhD** (2013-2015) - Biology of PDZ-RhoGEF, a key regulator of adipose tissue development and a modulator of cancer development and metastasis. **Rama Khokha** *Trainees*: • **Alison Casey, PhD** (2012-present) - Targeting mammary progenitor cell activity for chemoprevention • **Purna Joshi, PhD** (2012-present) - Host factors in mammary stem cell niche, breast cancer risk • **Pirashaanthy Tharmapalan, PhD Candidate** (2015-present) - Role of progesterone receptor in mammary subsets • **Hyeyeon Kim, PhD Candidate** (2015-present) - DNA damage response in the mammary gland
Program Staff	**Linda Bennett**, Project Manager (2014-current) **Elma Lee**, Project Coordinator **Karman Fazaee**, Clinical Research Coordinator	Program Staff provide support at PMH for trials led by MSH and PMH.

	Lakshmi Rao, Clinical Research Coordinator **Bee Ling Lu,** Clinical Research Coordinator **Olivera Jugovic,** Clinical Research Coordinator **Maria Chu,** Data Entry **Cary Greenberg** (2012-2014)	

Appendix 2: SAB Report 2016

February 26, 2016

Dr. Jim Woodgett
Director of Research
Lunenfeld-Tanenbaum Research Institute
600 University Avenue, Rm 982
Toronto, Ontario M5G 1X5

Dear Dr. Woodgett:

Re: Hold'em for Life Translating Discoveries into Breast Cancer Cures
Scientific Advisory Board Meeting January 27, 2016

Further to the teleconference call held on January 27, 2016, we are writing to provide our assessment of the Hold'em for Life Translating Discoveries into Breast Cancer Cures program.

The Scientific Advisory Board (SAB) is very impressed with the achievements of this program to date. The Hold 'Em for Life (HEFL) research team has accomplished an enormous amount organizationally and scientifically. The program has a well-developed infrastructure, is responsible with its resources and is conducting high quality science. We believe the HEFL fundraisers have made a good choice in funding this initiative.

Hold'em Research Team

The scientists have really come together as a team. It is exciting to see the highly committed clinician-scientists and basic researchers working closely with this level of synergy.

The program encourages and facilitates broad-based scientific input and shared decision making. It has been successful at breaking down institutional barriers to link Mt. Sinai Hospital (MSH)/Princess Margaret Hospital (PMH) breast cancer research in a productive manner. These collaborations leverage both the breadth and depth of expertise and additional funding that the scientists bring to the Hold'em breast cancer research program.

Hold'em has engaged 20 trainees over the last 5+ years and they are exposed to the full spectrum of research. Some of the trainees are now beginning to play a leadership role in their own studies and two former trainees have accepted staff positions and continue to work with the group.

Hold'em Research Activities

The overall focus of the Hold'em program is the on the interface between the patient (host) and her cancer, with research spanning molecular biology to physiology to lifestyle and clinical management.

There are four inter-related planks that reflect robust interaction between preclinical and clinical researchers, with a focus on host hormonal factors (e.g. insulin, estrogen, progesterone) and tumor development and progression.

1. **Breast Translational Research Resource (BTRR)**

   The BTRR is an established research resource available to the Hold'em researchers and other University of Toronto investigators. The BTRR currently includes tumor tissue, normal breast tissue, blood and clinical information.

   A total of 425 breast cancer patients have been enrolled into the BTRR. Pathology slides and full clinical annotations have been registered for all patients; key patients (primary invasive cancer and followed clinically at MSH) are being followed for clinical outcomes.

One researcher has completed a research project using BTRR samples; two other applications are in progress and there have been 5 additional requests and applications are anticipated this year. The plan is to continue to enroll subjects onto the BTRR and promote and facilitate access to this resource.

We were pleased to see the significant progress made. Over time, as key BTRR subjects reach 3-5-10 years of follow-up, these tissues will become increasingly more valuable as a research resource. The SAB cautioned the group to set up a process to safeguard against depleting these particularly valuable tissues and blood.

We have recommended that the group establish a policy whereby some tissues from key patients are preserved for asking important research questions in the future. We would encourage a process whereby the tissues from non-key participants are utilized first, wherever possible.

2. Circulating Tumour Cells / CTC Characterization Using the Amnis Imagestream/ Circulating tumour-derived DNA (ctDNA)

With HEFL funding, Dr. Martin Chang established the first clinical measurement facility in Canada at MSH. The group has recently completed an observational study involving 96 metastatic breast cancer patients that examined patient metabolic host factor correlates of CTCs; results were presented in two posters at the San Antonio Breast Cancer Symposium (SABCS) in December 2015. In total, three of the trials undertaken by the group have included CTC measurements.

In conjunction with this work, the Woodgett lab, in collaboration with Drs. Dowling and Chang, is developing assays to detect and further characterize CTCs using the Amnis ImageStream platform, with a particular focus on insulin receptor and viability assays.

