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Phase II Clinical Trial of Concurrent Neoadjuvant Chemotherapy With Radiotherapy in Locally Advanced Breast Cancer

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A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

PHASE II CLINICAL TRIAL OF CONCURRENT NEOADJUVANT CHEMOTHERAPY WITH RADIOTHERAPY IN LOCALLY ADVANCED BREAST CANCER

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by

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ABSTRACT

Locally advanced breast cancer (LABC) represents 15% of all non-metastatic breast cancers, with an overall poor prognosis, despite current guidelines that recommend neoadjuvant chemotherapy followed by surgery and adjuvant radiation. Therefore, a novel treatment paradigm using concurrent neoadjuvant chemoradiotherapy was proposed. A clinical trial was designed, where 32 LABC patients were treated with q3 weekly 5-fluorouracil, epirubicin and cyclophosphamide for three cycles, followed by weekly docetaxel for 9 weeks with concurrent regional radiation (45+5.4Gy) for the first 6 weeks. Patients subsequently underwent modified radical mastectomies. Pathological complete responses (pCR) and 3 year overall survival rates were compared to a matched concurrent control cohort. The concurrent chemoradiation cohort saw a significant increase in pCR rate and a trend toward 15% improvement in overall survival that failed to reach statistical significance. This regimen was not without toxicity, and 25% of patients experienced grade 3 or greater dermatitis and 25% experienced grade 3 or greater pneumonitis, resulting in one death. Tumour biomarker, plasma osteopontin, prior to chemotherapy was found to significantly predict for overall survival. In conclusion, LABC is an aggressive subset of breast cancer for which novel regimens must continue to be developed, taking advantage of the improved response to treatment with radiosensitivity seen in

this concurrent chemoradiation regimen, but using alternative radiosensitizing agents to minimize toxicity.

Keywords: *breast adenocarcinoma; locally advanced breast cancer; neoadjuvant; chemoradiotherapy; clinical trial*

THE CO-AUTHORSHIP

While each of the co-authors listed below made important contributions to this work, I am the principal author who designed all the projects, performed all of the experimental design, data collection and analysis. All manuscripts presented in this thesis were prepared by me, with the consultation and critical review by the co-authors.

Ann Chambers, PhD, in her role as my research supervisor, provided leadership and mentoring on the project, offering direction and guidance on data interpretation.

Alan Tuck, MD, PhD, FRCPC, in his role as my research co-supervisor, provided direction and guidance on data interpretation.

David Palma, MD, PhD, FRCPC, in his role as the project advisor, provided direction and guidance on data interpretation.

All three were crucial in critical review of all the manuscripts.

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DEDICATION

Everything I do, academic, clinical and otherwise, is in dedication to my children: Ella, Nathan, Nicholas and Daniel, and to my husband, Chris.

I dedicate this thesis to my parents, who have always believed in me, supported all of my aspirations, and exemplified passion in the pursuit of learning.

To God be the glory, great things He has done…

Fanny Crosby, 1872

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LIST OF ABBREVIATIONS

5-FU, 5-fluorouracil

AC, adriamycin-cyclophosphamide

AC-T, adriamycin-cyclophosphamide followed by taxane

ALND, axillary lymph node dissection

ARDS, acute respiratory distress syndrome

BCRP, breast cancer resistance protein

BCS, breast conserving surgery

Bkg, background

CCO, Cancer Care of Ontario

cCR, clinical complete response

CCRT, concurrent chemo-radiotherapy

CI, confidence interval

CEF, cyclophosphamide-epirubicin-fluorouracil

CMF, cyclophosphamide-methotrexate-fluorouracil

CNCR, concurrent neoadjuvant chemotherapy and radiation

CNF, cyclophosphamide-mitoxantrone-fluorouracil

CT, computerized tomography

CVAP, cyclophosphamide-doxorubicin-vincristine-prednisone

Cyclo, cyclophosphamide

Cyclodox, cyclophosphamide/docetaxel

DCIS, ductal carcinoma *in situ*

DFS, disease-free state

DOC, docetaxel

Dox, doxorubicin

DSG, disease site group

ER, estrogen receptor

FEC, fluorouracil-epirubicin-cyclophosphamide

FEC-D, fluorouracil-epirubicin-cyclophosphamide followed by docetaxel

FEC-wD, fluorouracil-epirubicin-cyclophosphamide followed by weekly docetaxel

FISH, fluorescent *in situ* hybridization

HER2, human epidermal growth factor *erb2*

HR, hazard ratio

IDC, invasive ductal carcinoma

IDSMC, independent data safety monitoring committee

IMRT, intensity-modulated radiation therapy

LABC, locally advanced breast cancer

MDP, multidrug resistance proteins

MIBI, ^{99m}Tc-labelled sesta-methoxyisobutylisonitrile

MRM, modified radical mastectomy

NACT, neoadjuvant chemotherapy

NIH, National Institute of Health Research

NR, no response

OAR, organ at-risk

OS, overall survival

pCR, pathological complete response

PEBC, program in evidence-based care

Pgp, P-glycoprotein

pPR, pathological partial response

PR, progesterone receptor

pSPR, pathological significant partial response

RAP, report approval panel

RCT(s), randomized control trial(s)

RECIST, response evaluation criteria in solid tumours

RP, radiation pneumonitis

RILI, radiation-induced lung injury

RIN, RNA integrity number

RFS, radiation fibrosis

ROI, region of interest

RT, radiotherapy

SD, stable disease

SLNB, sentinel lymph node biopsy

Tax, paclitaxel

Vin, vincristine

CHAPTER 1

General Introduction and Literature Review

Portions of this chapter were adapted from a published manuscript in which M. Brackstone was a co-author: Mandilaras V, Bourginam N, Spayne J, Dent R, Aranaout A, Boileau FJ, ***Brackstone M****, Meterissian S, Clemons M. Current Oncology 2015, vol. 22(1): 25–32. Reproduced with permission.*

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 BREAST CANCER

Breast cancer is the most common non-cutaneous cancer diagnosis for women in Canada, with an anticipated 24,400 Canadian women diagnosed in 2014, and 5,000 women dying of the disease [1]. Although newer treatments have improved both overall survival and progression-free survival for early and metastatic cancer patients respectively [2], there remains a subgroup of women with Locally Advanced Breast Cancer (LABC) who do poorly.

