Marnie Newell PhD Seminar Abstract:
The role of the dietary long chain polyunsaturated fatty acid, docosahexaenoic acid, in prevention of breast cancer and its efficacy during neoadjuvant breast cancer chemotherapy

Breast cancer (BC) is the most frequently diagnosed and the second leading cause of cancer related death in Canadian women. Docosahexaenoic acid (DHA) is an n-3 long chain polyunsaturated fatty acid (LCPUFA) that has shown efficacy in reducing BC cell growth, however its' role in prevention of BC or how it improves the efficacy of standard chemotherapy and the mechanisms involved have not been established. The overall objective of this thesis was to determine the efficacy of DHA in prevention and treatment of BC.

The relationship between plasma phospholipid fatty acid status and BC risk in a nested-case control study of women with BC (n=393) and age-matched controls (n=786) from Alberta's Tomorrow Project (ATP) and British Columbia Generations Project (BCGP) was determined. Women from BCGP had higher n-3 LCPUFA status compared to ATP (6.4±0.08% vs. 5.3±0.06%, P<0.001). Fatty acid status was not consistently associated with risk. In ATP among premenopausal women, total n-3 LCPUFA were positively associated with BC risk, while in BCGP, DHA and n-3 LCPUFA were associated with decreased cancer risk when the waist-to-hip ratio was <0.85. This study highlights the difficulty in using a single measure of fatty acid status to predict BC risk in diverse populations.

In a series of *in vitro* and *in vivo* experiments with immortalized BC cells, we sought to establish the efficacy and mechanisms for how pre-treatment of BC cells with DHA improves the action of chemotherapy. First, we determined that DHA is differentially incorporated into whole cell and lipid raft membranes of BC cell lines, with higher incorporation occurring in MDA-MB-231 triple negative BC (TNBC) compared to estrogen receptor positive MCF-7 BC cells. Doxorubicin (DOX) chemotherapy treatment did not alter this incorporation. Microarray analysis indicated that DHA+DOX treated MDA-MB-231 cells had upregulated expression of apoptosis genes (*RIPK1, Caspase-10*) and down regulated cell cycle gene expression (*Cyclin B1, WEE1, CDC25C,* all $P<0.05$). Mice fed a 2.8% w/w DHA diet and treated with 5 mg/kg DOX had 50% smaller MDA-MB-231 tumours compared to control (0% DHA) fed mice and increased expression of apoptotic proteins (Caspase-10 and Bid) combined with decreased cell cycle proteins (Cyclin B1 and Cdc25c, $P<0.05$).

We then employed a heterogeneic, drug resistant patient derived xenograft (PDX) model of TNBC. Mice bearing MAXF574 TNBC PDXs fed a 3.8% w/w DHA diet in combination with 5 mg/kg docetaxel (TXT) had a 57% reduction in tumour weight compared to mice fed a control diet ($P<0.004$) and a 64% reduction compared to control diet +TXT ($P<0.01$). DHA+TXT resulted in higher expression of proapoptotic proteins: RipK1 and Bid, lower expression of Ki67 proliferation marker, Bcl-2 and Parp and increased cell cycle arrest compared to control or Control+TXT mice ($P<0.05$). Next, to assess the efficacy of DHA at a lower dose, high DHA (HDHA 3.8% w/w) and low DHA (LDHA, 1.8% w/w) diets were fed to MAXF401 TNBC PDX bearing mice. Tumours from mice fed HDHA+TXT or LDHA+TXT were similar in size to each other, but 36% and 32% smaller than tumours from mice fed control+TXT, respectively ($P<0.05$). Both DHA doses resulted in increased necrotic tissue and decreased NFκB protein expression compared to control tumours, however only HDHA+TXT had increased expression of necroptosis related proteins: RIPK1, RIPK3 and MLKL ($P<0.05$). This work confirms the efficacy of DHA supplementation with TXT in two TNBC PDX models.

Our final translation was to determine the efficacy of supplementing 4.4 g/ day DHA in women undergoing neoadjuvant chemotherapy. A randomized, placebo-controlled trial was planned, received ethics and Health Canada approval and 49 women have been enrolled (80% of total patients required). Women entering the study had an average age of 52 years with a BMI=28.5±1.0. Half the women are post-menopausal and at baseline, women had 2.3±0.1% DHA content in plasma phospholipids.

In summary, while we did not find that DHA phospholipid status reduced the risk of future BC, our data provided strong pre-clinical evidence of efficacy of DHA in combination with chemotherapeutics in reducing BC cell growth. The mechanisms of action through which DHA works include increased apoptosis, necroptosis and cell cycle arrest and decreased cellular proliferation. Collectively the evidence obtained from these studies details the role of DHA in a neoadjuvant setting that we hypothesize will be confirmed in the clinical trial.