With the addition of Dr. Scott Bratman (radiation oncologist; clinician scientist, PMH) to the group, an increasing area of focus has been circulating tumor derived DNA ("liquid biopsy" or ctDNA). Drs. Bratman and Chang plan to examine CTC and ctDNA spikes post chemotherapy in the neoadjuvant and metastatic setting as potential early markers of tumor response to treatment.

The SAB supports the continuation of work with the CellSearch and the other platforms. Heterogeneity in the Hold'em research platforms is important in the event that the CellSearch technology ceases to be available. We suggest that the team continue with exploratory analyses, phenotyping and genotyping cells. The researchers are encouraged to also think about clinical questions, beyond the utilization of the CTC platform in practice that they could address.

3. Clinical Metabolic Studies: Focus on Obesity, Insulin and Metformin

The research investigating metabolic factors in breast cancer, with an ongoing focus on insulin, metformin and exploration of other potential obesity-associated mechanisms, represents a major theme of our Hold'em work. This area has been led by Drs. Pam Goodwin (MSH/ Lunenfeld-Tanenbaum Research Institute-LTRI) and Vuk Stambolic (Princess Margaret Hospital Research Institute-PMHRI). HEFL funding has contributed to the correlative work in MA.32, a large adjuvant trial of metformin; this work is important as it will provide information regarding which specific breast cancer patients may benefit from metformin. Initial results demonstrate improved metabolism in women receiving metformin (vs placebo); baseline and 6 month blood assays have been completed on all participants. Currently, ongoing work investigates a SNP (rs1121617, located at a locus associated with the ataxia telangiectasia mutated (ATM) gene) that has been associated with metformin benefit in diabetes.

Dr. Martin Chang is conducting additional investigations on tissue microarrays (TMAs) involving over 3500 MA.32 samples; the team (led by Dr. Ryan Dowling) is developing and testing key immunohistochemistry assays (including identification of clinically relevant cut-points) for markers in the PI3K, ras and other pathways using material from MA.21 (an older RCT of anthracycline based chemotherapy).

In parallel, the team has been evaluating these markers in samples obtained from a metformin neoadjuvant window of opportunity study, obtaining results that suggest metformin acts indirectly on breast cancer cells through improvement in patient metabolism. Accrual to a randomized trial of chemotherapy +/- metformin in the metastatic setting has recently been completed and results are anticipated in the next 6-9 months. Additionally, Drs. Vuk Stambolic and Ryan Dowling have developed an insulin receptor (IR) knockout mouse that is associated with reduced mammary tumor burden and metastatic potential; they plan to investigate molecular mechanisms and relationships with diet induced obesity using this mouse model.

Dr. Martin Chang has explored the association of crown-like structures of the breast (CLS-B) and altered patient metabolism/obesity in women with early breast cancer, reported in a poster presentation at SABCS 2015. CLSB were associated with obesity but not obesity associated physiologic factors that have been linked to poor breast cancer outcomes.

Finally, the relative contributions of estrogen, insulin and inflammatory signaling in obese and non-obesity associated estrogen receptor positive breast cancer are being examined using BTRR resources.

At the request of the HEFL scientific team, the SAB discussed at length whether the program should be more scientifically heterogeneous. It was noted that a focused program is required to determine the relationship between obesity and breast cancer. The funding environment is such that it is difficult to secure funding for comprehensive work such as this; ultimately it was agreed that a good balance has been struck and that diluting the focus too much would weaken the potential of the program to succeed.

4. Hormonal Factors

Drs. Khokha and Cescon are investigating factors such as estrogen and progesterone in the development and treatment of breast cancer.

At SABCS 2015, Dr. Cescon presented results of an intervention study of the impact of obesity on the ability of standard dose vs double dose letrozole to effectively lower estrogen levels in obese breast cancer patients (AI Host Factors Study) – both doses of letrozole had similar effects on estrogen levels.

Dr. Khokha's group is focusing on the role of hormones in the development of breast cancer – her group has demonstrated that progesterone generates expansion of mammary stem/progenitor cells and is investigating underlying molecular targets. Her work led to an editorial in JAMA Oncology written by Drs. Khokha, Goodwin and Purna Joshi (a post-doc in Dr. Khokha's lab) addressing mechanisms underlying observations of increased breast cancer risk with estrogen plus progesterone vs. estrogen alone in the Women's Health Initiative trials, and two publications in Stem Cell Reports. Ongoing and planned work focusses on single cell analysis of mammary progenitor cells, as well as matched proteome & methylome profiling of mouse mammary stem/progenitor cell subsets where new epigenetic inhibitors are being identified to target mammary progenitors as a prevention strategy. This work is ripe for clinical translation and this translation has been prioritized by the Hold'em group. Dr. Khokha's team is extending this preclinical work to study patient samples in the BTRR and the group is exploring the potential for proof of principle clinical interventions studies that may ultimately move agents into more advanced clinical testing.

Dr. Khokha's work has enhanced the focus on breast cancer risk and the SAB is strongly supportive of its ongoing inclusion in this program. Her work does provide some of the recommended breadth, suggested earlier, to the Hold'em program focus.