1.2 PATHOPHYSIOLOGY OF CANCER

Cancer is a multi-step process that occurs because of an interaction between an environmental factor(s) and a host that is genetically susceptible [3,4]. Cell division is a physiological process that occurs in almost every tissue type in the body. Under normal circumstances, the balance between proliferation and programmed cell death (usually in the form of apoptosis) is maintained by regulation of both of these processes, to ensure the integrity of tissues and organs. Any alteration by mutation of the genes responsible for the control of either of these leads to cancer. Cancer cells, therefore, behave as cells that have lost the control over their growth rate, spreading to other tissue types and invading other areas of the body, as well as interfering with their function. This can lead to death if not treated or removed.

DNA damage is considered to be the primary cause of any cancer. Normal damage to DNA is common, but inherent repair machinery is available and able to fix it. Thus, a deficiency in DNA repair would cause more DNA damage to accumulate and increase the risk of cancer. There are two main types of genes that regulate cancerous cell growth and differentiation: oncogenes (normal genes that regulate cellular growth are called proto-oncogenes, while mutation of these genes results in constitutive activation; these genes are then called oncogenes, which are either present in inappropriately high numbers, or altered, to then exhibit new tumour promoting properties as a result of their alteration) and tumour suppressor genes (genes that inhibit cell division or survival of cancer cells; mutations in these genes result in a loss of function and resultant cancer [3,4].

Breast cancer refers to several types of neoplasm arising from breast tissue. The most common one is adenocarcinoma, originating from the epithelial cells lining the terminal duct lobular unit. Over 80% of breast adenocarcinomas are derived from the epithelial cells lining the ducts specifically, termed mammary ductal carcinoma. *Carcinoma in situ* (DCIS) is proliferation of cancer cells within the duct itself but without invasion through the myoepithelial and basement membrane lining of the ducts; *invasive carcinoma* (IDC) is composed of cancer cells that have invaded through the myoepithelial lining of the ducts into the surrounding stromal tissue of the breast. Lymphovascular space invasion is usually associated with a more aggressive phenotype. Although DCIS is believed to be a non-obligate precursor of IDC in most instances, approximately 40% of

DCIS will progress to IDC if left untreated, evidenced by DCIS and IDC having very similar gene expression patterns, although both display intra-tumoural heterogeneity [5]. The drivers of invasion, or epithelial-to-mesenchymal transition, remain unknown, but epigenetic (DNA methylation) or histone modification events have been implicated [5]. The breast microenvironment, as well as genetic and epigenetic factors have been also implicated in driving tumour progression from invasive to metastatic breast cancer.

1.2.1 Risk Factors for Developing Breast Cancer

There are many risk factors associated with the development of this disease; these include gender (females are more prone than males), age (the risk of developing breast cancer increases with age, particularly after menopause), reproductive history (risk increases with higher number of ovulatory cycles and nulliparity), lactation (lactational changes related to breast feeding reduces risk of developing breast cancer), exposure history (ionizing radiation exposure, alcohol intake and hormone replacement therapy all increase the risk of having breast cancer), height and weight (taller women and those of higher BMI have a higher chance of developing breast cancer), family history and Breast Related Cancer (BRCA-1/BRCA-2) gene mutation (these significantly increase the chance of breast cancer) [6,7].

A number of inherited tumour suppressor gene mutations can lead to breast cancer, the most common of which are within the BRCA1 and BRCA2 genes. BRCA gene mutations significantly increase the risk of breast cancer

development from a 1 in 9 risk for average women to 65% lifetime risk for BRCA gene mutation carriers [8]. BRCA genes are DNA repair genes, responsible for identifying and repairing erroneous double-stranded DNA breaks. BRCA gene mutation carriers have inherited a defective copy of this gene from one parent and therefore rely on the other functioning inherited copy. A secondary somatic mutation in this gene leads to cancer development, consistent with the 2-hit hypothesis [9]. As a result, these gene mutation carriers develop breast and/or ovarian cancer (tissues where BRCA proteins are most responsible for DNA damage repair) at an earlier age than is seen in women who develop somatic mutations in each of their normal genes as a result of exposure or other unknown factors. Mutation carriers can develop cancer in their early 20s, are much more likely to be pre-menopausal at diagnosis, and develop more aggressive cancers (typically estrogen negative in 80% of cases) [10].

Hormones appear to have an important influence over the development of breast cancer, particularly estrogen and progesterone. Estrogen and progesterone are steroidal sex hormones that are produced by the ovaries in premenopausal women, whereas in postmenopausal women, they are derived from the conversion of androgens to estrogen by aromatase in the adrenal glands and to a lesser degree in peripheral tissues such as adipose tissue [11]. Progesterone is derived from pregnenolone that is itself derived from cholesterol [11]. Circulating estrogen (estradiol or its weaker counterpart, estriol) promotes the upregulation of progesterone receptors, particularly in breast tissue [11]. Both estrogen and progesterone have a number of roles, including female sexual

development, maintenance of sex characteristics and fertility. Two different types of estrogen receptors (ER) exist: alpha (α) and beta (β) (ERα and ERβ respectively). Various tissues express ER (breast, ovaries and the endometrium express ERα, while the kidneys, brain, lungs and several other organs express ERβ). The role of ERβ in carcinogenesis remains controversial, whereas, a clear link between ERα protein and breast cancer has been established [12]. Both ER subtypes carry a DNA binding domain and exist in the nucleus and the cytosol. When estradiol enters the cell, it binds the ER, and the complex migrates into the nucleus, where it functions as a transcriptional activator and promoter of cell growth. Most breast cancers (at least 80%) are ER positive and/or PR positive, meaning that these cancer cells proliferate in the presence of circulating estradiol, and are, therefore, inhibited by ER antagonists or aromatase inhibitors [11].

1.2.2 Prognostic Factors or Markers

A number of tumour and patient factors determine the risk of recurrence or death from recurrence of breast cancer [13]. These include tumour stage (outlined below, in section 1.4.1), menopausal status (worse prognosis with pre-menopausal status), tumour grade (worse prognosis with higher grade), and histological subtype (some breast cancer subtypes exhibit more favourable prognoses as a function of slower tumour growth and lower risk of developing metastases; favourable types include mucinous, medullary and tubular carcinomas) [14].