❖ **Summary of Clinical Studies and Clinical Trials Infrastructure Supported by Hold'em**

The program also supports clinical trials infrastructure that facilitates participation in multicenter clinical trials. In total – 8 Hold'em funded studies have been initiated; 3 are completed; 3 are ongoing and two will be open for recruitment in the next few months. The preliminary results of the completed trials have been presented and manuscripts detailing the final results will be prepared this year.

Much of the full complement of trials work (25 additional projects) has taken advantage of funding from other sources and/or has been layered onto existing infrastructure for clinical trials – this has allowed the program to have an impact that is greater that would have been possible using only Hold'em funding. The enhanced level of clinical trial activity has recently facilitated membership of MSH in the Canadian Cancer Clinical Trials Network, which provides additional infrastructure support for clinical trials.

Overall Impressions

In summary, HEFL funding has led to the establishment of an integrated, collaborative, multidisciplinary research team that engages and supports the development of the next generation of researchers. The strength of this interaction drives a focused research agenda aligned with the program goal to better understand the biology and enhance the outcomes for women with breast cancer. The research team has cultivated a competent research infrastructure and core resources with which to conduct basic, translational and clinical studies. The research team is now generating, presenting and publishing the results of work funded and enabled by the Hold'em for Life Charity (23 publications; 12 abstracts).

We believe they should continue to stay focused and remain true to the Hold'em for life program mission. Although we have suggested a slight broadening of the research emphasis (in particular, enhanced clinical translation of Dr. Khokha's work) to enhance the long-term sustainability of the program, we do not recommend they drop any of the projects that are ongoing or planned. This group is well-placed to explore the impact of obesity, metabolism and hormonal factors on the incidence and outcomes of breast cancer. Funding for research of this type is not easily found and important questions such as these can only be answered with funding from groups such as the Hold'em for Life Charity.

The addition of emerging technologies or the integration of new areas, such as metabolomics, genetics or immune regulators, would strengthen the program without detracting from its focus.

Finally, it was noted that it would be desirable to have more imaging involvement in the HELF program. Given the synergies between the Hold'em research activities and those of the Sunnybrook Research Institute imaging group, it was agreed that establishing a research collaboration might be mutually beneficial.

Again, we are very impressed by the progress of the group and wish to recognize how responsibly and intelligently the Hold'em funds have been utilized to build this exciting program.

Sincerely,

Daniel F. Hayes, MD

Clinical Director, Breast Cancer Oncology

Stuart B. Padnos Professor in Breast Cancer

University of Michigan Comprehensive Cancer Centre

Morag Park, PhD

Professor in the Departments of Biochemistry, Medicine and Oncology, McGill University

Diane and Sal Guerrera Chair in Cancer Genetics

Scientific Director, CIHR Institute of Cancer Research

W. Fraser Symmans, MD

Professor, Department of Pathology

The University of Texas MD Anderson Cancer Center

Appendix 3: Hold'em Program Timelines and Progress

Hold'em Supported Research Activities	July 2012 - Mar 2013 (8 mths)				Apr 2013 - Mar 2014				Apr 2014 - Mar 2015				Apr 2015 - Mar 2016				Apr 2016 - Mar 2017				Apr 2017-Mar 2018			
	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sept	Oct-Dec	Jan-Mar
Basic Studies																							
Khokha Lab	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O		
Stambolic Lab	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O		
Bratman Lab																				P	P		
Translational Studies																							
MA.21 Correlative - Assays									B	B	B/S	Pu											
MA.21 Correlative - Tissue	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T		
MA.32 Translational - DNA Extraction & SNP Analysis																		B	B	B	B		
MA.32 Translational - Inflammatory & Metabolic Factors		B/S	B/S	B/S	B/S	B/S	B	B	B	B	B	B/S											
MA.32 Translational - Tumour Marker Ca 15-3/MUC-1																	B	B	B	B	S		
TRR Obesity									P	P	E/C	A	B	B	S	S	S	Pu					
NRF Additional Analysis - Inflammatory Markers																P	E	B	S	S			
NRF Additional Analysis - Iron Studies																P	P	B	S	S			
NRF Additional Analysis -CLSB & Obesity Protocol	T	T	T	T	T	T	T	T	T	T	T	T	S	Pr								Pr	
GENomic Investigation of UnuSual responders (GENIUS)																	C	C	C	O	O		
Clinical Studies																							
AI Host Factor	E/C /A	R	R	R	R	R	R	O	O	O	R/B	B/S	S	S/P r				Pr			Pu		
OZM-027	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	O	O	CS		
CTC Host Factors	P	E/C /A	R	R	R	R	R	R	R	R	CS	R/B	B/S	Prx 2									
COMETI-P2	P	P/E	E/C	C	R	R	R	R	R	R	R	R	O	CS				Pr					
Pre-Peri						P	P	E/C	A	R	R	R	R	R	R	R	R	R	R	R	R	R		

Hold'em Supported Research Activities	July 2012 - Mar 2013 (8 mths)				Apr 2013 - Mar 2014				Apr 2014 - Mar 2015				Apr 2015 - Mar 2016				Apr 2016 - Mar 2017				Apr 2017-Mar 2018			
	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sept	Oct-Dec	Jan-Mar	
									& R															
Late Rec Questionnaire														P	P	E/C	R/A	R	R	R	R			
ctDNA in Adv BC																		P	P	P	E/C			
Investigation of Late Recurrence in ER+ HER2- BC																					P			
Biospecimen Repository																								
BTRR - Cancer	P	P	E/C	A	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R					
BTRR - Benign						P	P	E/C	A	R	R	R	R	R	R	R	R	R	R					
TRR Obesity Study									P	P	E/C	B/S												
Clinical Trials Infrastructure																								
Endocrine Therapy & Cognition_2015 Sept										EC	EC	R	R	R	R	C/S	F	F	F	F	F			
MA 17.R	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F			
MA.24 HERA BO16348_Roche NCIC	R	R	R	R	R	R	R	R	F	F	F	F	F	CS										
MA.32 (activated Oct2010; recruit closed 01-2013)	R	R	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F			
MA.32 F (activated dec2011; recruit closed 01-2013)	R	R	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F			
MA.34 Hoffmann-La Roche APHINITY Study	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O			
MAC 15 / SWOG 1007	R	R	R	R	R	R	R	R	R	R	F	F	F	F	F	F	F	F	F	F	F			
MAC 18 A221405 POSITIVE NCIC																	E/C	A	R	R	R			
MAC 20 A011401 BWEL NCIC																	E/C	E	P	P	R			
OCOG-2007-LISA	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F			
OCOG-2015-AToM															E	R	R	R	O	O	CS			
Metformin in Early BC (KG080358)	CS																							
NSABP B-36 (activated June 2006; recruit closed 07-2008)	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F			
NSABP B-42 (activated 2007Aug; recruit closed 01-2010)	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F	O/F			
REFLECT PDX post-neoadj TNBC w residual disease																P	P	E	E	P	E			
Survivorship & NRF Studies	CS																							
EMBRACE-MRI Study																				P	E			

Hold'em Supported Research Activities	July 2012 - Mar 2013 (8 mths)				Apr 2013 - Mar 2014				Apr 2014 - Mar 2015				Apr 2015 - Mar 2016				Apr 2016 - Mar 2017				Apr 2017-Mar 2018			
	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sept	Oct-Dec	Jan-Mar	
Autofluorescent Imaging of BC Using Microendoscopy																				P	E			
Wide-Field Optical Coherence Tomography																				P	E			

Appendix 4: Hold'em Funded Clinical Studies

Study	Description	Enrollment/Target	Centres	Status
Host Factor – CTC Goodwin/Chang	Observational study examining the association of CTCs with host factors (notably BMI, insulin, glucose) in metastatic BC with progressive disease.	96 evaluable/100	MSH (Mar 2013); PMH (Feb 2013); LHSC (Aug 2013); SMH (Mar 2014)	study completed; assays and analysis ongoing
Host Factor – AI Cescon/Sridhar	Two step study in women receiving adjuvant letrozole. Part A- association of Vitamin D, BMI and estrogen levels at standard dose. Part B (BMI > 25) - change in estrogen levels with double-dose letrozole.	Total 113 evaluable/106 **Grp A** (BMI ≤ 25) = 65 completers **Grp B** (BMI>25) = 34 completers Std dose = 113; Dbl dose = 34	MSH (Nov 2012); PMH (Oct 2012); WCH (Feb 2013); SHSC (Apr 2014)	Mar 2015 – interim (futility) analysis completed study completed; analysis ongoing & manuscript preparation
OZM027 Goodwin	Phase II RCT of Metformin vs placebo in addition to 1st, 2nd, 3rd or 4th line CXT in metastatic BC; endpoint PFS.	40/40	MSH (Aug 2011), PMH, London, Windsor, SMH	Study completed; assays & analysis completed. Manuscript in preparation
COMETI Goodwin/Amir	Phase II multicentre trial of CTC characterization in metastatic ER+, Her2 neg BC	6 at MSH (1 screen fail; 4 ET) 6 at PMH (0 active; 6 ET) Study recruitment ~110 subjects	MSH (open Aug 13) PMH (open May 2014)) Multiple USA sites	study completed
Pre-Peri - Chang, Escallon, Goodwin, Stambolic, Dowling	Biomarker evaluation in pre-operative and peri-operative tissues	22/80 12-completed; 1 active; 5 IDL; 4 early term (surgery elsewhere; no core; pt withdrew)	MSH (open Jul 2014)	recruitment ongoing
MA 32 Correlative Stambolic, Dowling, Chang, Goodwin	Phase III Intergroup Adjuvant Trial of Metformin vs placebo in early BC Blood – metabolic factors Tissue – TMA construction & IHC analysis DNA extraction and SNP analysis	3649 Pts Paired Blood Specimens: 2,586 Pts Baseline Only Blood Specimens: 370	> 200 sites in 4 countries	study completed; assays and analysis ongoing
CLSB & Obesity Protocol NRF 2014 Chang	Tissue Biomarkers Associated With Obesity: A Retrospective Pathology Analysis for Crown-Like Structures of the Breast and Adipocyte Size	99	MSH	study completed; scoring ongoing