Another poor prognostic factor is HER2/neu (HER2) status. HER2 is a receptor that belongs to the human epidermal growth factor receptor (HER) family of proto-oncogenes including epidermal growth factor receptor (EGFR, HER1), one of four plasma membrane-bound receptor tyrosine kinases. HER family proteins have an extracellular ligand binding domain, a transmembrane domain and an intracellular domain that can bind and activate signaling molecules through its tyrosine kinase as a result of, or independent of, ligand binding [15]. HER2 can heterodimerize or homodimerize for activation and is, therefore, the preferred partner in dimerizing of the HER family receptors. Its constitutive activation by dimerization results in it functioning as an oncogene by autophosphorylating tyrosine residues, thus activating those signaling pathways [15]. Up to 30% of all breast cancers are comprised of tumour cells that overexpress HER2, and this overexpression allows for homodimerization and activation of HER2, promoting oncogenic features such as rapid tumour growth as well as an increased risk of locoregional and distant recurrence after surgery. As a result, HER2 overexpression is an independent poor prognostic marker and these breast cancers are associated with worse survival than HER2 normal tumours.

Overall, the integration of ER, PR and HER2 status, as well as proliferation markers such as Ki67, allow for the prognostication of tumours based on a rough molecular subtype [16,17]. The molecular classification was first described as a genomic DNA classification of tumours into 5 main subtypes, roughly defined by the following: Luminal A (low grade, ER positive, HER2

negative); Luminal B (ER positive, Ki67 high, usually high grade, HER2 positive or negative); HER2 positive (typically ER negative and HER2 positive); Triple negative (ER negative, PR negative and HER

2 negative) and Basal-like (high grade, poorly differentiated) [16]. Since then, a number of other classification systems have been reported, some using DNA expression patterns, others using mRNA expression, others still with molecular phenotype as a surrogate for gene expression profiling. This differentiation remains the most clinically relevant prognostic marker for breast cancer.

1.3 CLASSIFICATION

Breast cancers can be classified and substratified using a number of clinically relevant features. The purpose of classification is to select the best therapy and treatment algorithms, as well as to prognosticate. The major classification features include histopathological type, the grade of the tumour, the stage of the tumour, and the molecular (ER/PR/HER2) subtype (a surrogate for gene expression profile classification).

Histopathological classification involves the differentiation between *in situ* and invasive breast cancers, as well as their histologic type and grade. Histological features roughly stratify the invasive cancers as either no special type (infiltrating ductal) or special type (medullary, mucinous, lobular, tubular, cribriform), although there are also other more rare forms (e.g. metaplastic, apocrine, adenosquamous, etc.) [18]. Tumours showing mixed ‘no special type’

and ‘special type’ features usually behave and are classed as a ‘no special type’ tumour of the same histologic type and grade.

Grading focuses on the differentiation of the breast cancer cells compared to that of the normal breast cells. As the cell division becomes uncontrolled, nuclei become less uniform and cell arrangement more disorganized. The grade of an invasive carcinoma is assessed using the Scarff-Bloom-Richardson grading system, which involves three criteria: tubule formation (the percentage of tumour made up of tubular structures (1 point for >75% tubules; 2 points for 10-75% tubules and 3 points for <10% tubules)); nuclear pleomorphism (the degree of change in the shape and size of the cells’ nuclei (1 point for small and uniform nuclei; 2 points for medium to large nuclei but they remain consistent in shape); and 3 points for large and varied nuclei)); and mitotic count (number of cells under microscope that are actively dividing (1 point for slow mitotic rate; 2 points for medium mitotic rate and 3 points for rapid mitotic rate)) [19]. Thus every tumour is graded out of a possible 9 points. This is then further collapsed into a score for grade out of three (grade 1=1-5 points; grade 2=6-7; grade 3=8-9 points). These could also be described as well differentiated (low-grade), moderately differentiated (intermediate-grade) and poorly differentiated (high-grade) as the cells progressively lose the features and arrangement of normal breast cells. The poorer the differentiation is (or the higher the grade), the worse the prognosis for the patient [19].

Staging of breast cancer is based on the size where it originally started, and the locations to which it has travelled. The tumour, lymph nodes, metastasis

(TNM) staging system from the American Joint Committee on Cancer is used (see section 1.4.1 on Staging) [20].

Receptor status (ER, PR, HER2) is also a clinically-relevant classification stage, given that it determines treatment with hormonal therapy (selective estrogen receptor modulator, for premenopausal women to function as an ER antagonist (such as anastrazole, letrozole or aromasin drugs) breast cells including cancer cells, or an aromatase inhibitor (to block the conversion of steroidal molecules into estrogen for postmenopausal women to virtually eliminate any estrogen which could otherwise bind to ERs in cancer cells). HER2 overexpressing tumours are treated with a targeted monoclonal antibody called trastuzumab following chemotherapy, with other anti-HER2 targeted therapies currently under investigation.

1.4 DIAGNOSIS

Patients present with cancer in one of two ways: a palpable breast mass or change in breast appearance, or an abnormality such as a mass or microcalcifications seen on screening mammogram. Appropriate diagnostic imaging for any suspicious finding requires both a mammogram and an ultrasound [21]. If the finding persists on imaging, then an image-guided core needle biopsy is performed. If the clinical finding persists but the mammogram and ultrasound are negative, a surgical consultation is obtained to determine whether this is an abnormal finding requiring an excisional biopsy procedure, or whether further imaging (such as magnetic resonance imaging (MRI)) would be

warranted for the few cancers which present as mammogram and/or ultrasound occult [21].

Once a biopsy is done using image guidance, the specimen is processed by the pathology team, using formalin fixation and paraffin embedding for microscopic examination, with hematoxylin and eosin staining. The pathologist determines whether the cancer is *in situ* or invasive, and if invasive, its histologic type and grade, as described above. Immunohistochemical staining is done to determine whether the cancer cells are ER and/or PR positive and whether the cells are HER2 overexpressing [22]. If the tumour is HER2 equivocal by immunohistochemistry, testing for the HER2 gene may be performed by fluorescent *in situ* hybridization (FISH), to determine whether or not the HER2 gene is amplified.

Distant staging investigations (searching for distant metastases using imaging tests) are not recommended for early breast cancers; however, as the risk of distant metastases rises, then staging investigations are recommended prior to any systemic therapies. These standardly include a computerized tomography (CT) scan of the chest, abdomen and pelvis, and a full body bone scan. Imaging of the brain is not indicated in the absence of symptoms, as the yield for detecting metastases is otherwise low [21].

1.4.1 Staging

There are two different staging methods that can be applied to breast cancer. These are the Roman numeral staging system, as well as TNM staging,

which is most clinically utilized [20]. The TNM staging system can be collapsed into the Roman numeral staging system for ease of use prognostically.

The TNM system [20] is used for staging breast cancer in order to roughly prognosticate survival and determine need for adjuvant therapies based on statistical likelihood of distant recurrence.

Tumour: tumour classification (TX, T0, Tis, T1, T2, T3 or T4) depends on the cancer site. TX refers to an inability to assess that site; Tis refers to ductal *in situ* carcinoma, lobular *in situ* carcinoma or Paget's disease of the nipple; T1 represents tumours up to 2cm in size; T2 represents tumours more than 2cm but less than 5cm; T3 represents tumours 5cm or greater; T4 represents tumours invading surrounding structures including chest wall, skin, both or infiltrating dermal lymphatics resulting in a clinical diagnosis of inflammatory breast cancer.

Lymph Node: lymph node involvement with cancer (NX, N0, N1, N2 or N3) depends on the number and location of the involved lymph nodes, whether axillary lymph nodes, the infra or supraclavicular lymph nodes, or the internal mammary lymph nodes are affected. The axilla is designated as having three levels: lateral, deep and medial to the pectoralis muscle. In the axilla, NX designation means the lymph nodes have not been assessed; N0 signifies no lymph node metastases; N1 means 1-3 lymph nodes are involved with cancer (either microscopic or macroscopic nodal involvement); N2 means 4-9 nodes are involved and N3 means 10 or more nodes are involved. Clinically, all nodal basins are examined and any positive nodes identified clinically are classed based on their location: N1 means palpable but mobile axillary nodes; N2

represents matted nodes in the axilla or infraclavicular or internal mammary nodes; N3 represents nodes found in the supraclavicular nodal basin.

Metastases: The clinically relevant classification for distant metastases for breast cancer are M0 and M1, which refers to distant detectable metastases or absence thereof. The most likely areas for breast cancer cells to harbour clinically visible or relevant metastases are bone, lung, liver and brain.

Roman numeral staging involves assigning a number to describe the progression of cancer. The following stages are recognized:

- Stage 0: carcinoma *in situ*.
- Stage I: T1 tumours that are lymph node negative.
- Stage II: tumours up to T2 in size, with up to N1 nodal metastases, or T3 in size but no nodal metastases. This is the most common stage at diagnosis for breast cancer, and typically involves the addition of systemic therapies such as chemotherapy, and hormonal or antibody-based therapies if indicated. Distant staging is indicated from this stage forward.
- Stage III: This stage is considered locally advanced. These cancers are all lymph node positive (N1-N3) or invading surrounding structures (T4). The patients all benefit from systemic therapy and are usually given neoadjuvant chemotherapy prior to surgery, in order to cytoreduce the tumour burden and increase the likelihood of successful resectability.
- Stage IV: cancer has metastasized to other organs or throughout the body. Management of stage IV cancers is palliative, and treatments are

aimed at lengthening quality of life and reducing negative symptoms of the disease.

1.4.2 LABC

Locally advanced breast cancer (LABC) is a mix of neglected and aggressive cancers [23]. In developing nations, most patients present with LABC, but it appears that the majority of these are neglected due to patient, societal and care access factors, allowing the tumour to grow and advance in stage. LABC in North America can present as a neglected cancer in patients with denial and/or fear of the diagnosis, but more commonly represents an aggressive subset of breast cancer which can present as a large burden of disease that arises within a year from an otherwise normal screening mammogram (called an interval cancer). Neglected tumours tend to be seen in older patients, with the tumours displaying lower grade, and are more commonly ER positive, while interval LABC cancers tend to present in younger patients, with the tumours displaying high grade features and ER negativity. LABC is most commonly defined as stage IIB (T3N0) and Stage IIIA/B/C from the TNM classification (AJCC 2009) [20,24-26]; clinically, these tumours being greater than 5cm in size and/or extend beyond the breast tissue into the surrounding skin or muscle, with/without matted axillary lymph nodes (N2), internal mammary nodes or ipsilateral supraclavicular lymph node involvement (N3). LABC represents approximately 10-15% of all breast cancer cases, and the overall survival has historically been estimated at 30-42% at 5 years [27] a significant portion of whom will be living with metastatic disease.

However, a small subset of women receiving neoadjuvant chemotherapy who achieve a complete pathological response, or pCR, (defined as no residual invasive breast cancer pathologically following neoadjuvant treatment) to treatment are projected to have a vastly improved 5 year disease free survival rate of 87% [27] with 5 year overall survival rates of 89% to 90% [28]. As such, pCR rates have become the surrogate measure for favorable long-term outcomes in trials involving neoadjuvant treatment [29], particularly since the efficacy of systemic therapy can only readily be evaluated with the tumour *in vivo*.

Historically, pCR was defined as no residual invasive breast cancer within the breast [30], but has since been more commonly used to denote the absence of residual invasive breast cancer either within the breast or axillary nodes [31]. More recently, it has also been demonstrated that pCR is significantly associated with tumour subtype (also termed intrinsic subtype, roughly classified by a combination of estrogen receptor (ER) or progesterone receptor (PR) positivity, tumour grade and epidermal growth factor receptor HER2 over-expression/amplification as described above), based on a meta-analysis of 30 trials [32]. A review of 7 German neoadjuvant trials also showed that pCR rates rose with increasing number of chemotherapy cycles, increasing anthracycline dose, cumulative taxane dose and capecitabine-containing regimens [33]. The most significant predictor of response to neoadjuvant chemotherapy (as measured by pCR) was found to be molecular subtype status [33]. Based on this, pCR is being increasingly considered a suitable surrogate for all tumour subtypes

except Luminal A (clinical proxy for genotypic classification of Luminal A is made using ER positive, HER2 negative, absence of high grade) [34].