Study	Description	Enrollment/Target	Centres	Status
BTRR-Cancer	Translational Research Resource for Breast Cancer	Consented 684 Surgery completed 653	MSH UHN (recruitment only)	recruitment ongoing
BTRR Benign	Translational Research Resource for Benign Breast Cases	Consented 55 Surgery completed 47	MSH UHN (recruitment only)	recruitment ongoing
BTRR-Obesity Goodwin	Association of Obesity and Associated Physiologic Factors with Estrogen, Insulin and Inflammatory Signaling in Estrogen Receptor Positive, HER2 Negative Primary Breast Cancer	120/120 Tissue 129/120 Bloods	2014Oct1-2015Sept30	blood assays completed; data extraction completed; tissue assays completed; analysis ongoing

Appendix 5: All Hold'em Supported Clinical Studies

HOLD'EM SUPPORTED PROGRAMS	DESCRIPTION	Status
CTC Platform & ctDNA		
Chang-Bratman - CTC & cDNA in Adv BC_2015Sept	Characterization of Circulating Cell-Free DNA (cfDNA) as a Biomarker of Chemotherapy Response in Advanced and Locally Advanced Breast Carcinoma	protocol written REB review
Goodwin - Janssen COMETI P2 Trial	CTC characterization in metastatic ER+, Her2 neg BC	study completed
Goodwin - CTC Host Factors Study	CTCs with host factors (notably BMI, insulin, glucose) in metastatic BC with progressive disease	study completed assays/analyses ongoing
Metabolism - Clinical & Signaling		
Goodwin-Cescon – Host Factors in ER+ Late Recurrence	Investigation of Late Recurrences in Hormone Receptor Positive, HER2 Negative Breast Cancer	DRAFT protocol under review
Goodwin - Host Factors in ER+ Late Recurrence	Patient (Host) Factors Associated with Late Recurrence in ER+ Breast Cancer	8 pts test group; questionnaire revised; 44 recruited; 37 completed paired testing
Lohmann - Inflammatory Markers - NRF Additional Analysis	Inflammatory Markers [Nutrition Related Factors in Breast Cancer: Continuation of Survivorship Study (CT158) Clinic In LIS: CT318]	assays completed; analysis ongoing
Jerzak – Hepcidin - NRF Additional Analysis	Hepcidin in Breast Cancer [Nutrition Related Factors in Breast Cancer: Continuation of Survivorship Study (CT158) Clinic In LIS: CT318]	assays completed; analysis ongoing
Chang-Biomarkers in Pre- and Peri-Op tissues Study	Biomarker evaluation in pre-operative and peri-operative tissues	study ongoing
Goodwin - OZM 027	RCT Metformin vs placebo plus 1st, 2nd, 3rd or 4th line CXT in metastatic BC; CTC measurement added June 2013	recruitment completed; assays & analysis ongoing
Cescon-Sridhar - AI Host Study	Vitamin D, BMI and estrogen levels in women receiving adjuvant letrozole	study completed assays/analyses completed. Manuscript in preparation.
Chang-CLSB & Obesity Protocol NRF 2014	Tissue Biomarkers Associated With Obesity: A Retrospective Pathology Analysis for Crown-Like Structures of the Breast and Adipocyte Size	study completed assays/analyses ongoing
Goodwin - MA 32 Correlative	Metformin Versus Placebo on Recurrence and Survival in Early Stage Breast Cancer	study completed