1.5 THERAPEUTIC APPROACH TO LABC

1.5.1 Systemic Chemotherapy

Systemic chemotherapy can be delivered in two main regimens: neoadjuvant (prior to surgery) and adjuvant (following surgery). Multiple chemotherapeutic agents may be used in combination. Determining the appropriate regimen depends on the character of the tumour (i.e. its hormonal status), lymph node status, and the age/health of the patient. Many regimens have been clinically evaluated and proposed, but for the majority of breast cancers, the regimens typically contain an anthracycline and a taxane, as these have demonstrated superior survival to regimens not containing these classes of drugs [26]. Six international randomized controlled trials (RCTs) that have compared neoadjuvant versus adjuvant chemotherapy have included patients diagnosed with breast cancer from stages T1-T4 [35-40]. All failed to demonstrate a survival advantage with neoadjuvant chemotherapy, although an individual level pooled analysis of the LABC subset of these trials is currently underway (Brackstone, unpublished). For more detailed discussion regarding specific regimens, see the neoadjuvant chemotherapy section below. Tumours that are HER2 overexpressing benefit from the addition of trastuzumab to reduce recurrence and improve survival. Its use was validated in the adjuvant setting concurrently with the taxane component of chemotherapy [26]. As a

monotherapy, it is less efficacious in improving survival, therefore is not delivered to patients not also receiving cytotoxic chemotherapy [41]. Since trastuzumab is a monoclonal antibody-targeted therapy, it is delivered intravenously, with an initial loading dose of 18mg/m^2, followed by a maintenance dose of 9mg/m^2 every 3 weeks for 18 doses (one year) [26]. Therefore, it overlaps with the administration of adjuvant radiation. It was tried as a concurrent regimen with anthracyclines, but this was found not only not to improve pCR rates, but also an increase in cardiac toxicity (in the metastatic setting) [41]; therefore, most regimens are designed to deliver the antracyclines first, followed by taxane with trastuzumab and radiation.

The most common regimens used to treat breast cancer include: AC-T (anthracycline and cyclophosphamide IV, q3 weekly x 4 or dose-dense as q2 weekly x 4) followed by taxane (paclitaxel or docetaxel, either q3 weekly x 4 or q-weekly x 9-12); FEC-D (5-fluorouracil, epirubicin and cyclophosphamide IV q3 weekly x 3) followed by docetaxel IV q3 weekly x 3. The dosages and more specific details regarding these regimens can be found below (Section 1.6).

Systemic hormonal therapy is recommended for all ER and/or PR positive breast cancers where there is a significant risk of distant relapse, balanced against the toxicity profile of these agents for each individual patient. Current recommendations support the use of a selective estrogen receptor modulator for 10 years in premenopausal patients, and an aromatase inhibitor for 5 years (10 year versus 5 year RCT, NCIC-MA17R, results still pending).

1.5.2 Surgery

The goal of surgery is to remove all of the cancerous tissue (tumour), plus some of the margins. The extent of surgery is dictated by the staging and the type of tumour, and may include lumpectomy (removal of the lump) or mastectomy (removal of the whole breast). Most early breast cancers (stage I and II) consist of small primary breast cancers that are, therefore, easily resectable by lumpectomy (termed ‘breast conserving surgery’), whereas stage III advanced cancers tend to occupy a larger portion of the breast and, therefore, require a mastectomy for successful removal of the entire involved area. Standard practice requires the surgeon to establish margins clear of cancer, indicating that the cancer has been completely excised. If the removed tissue does not have clear margins, further operations to remove more tissue may be necessary. Therefore, in an effort to minimize the risk of margin positivity while reducing the amount of normal breast tissue that needs to be resected, neoadjuvant chemotherapy has increasingly been used to downsize the primary breast cancer, in order to render operable breast cancers amenable to breast conserving surgery [40]. These tumours would otherwise require a mastectomy for complete removal if chemotherapy were to be delivered in the adjuvant setting.

For larger breast cancers that remain extensive despite neoadjuvant chemotherapy, or which are multicentric in nature (separate tumours distributed throughout different quadrants of the breast), a mastectomy remains the standard of care. This involves removing the glandular breast tissue from the

pectoralis fascia, resecting overlying skin and nipple-areolar complex and achieving primary skin closure over the chest. A drain is left beneath these large skin flaps, as seromas tend to accumulate over the ensuing 1-2 weeks, until the wound heals.

During the operation, the lymph nodes in the axilla must be sampled or removed entirely, in order to stage the patient for regional metastases. Until the early 2000s, the standard of care for staging the axilla involved resection of all axillary lymph nodes in the level I and II zones, resulting in reduced arm mobility, dysesthaesias of the upper arm and a 10-20% risk of permanent lymphoedema of the arm (lymphatic drainage was severely affected by the removal of so many lymph nodes, resulting in a swollen upper extremity). More recently, the technique of sentinel lymph node (SLN) dissection has become popular, as it requires the removal of far fewer lymph nodes (i.e. fewer side effects) [26,42]. The SLN dissection involves resection of the first tier of axillary lymph nodes that drain the breast, mapped functionally using dual tracer modality (blue dye injected into breast parenchyma preoperatively and technetium 99-m ($^{99\text{-m}}$Tc) radiocolloid bound to sulphur protein injected intradermally preoperatively). SLN mapping can spare 65-70% of patients with breast cancer from having a complete lymph node dissection, for what could turn out to be a negative nodal basin, but is indicated for early breast cancers felt clinically to be lymph node negative. In LABC, the vast majority of these patients are lymph node positive (either clinically or pathologically) and, therefore, the standard of care remains an axillary lymph node dissection [26], although this is a rapidly evolving field.

Removal of the breast with the axillary lymph nodes is called a modified radical mastectomy (radical mastectomy refers to removal of the breast, axillary lymph nodes and pectoralis muscle, a surgical technique that is no longer performed).

Patients with Stage IV breast cancer are deemed incurable and, therefore, goals of care are shifted to extension of quality life years. As a result, there is great debate whether the patient should undergo surgery to remove the primary cancer if it has already metastasized, particularly if the primary tumour appears to be well-controlled by the systemic therapies being given to control the distant disease. This is being addressed by a current clinical trial, the results of which remain outstanding (NCIC MAC14). Certainly there is a tendency to treat distant disease with monotherapies rather than combined regimens of chemotherapies and hormonal therapies, in an effort to maximize the duration of treatment response from each.

1.5.3 Radiation Therapy

Radiation therapy (RT) is used to reduce the risk of locoregional recurrence, and is almost always delivered in the adjuvant setting to the surgical field. It is the standard of care for reducing by more than 50% the risk of local recurrence in the breast following lumpectomy, or following mastectomy for lymph node positive breast cancers [43].

RT involves the delivery of high-energy X-rays that target the tumour, or post-surgery tumour site. It can be delivered in the form of external beam radiotherapy (linear accelerator), or brachytherapy (radiation source is placed

directly at the treatment site, which for breast remains largely confined to select subspecialty cancer centers or through clinical trials). This high energy therapy results in double-stranded DNA breaks for rapidly dividing cells, felt to target any residual cancer cells which might be present in the surrounding normal tissues or pre-invasive cancer cells at risk of progressing to invasive and resulting in a local recurrence.

RT delivered by tangents (or 2-field) refers to radiation to the breast alone, although the radiation beams do overlap and, therefore, treat approximately 80% of the lower axilla. Four-field or regional radiation refers to additional fields or radiation to the upper axilla (level III nodes, clavicular and internal mammary nodal basins).

The dose of radiation must be strong enough to be cytotoxic to proliferating cancer cells, but tolerable by surrounding normal cells, since all tissue types are susceptible to this damage. Therefore, the radiation delivery is planned using a 3-dimensional conformal CT scan where radiation oncologists and physicists calculate dosage to deliver even radiation to the area in question while constraining doses to critical structures. Damage is then minimized by delivering the treatment over many fractions at a low dose per fraction (typically 2Gy per fraction).

For LABC, the standard post-mastectomy dosage of 50Gy in 25 fractions is typically delivered, plus a boost of up to 9Gy in 5 fractions to regional nodes or surgical site for margin positivity or heavy nodal involvement [26]. Radiation is standardly delivered 5 days per week, excluding weekends, for administrative

convenience rather than for therapeutic reasons. It remains controversial whether patients who have achieved a pCR with neoadjuvant chemotherapy still require regional radiation after surgery, given the vastly improved survival rates in this subset, but at present it remains the standard recommendation [26].

1.6 NEOADJUVANT TREATMENT

1.6.1 Neoadjuvant Chemotherapy

Regardless of differential sensitivities between breast cancer subtypes, and despite an absence of survival benefit, neoadjuvant chemotherapy has become a standard of care for locally advanced breast cancer. The NSABP-18 trial was the first large randomized study to demonstrate the efficacy of neoadjuvant chemotherapy. In the study, 1523 patients with T1-3, N0-1 were randomized to four cycles of AC (adriamycin 60mg/m^2 and cyclophosphamide 600mg/m^2) intravenously every 3 weeks either pre- or post- operatively [44]. There was a significant improvement in clinical complete response (cCR), pathologic complete response (pCR) and the rate of breast conserving surgery with neoadjuvant treatment, but no difference in disease-free (DFS) or overall survival (OS) across the study population. However, in analyzing the 13% of patients who achieved a pCR, there was a significant improvement in DFS ($p=0.014$), distant DFS ($p=0.0004$) and a trend to OS ($p=0.06$) when compared to patients who did not achieve a pCR. At sixteen years of follow-up, these patients continued to show a significant improvement in DFS (Hazard Ratio (HR) 0.47, $p<0.0001$) with OS (HR 0.32, $p<0.0001$) now reaching statistical significance [45].

The correlation between improved survival from locally advanced breast cancer and pCR has been identified in other studies, mainly using anthracyclines [46-48].

In order to improve survival from breast cancer, novel cytotoxic agents such as taxanes were evaluated. Docetaxel is a microtubule-stabilizing agent that induces cell-cycle arrest and apoptosis [49,50]. It has demonstrated response rates up to 50% in anthracycline-resistant metastatic breast cancer [51-53], and superior survival when used first-line in randomized studies in the metastatic setting [54,55]. Docetaxel is most commonly given intravenously every 3 weeks. However, a randomized phase III study in the metastatic setting compared docetaxel 100mg/m^2 every 3 weeks, to 35mg/m^2 weekly for 3 of every 4 weeks [56]. Although response rates were lower on the weekly arm, there was no difference in progression-free survival (5.7 months vs 5.5 months; p=0.46) or OS (18.3 months versus 18.6 months; p=0.34). There were higher rates of clinically significant toxicity in the q 3-weekly arm, (88.1% versus 55.9%; p=0.0001).

Based on its activity in the metastatic setting, docetaxel has been tested in randomized trials in early stage breast cancer, and demonstrated superior survival when added to anthracycline-based regimens compared to the regimens alone [57,58]. FEC-D, (fluorouracil 500mg/m^2 IV, epirubicin 100mg/m^2, cyclophosphamide 500mg/m^2 IV every 3 weeks x 3 cycles, followed by docetaxel 100mg/m^2 IV every 3 weeks x3) is currently one of the more commonly employed regimens in the adjuvant post-operative setting. In the PACS-01 study, this regimen was compared to 6 cycles of FEC in 1999 women with node-positive

operable breast cancer. With a median follow-up of 60 months, there was a trend toward improvement in DFS (73.2% versus 78.4% p=0.12) and overall survival (86.7% versus 90.7%, p=0.17) favouring the docetaxel arm [57]. Toxicity was considered favourable, with more grade 3 and 4 neutropenia in the FEC arm (33.6% versus 28.1%, p=0.008), as well as higher use of colony-stimulating factors to maintain blood counts (27% versus 22%, p=0.01). There was a slightly higher incidence of febrile neutropenia in cycle number 4 with docetaxel compared to FEC (1.0% versus 4.6%, p=0.005), as well as significantly more edema and nail changes with the docetaxel. Similar improvements in DFS and OS were also found in the Breast Cancer International Study Group 001 which compared 6 cycles of TAC (docetaxel 75mg/m^2, adriamycin 50mg/m^2, cyclophosphamide 500mg/m^2 IV every 3 weeks) to FAC (5-FU 500mg/m^2, adriamycin 50mg/m^2, cyclophosphamide 500mg/m^2 IV every 3 weeks) in a similar population of women [58].

Several nonrandomized studies of docetaxel have also shown activity in the locally advanced setting either as a single agent, concurrent, or sequentially with other agents [59-63]. In order to determine whether the addition of docetaxel improves outcomes in the pre-operative setting, several randomized studies have been conducted. The largest, the NSABP-27, randomized 2411 women with T1c – T3 N0-N1 disease to receive 4 cycles of adriamycin and cyclophosphamide (AC) pre-operatively, versus 4 cycles of AC followed by 4 cycles of docetaxel preoperatively, or 4 cycles of AC preoperatively followed by surgery and 4 cycles of post-operative docetaxel. Compared to preoperative AC

alone, the addition of docetaxel significantly improved cCR (40.1% versus 63.6%; p<0.001), pCR (13.7% versus 26.1%; p<0.001) and proportion of patients with negative nodes (50.8% versus 58.2%; p<0.001) [64]. With 8.5 years of follow-up, across all 3 groups, there was no difference in DFS or OS (2). However, in the patients achieving a pCR, there was a significant improvement in DFS (HR=0.49, p<0.0001) and OS (HR=0.36, p<0.0001).