HOLD'EM SUPPORTED PROGRAMS	DESCRIPTION	Status
		assays/analyses ongoing
Goodwin - MA.32 – DNA extraction/SNP	Correlative Research in NCIC MA.32 (Adjuvant RCT of Metformin vs Placebo): DNA extraction and known SNP analysis	study completed assays completed; analyses ongoing
Goodwin-Dowling - MA.21 Correlative	Combination Chemotherapy With or Without Colony-stimulating Factors in Treating Women With Breast Cancer	study completed assays/analyses ongoing
Goodwin - Metformin in Early BC (KG080358)	Clinical and Biologic Effects of Metformin in Early Stage Breast Cancer	study completed assays/analyses completed
Hormone Factors		
Khokha (Elser) Proteomics/progesterone	Reproductive hormones; identify in radiology; blood testing	planning
Genetics		
Cescon - GENomic Investigation of UnuSual responders (GENIUS)	Sequencing "exceptional responders" or those who responded poorly will elucidate functional mechanisms of tumour behavior and drug response	ongoing
Cescon - REFLECT PDX post-neoadjuvant TNBC with residual disease	PRospective Evaluation of Freshly ImpLantEd Cancers in Mice to Test Drug Response in Matching Host	Open (UHN); REB submission (MSH)
BTRR - Translational Research Resource		
*BTRR - Cancer (including PABC)	Translational Research Resource for Breast Cancer	ongoing
*BTRR – Benign	Translational Research Resource for Benign Breast Cases	ongoing
Participation – Hold'em Enabled		
Elser - MAC 18 A221405 POSITIVE NCIC	A Study Evaluating the Pregnancy Outcomes and Safety of Interrupting Endocrine Therapy for Young Women with Endocrine Responsive Breast Cancer who Desire Pregnancy	ongoing
Elser - Endocrine Therapy & Cognition_2015 Sept	Cognitive Sequelae of Adjuvant Endocrine Therapy for the Treatment of Breast Cancer in Older Women [Cognition in 60+ on Tam]	completed; analysis ongoing
Elser- Decision Aid Incidental Genomic Findings_2015Dec	Randomized Controlled Trial of a Decision Aid for Incidental Genomic Findings	planning
Goodwin - A011401-BWEL	Phase III Trial Evaluating the Role of Weight Loss in Adjuvant Treatment of Overweight and Obese Women with Early Breast Cancer	initiation
Elser - OCOG-2015-AToM_2015Aug10	Pragmatic Cluster-Randomized Trial of Ambulatory Toxicity Management in Patients Receiving Adjuvant or Neo-adjuvant Chemotherapy for Early Stage Breast Cancer (AToM)	completed

HOLD'EM SUPPORTED PROGRAMS	DESCRIPTION	Status
OCOG-2007-LISA	Lifestyle Intervention Study in Adjuvant Treatment of Early Breast Cancer (LISA)	ongoing
Elser - B-42	A Clinical Trial to Determine the Efficacy of Five Years of Letrozole Compared to Placebo in Patients Completing Five Years of Hormonal Therapy Consisting of an Aromatase Inhibitor (AI) or Tamoxifen Followed by an AI in Prolonging Disease-Free Survival in Postmenopausal Women with Hormone Receptor Positive Breast Cancer	ongoing
MA.34 APHINITY	A randomized multicenter, double-blind, placebo-controlled comparison of chemotherapy plus trastuzumab plus placebo versus chemotherapy plus trastuzumab plus pertuzumab as adjuvant therapy in patients with operable HER2-positive primary breast cancer	ongoing
B36	A Clinical Trial of Adjuvant Therapy Comparing Six Cycles of 5-Fluorouracil, Epirubicin and Cyclophosphamide (FEC) to Four Cycles of Adriamycin and Cyclophosphamide (AC) in Patients With Node-Negative Breast Cancer	ongoing
MA.17R	A Double Blind Randomization to Letrozole or Placebo for Women Previously Diagnosed with Primary Breast Cancer Completing Five Years of Adjuvant Aromatase Inhibitor Either as Initial Therapy or After Tamoxifen	ongoing
Goodwin - MAC 15 Study	RCT ADJUVANT ENDOCRINE THERAPY +/- CHEMOTHERAPY IN PATIENTS WITH 1-3 POSITIVE NODES, HR+ & HER2-NEGATIVE BREAST CANCER WITH RECURRENCE SCORE (RS) OF 25 OR LESS. RXPONDER	enrollment closed; study ongoing
Goodwin - Survivorship & NRF Studies	Survivorship in a Long-Term Breast Cancer Cohort: Integration of Biologic, Psychological and Health-Related Quality of Life Factors / Nutrition Related Factors in Breast Cancer	study completed assays/analyses ongoing
Elser - MA24 HERA BO16348_Roche	Randomised Three-Arm Multi-Centre Comparison of 1 Year and 2 Years of Herceptin* Versus no Herceptin* in Women with HER2-Positive Primary Breast Cancer Who Have Completed Adjuvant Chemotherapy	study completed assays/analyses ongoing