The Aberdeen Breast Group also tested the efficacy of the addition of docetaxel to an anthracycline-based regimen in the preoperative setting [65]. One hundred and forty-five women with newly diagnosed T3, T4 or TxN2 disease received 4 cycles of CVAP (cyclophosphamide 1,000mg/m^2, doxorubicin 50mg/m^2, vincristine 1.5mg/m^2 and prednisone 40mg). Those who achieved a pCR or cCR were then randomized to either 4 more cycles of CVAP or 4 cycles of docetaxel (100mg/m^2). Those who did not respond to the initial 4 cycles of chemotherapy were treated with docetaxel in a nonrandomized fashion. Intention-to-treat analysis demonstrated a higher clinical cCR (94% versus 66%, p=0.03) and pCR (31% versus 16%, p=0.04) with the addition of docetaxel compared to 4 more cycles of CVAP. At 38 months median follow-up, docetaxel significantly improved DFS (90% versus 77%, p=0.03) and OS (97% versus 84%, p=0.05) [66]. A third study, the GEPARDUO, compared AC for 4 cycles followed by docetaxel for 4 cycles (AC-DOC) to dose-dense doxorubicin 50mg/m^2 plus docetaxel 75mg/m^2 every 14 days for 4 cycles, with filgastrim support (ADOC) preoperatively in 913 women with T1-3 N0-2 breast cancer [67]. All endpoints, including pCR (22.4% versus 11%) and breast-conserving surgery rates (75%

versus 66%) were significantly improved with the sequential AC-T over the dose-dense arm [67]. Survival endpoints have not yet been reported. Based on these findings, the use of taxanes in the neoadjuvant setting has been recommended for non-metastatic advanced breast cancer [68]. For HER2 overexpressing tumours, the GerparQuattro trial (combining trastuzumab with anthracycline-based neoadjuvant chemotherapy regimens) demonstrated a significant increase in pCR with the addition of trastuzumab and recommended it also be initiated in the neoadjuvant setting [69].

In spite of the improved outcomes with neoadjuvant use of chemotherapy, the gains are modest in terms of impact on overall survival. Furthermore, the only patient groups experiencing a survival advantage are those who achieve a pathologic complete response, which represent a small proportion of women treated with a neoadjuvant approach (historically 10% in our institution as well as in our provincial consortium database, unpublished data).

1.6.2 Concurrent Chemo-Radiotherapy (CCRT)

Concurrent chemoradiation is an increasing form of effective therapy for a variety of cancers. The mechanisms by which various chemotherapeutic agents interact with radiation effects to produce supra-additive or synergistic effects (i.e. treatment response that is more than additive with what would have been seen with either treatment alone) differ widely [70]. There remains no one universal mechanism to explain the interaction between these drugs and radiation effects. Rather, the molecular class of drugs determines the mechanism of

radiosensitization; this can include modification of DNA damage by radiation, interference with DNA repair processes, cytokinetic cooperation, inhibition of proliferation, enhancing apoptosis, inhibition of angiogenesis, modifying hypoxia, and interference with signal transduction pathways [70].

Some classes of drugs exhibit biological cooperation, where the chemotherapy targets disease in one area, while radiation targets disease in another area. Others exhibit kinetic cooperation, meaning that both the chemotherapy and radiation modulate cell cycle and proliferation mechanisms [70].

A common feature of radiosensitizing drugs is that the interaction is dose and time dependent rather than tumour cell specific. For example, cisplatin must be present prior to radiation to produce any radiosensitizing effects [70]. Wilson and colleagues speculated that the presence of cisplatin molecules in the tissues receiving radiation inhibits sub-lethal damage repair from occurring after radiation-induced DNA damage [70]. 5-fluorouracil, on the other hand, inhibits DNA synthesis. It is not currently known what the specific mechanism is by which taxanes (docetaxel and paclitaxel) produce a supra-additive effect when present during radiation.

For breast cancer, radiation is recommended in patients who undergo breast conserving surgery for early disease, as well as for patients with advanced or lymph node positive disease. The recommended timing for the delivery of radiation for breast cancer is in the adjuvant setting; however, there is evidence to suggest that neoadjuvant radiation may improve patient outcome and should

be considered. The Stockholm Breast Cancer Trial, conducted between 1971-1976, randomized 960 early breast cancer patients to neoadjuvant radiation, adjuvant radiation (both delivered as 4500Gy in 25 fractions, 4-field) or surgery only (modified radical mastectomy for all patients) [71]. No systemic therapy was given. The study demonstrated a significant improvement in survival among patients treated in the preoperative setting, suggesting a potential benefit from receiving radiation therapy with the tumour *in vivo*. Although this study was completed a long time ago in what might be considered another era of care, it is a pure investigation, in the sense that it was a RCT where no other treatments were delivered (hormonal or systemic chemotherapy), thereby allowing the effects of the radiation to be evaluated alone in terms of impact on recurrence and survival.

In other cancers diagnosed elsewhere in the body, a combined modality approach of local neoadjuvant radiation given concurrently with radiosensitizing chemotherapy has been employed, in order to improve outcomes (both the locoregional control and overall survival). This has become the standard of care for the treatment of head and neck, rectum, lung, cervix and other cancer sites. Furthermore, a significant improvement in pCR as a surrogate for DFS with preoperative chemo/radiotherapy has also been reported [72]. The concurrent approach has demonstrated improvements in organ preservation and survival over radiation alone in multiple randomized trials [73-76].

Several randomized trials have demonstrated improved local control and survival in non-small cell lung cancer with the use of concurrent versus

sequential chemoradiation, most commonly with platinum-based chemotherapy [77-80]. Multiple small studies have been done in this patient population adding docetaxel as the radiosensitizer [81-86]. Doses have varied from 20mg/m^2 - 40mg/m^2 weekly as a single agent, or in combination with cisplatin. The studies showed the feasibility of adding docetaxel, for favorable response rates and survival, with manageable toxicity.

Limited published data exist for the use of neoadjuvant chemo-radiotherapy in locally advanced breast cancer. The most common reported use of concurrent chemo-radiotherapy is in the metastatic setting or in locally advanced (inoperable) or inflammatory breast cancer patients who progress on first line anthracycline-based chemotherapy [87-90], all of these using 5-FU or capecitabine as the radiosensitizing agent. These trials all occurred prior to standard incorporation of taxane into breast chemotherapy regimens.