Appendix 6: Hold'em Funded Research – Peer Review and Funding Partners

Type of Study	Hold'em Role	Name of Project	PI PI(s) (Institution)	Current Status	Peer review	Other Funders
Clinical Study	Full Funder	ctDNA in Adv BC	Cescon (UHN), Bratman (UHN) Chang(MSH)	protocol written, REB submission prepared	SAB	CCSRI $450,000
Clinical Study	Full Funder (Breast pts)	GENomic Investigation of UnuSual responders (GENIUS)	Cescon (PMH)	up to 10 breast patients to be enrolled	SAB	PMRI
Clinical Study	Full Funder	MA.21 Correlative - Tissue**	Goodwin (MSH) - Chang (MSH)	TMA construction and scoring ongoing	CTEP, CCTG, CCSRI	CTEP (NIH), CCSRI
Clinical Study	Full Funder	NRF Additional Analysis - Inflammatory Markers	Lohmann	assays completed & analyses ongoing	SAB, UofT Grad Student Committee	BCRF, MRC (currently CHRI), NCIC (currently CCSRI) ($1.3Million)
Clinical Study	Full Funder	NRF Additional Analysis - Hepcidin	Jerzak (SHSC) Goodwin (MSH)	assays completed & analyses ongoing	UofT Grad Student Committee	See above
Clinical Study	Full Funder	NRF Additional Analysis - CLSB & Obesity Protocol	Chang (MSH)	assays completed; manuscript in preparation	SAB	See above
Clinical Study	Full Funder	Pre-Peri biopsy study	Chang, Escallon, Goodwin, (MSH) Stambolic, Dowling (PMH)	accrual ongoing	Hold'em SAB	
Clinical Study	Joint Funder	Investigation of Late Recurrences in Hormone Receptor Positive, HER2 Negative Breast Cancer	Goodwin (MDH; Cescon (UHN); Jerzak (SHSC)	protocol written, review ongoing	BCRF Hold'em SAB	BCRF $350,000
Clinical Trial	Joint Funder	OZM 027**	Goodwin (MSH)	study completed; analyses ongoing	BCRF, Health	BCRF $475,000)

Type of Study	Hold'em Role	Name of Project	PI PI(s) (Institution)	Current Status	Peer review	Other Funders
					Canada	
Laboratory	Full Funder	Human Atlas of Insulin Receptor Expression in Cancer	Stambolic (UHN)		PMHRI	
Clinical Study	Full Funder	BTRR - Benign	Goodwin (MSH) Chang (MSH)	ongoing accrual	SAB	The BTRR is a resource for researchers funded by Hold'em - researchers obtain external funding for specific projects that utilize the BTRR resources - those granting agencies review the specific projects - for example, see "Obesity associated signalling and pathway activation in ER+ breast cancer" below
Clinical Study	Full Funder	BTRR-Cancer	Goodwin (MSH) Chang (MSH)	ongoing accrual	SAB	See above
Clinical Study	Joint Funder	Obesity associated signalling and pathway activation in ER+ breast cancer - BTRR study	Goodwin	study completed; analyses ongoing	BCRF	BCRF ($135,000)
Clinical Trial	Joint Funder	MA.32 Translational - DNA Extraction & SNP Analysis	Goodwin (MSH)	assays & analyses ongoing	BCRF, NCI (US), CTEP, CCT-CCS, CBCF	BCRF $155,000 (in addition to MA.32 funding from BCRF, CTEP, CCSRI, CBCF, Apotex Ttl: $25million for conduct of the clinical trial)
Clinical Trial	Joint Funder	MA.32 Translational - Inflammatory & Metabolic Factors	Goodwin (MSH) Stambolic (UHN) Dowling (UHN)	assays & analyses ongoing	BCRF, NCI (US), CTEP, CCT-CCS	BCRF $125,000 (in addition to MA.32 funding from BCRF, CTEP, CCSRI, CBCF, Apotex Ttl: $25million for conduct of the clinical trial)
Clinical Study	Joint Funder	MA.32 Translational - Tumour Marker Ca 15-3/MUC-1	Goodwin (MSH)	translational assays & analyses ongoing	BCRF, NCI (US), CTEP, CCT-CCS	BCRF ($325,000 (in addition to MA.32 funding from BCRF, CTEP, CCSRI, CBCF, Apotex Ttl: $25million for conduct of the clinical trial)
Clinical Study	Joint Funder	REFLECT PDX post-neoadjuvant TNBC with residual disease	Cescon (PMH)	ongoing at UHN; awaiting protocol to open at MSH	PMHRI	PMHRI $50,000
Clinical Study	Joint Funder	Survivorship & NRF Studies	Goodwin (MSH)	study completed; assays/analyses ongoing	MRC, CCSRI, BCRF	BCRF $420,000 (in addition to NRF funding listed above)

Type of Study	Hold'em Role	Name of Project	PI PI(s) (Institution)	Current Status	Peer review	Other Funders
Laboratory	Joint Funder	Human Atlas of Insulin Receptor Expression in Cancer	Stambolic (UHN)	Study complete	CCSRI	CCSRI $200,000
Laboratory	Joint Funder	Insulin Receptor and Signalling Pathways in Human Cancer	Stambolic (UHN)	Complete	CCSRI	CCSRI $200,000
Laboratory	Joint Funder	Insulin Receptor and Human Breast Cancer	Stambolic (UHN)	Ongoing	CCSRI	CCSRI $449,910
Laboratory	Joint Funder	Modelling the Role of the Insulin Receptor in Breast Cancer	Stambolic (UHN)	Ongoing	CCSRI	Principal Applicant - "Insulin receptor signalling in human cancer" Funder - Canadian Cancer Society Research Institute, operating grant, Impact Period: 2016-19; Total Awarded $ 449,910
Laboratory	Joint Funder	Hormone signalling in mammary cells informs breast cancer risk and treatment	Khokha (UHN)	Complete	CCSRI	CCSRI $1222,500
Laboratory	Joint Funder	Identifying dependencies of normal and cancer breast stem cells	Khokha (UHN)	Complete	CCSRI	CCSRI $40,000
Laboratory	Joint Funder	Proteome-based target discovery to impact stem and progenitors in high risk and breast cancer	Khokha (UHN)	Ongoing	CBCF	CBCF $450,000
Laboratory	Joint Funder	Rationalized depletion of breast cancer precursor cells as a strategy for breast cancer prevention	Khokha (UHN)	Ongoing	CBCF	CBCF $450,000

Appendix 7: BTRR Holdings

BTRR Asset Summary - May/2013 to Jun/2017							
Baseline (entry) characteristics	**Cancer**	**Key Patients**	**Non-Key patients**	**Deceased**	**Status TBD**		**Benign**
Enrolled	**684**						**55**
IDL	**16**						**7**
Withdrawn from study	**4**						**0**
Active	**664**						**48**
OR completed	**653**	358	272	4	20		**47**
Current BC Dx							
Insitu Only	**73**	0	73				
Phyllodes	**1**	0	1				
Ipsilateral New Primary Invasive	**2**	0	2				
Loco-regional recurrence	**22**	0	22				
Primary IDC	**451**	290	147	3	11		
Primary ILC	**46**	32	13		1		
Primary Invasive Other	**20**	16	4				
Primary Mixed Invasive	**26**	17	9				
# subjects with Mets tissue	**4**	4					
Pre-diabetes & type 1 & type 2	**93**	42	45		3		
Currently Pregnant = Yes	**5**	2	2				
PRIOR Breast Ca at entry (Invasive or insitu)	**80**	8	70				
PRIOR Other Ca at entry	**88**	38	49				
Fam Hx (1st/2nd/3rd) BC = YES	**306**	153	134	1	1		
Fam Hx (1st/2nd/3rd) BC = NO	**339**	186	129	1	3		
Genetic Testing	**174**	99	65	1			
BRCA1 pos	**11**	7	3				
BRCA 2 pos	**15**	7	8				
BRCA 1&2 pos	**1**	1					
Deceased	**4**			2	0		

Primary invasive cases (543)*	N	Pre-/Peri-menopause	Post-menopause	Neo yes	Neo no	Blood	Fasting Blood	BSR Tissue	Tissue & Blood
HER2- & ER+ and/or PR+ (Key)	236	72	164	30	206	178	107	130	91
HER2- & ER+ and/or PR+ (Non-Key)	123	29	94	14	109	87	46	62	42
HER2-/ER-/PR- (TNBC) (Key)	27	12	15	11	16	25	15	10	9
HER2-/ER-/PR- (TNBC) (Non-Key)	18	4	14	3	15	17	12	10	9
HER2+ & ER+ and/or PR+ (Key)	42	15	27	16	26	33	22	20	17
HER2+ & ER+ and/or PR+ (Non-Key)	11	6	5	2	9	10	3	4	4
HER2+/ER- & PR- (Key)	16	6	10	9	7	13	8	5	4
HER2+/ER- & PR- (Non-Key)	6	1	5	2	4	4	1	2	1

May 2013- Jun 2017	Cancer	Benign
Completed Surgery	653	47
*Blood specimens stored in BSR	499	39
Fasting	286	24
Non-fasting	213	15
*Sufficient tissue for storage in BSR	297	37
Second tissue donation	6	4

Appendix 8: Hold'em Publications and Abstracts

Publications

1. Tsu, T., Ennis, M., Hood, N., Graham, M., Goodwin, P.J. Quality of life in long-term breast cancer survivors. Journal of Clinical Oncology 31:3540-3548, 2013.
2. Lega, I.C., Austin, P.C., Gruneir, A., Goodwin, P.J., Rochon, P.A., Lipscombe, L.L. Association between metformin therapy and mortality after breast cancer: A population-based study. Diabetes Care 36:3018-3026, 2013.
3. Goldhirsch, A., Winer, E.P., Coates, A.S., Gelber, R.D., Piccart-Gebhart, M., Thürlimann, B., Senn, H.-J. and Goodwin, P.J (Panel Member). Personalizing the treatment of women with early breast cancer: Highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Annals of Oncology 24:2206-2223, 2013.
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Appendix 9: CVs for Core Scientists and Trainees

FOR CORE SCIENTISTS (1-10 BELOW) AND TRAINEES (11-16 BELOW)

These will be provided under separate cover.

1. Dr. Pamela J. Goodwin
2. Dr. Martin Chang
3. Dr. Vuk Stambolic
4. Dr. Ryan Dowling
5. Dr. Rama Khokha
6. Dr. Jim Woodgett
7. Dr. David Cescon
8. Dr. Scott Bratman
9. Dr. Marguerite Ennis
10. Dr. Daniel Schramek

11. Dr. Ana Lohmann
12. Dr. Katarzyna Jerzak
13. Dr. Jennifer Gorman
14. Dr. Alison Casey
15. Dr. Purna Joshi
16. Ellen Langille