Among early stage II breast cancer patients, the sandwich approach to neaodjuvant chemotherapy was evaluated, with radiation delivered between cycles 1,2 and 3,4 [91] in 14 patients. The regimen was considered feasible, with a 7% pCR rate but no survival outcome data. For similar early breast cancer patients treated in the adjuvant setting, sequential was later found to be similar to concurrent chemo-radiotherapy (ARCOSEIN trial) in terms of overall and disease-free survival, with the caveat of having used older, less effective chemotherapy regimens [92]. Of note, among the higher risk patients (node positive), there was an improved relapse-free survival.

In high-risk breast cancer patients (LABC), a few studies were undertaken to explore the potential use of concurrent neoadjuvant chemoradiotherapy. The largest trial was a retrospective review of 1,117 LABC patients treated in South India from 1990-1999 [93], who were treated with cyclophosphamide, methotrexate and 5-fluorouracil (CMF) versus 5-fluorouracil, epirubicin and cyclophosphamide (FEC) chemotherapy, with ^{60}Co delivered as 40Gy in 20 fractions, followed by surgery and oophorectomy. A pCR rate of 34% was obtained with acceptable toxicity.

Prospectively, similar smaller trials were undertaken using CMF [94] and 50Gy in 25 fractions to breast, where a pCR rate of 44% was reported in 73 LABC patients but no survival data was available. Again, sandwich techniques were explored, where radiation was delivered halfway through neoadjuvant chemotherapy cycles in LABC patients, but did not seem to improve outcomes [95,96].

Data supporting sequential treatment derives mostly from studies in early stage breast cancer. From pooled data of 10 retrospective studies, delaying radiotherapy in favour of chemotherapy increased the risk of local relapse from 6% to 16% [97]. Furthermore, radiotherapy given more than 8 weeks after surgery has been shown to double the local recurrence rate [97]. The only prospective trial designed to answer this question in early breast cancer demonstrated that patients treated initially with radiotherapy had higher rates of distant relapse; in contrast, patients treated with initial chemotherapy had higher rates of local relapse [98]. These differences were no longer apparent at 10

years of follow-up [99]. A major limitation of these sequential studies is the fact that the systemic treatments used are not comparable to modern chemotherapy regimens (which typically include taxanes, or targeted agents like trastuzumab). It is, therefore, possible that the differences in local relapse rates seen in the above studies may over-estimate the clinical reality today.

A number of cytotoxic chemotherapy drugs have been demonstrated to have radiosensitizing features (through molecular mechanisms not fully understood) as assessed through improved clinical outcomes with increased locoregional toxicity, most notably fluoropyrimidines [93,95], mitoxantrones [100], taxanes (docetaxel and paclitaxel) [101,102] and platinum [87] drugs.

Concurrent chemotherapy with radiation has the potential to offer patients the combined benefits of improved local and distant disease control. In early breast cancer, CMF-based concurrent adjuvant chemo-radiotherapy has been studied in several trials. Although this treatment had an acceptable toxicity profile, and shortened overall treatment time, clinical benefit in terms of overall or disease free survival was not consistently shown [103-106]. Anthracycline-based CCRT has been associated with serious skin toxicity, including recall reactions and cardiac toxicities. Mitoxantrone ($12mg/m^2$) in combination with cyclophosphamide ($500mg/m^2$) and fluorouracil ($500mg/m^2$) every 21 days for 6 cycles (CNF) and radiotherapy starting during the first cycle of CNF was shown in the multicentre randomized Arcosein trial, to improve local control in lymph node positive subgroup patients compared to sequential CNF and radiotherapy [92,107]. Unfortunately, concurrent CNF and RT compared to sequential CNF

and RT failed to show any benefit in 5-year DFS and OS. Similar results were seen in a French multicentre trial comparing concomitant CNF and radiotherapy to CEF (cyclophosphamide, epirubicin, and fluorouracil) and sequential RT [100]. A benefit in local control and decrease in local recurrence rate by 2.8 fold was seen in the concurrent CNF and RT arm, compared to the sequential CEF and RT arm, with no significance difference in OS and DFS. Unfortunately mitoxantrone has been associated with high rates of leukemic transformation and, therefore, is now rarely used.

In exploring the role of CCRT in breast cancer, using pCR as a surrogate for increase in survival has its limitations. The correlation of pCR achieved with systemic therapy and survival has been well established. This may be due to the ability of systemic therapy to sterilize micrometatstases if capable of achieving a complete response in the primary tumour and lymph nodes. Therefore, in this setting pCR would be a reflection of the effect of the treatment on all cancer cells, including disseminated disease. The value of achieving pCR with CCRT is not known. In fact, if one views radiation therapy, in a minimalist fashion, as a locoregional treatment, then achieving a pCR might not reflect the systemic benefit. However, some authors have proposed an anti-tumour systemic effect of local breast radiation [108].

1.6.3 Neoadjuvants: Taxanes as Radiosensitizers?

Anthracycline and taxanes are the backbone of most modern breast chemotherapy regimens in North America. Since anthracycline-based CCRT has

been associated with serious skin and cardiac toxicity, there has been an interest in evaluating taxane chemotherapy use as a radiosensitizer in the neoadjuvant setting currently with neoadjuvant radiation [109]. One retrospective review of 44 patients receiving concurrent chemoradiation with taxanes for stage I – IV breast cancer as a second line regimen was reported [110]. The majority had stage IIIA disease (31%). Seven patients had received prior radiation for breast cancer. Twenty-nine patients received concurrent paclitaxel and 15 received concurrent docetaxel, given on a q3 weekly schedule for the majority. Treatment was generally well tolerated with no grade 4 toxicities. Nine patients (20%) experienced grade 3 skin toxicity (moist desquamation), leading to a delay in chemotherapy in 11% of patients, until radiation was completed. Only one patient experienced long-term toxicity with retraction and fibrosis of the breast. Overall, this study demonstrated the safety and feasibility or concurrent chemoradiation with taxanes in locally advanced breast cancer. Response rates and survival outcomes were not reported.

A second study reported on 44 women with stage IIB to III locally advanced breast cancer who received twice weekly intravenous paclitaxel 30 mg/m^2 for 8 – 10 weeks concurrent with radiation to total dose of 45Gy to the breast plus 14Gy in 7 fractions boost, followed by surgery [101]. For those who responded to the initial chemoradiation, post-operative doxorubicin/paclitaxel was given for 4 cycles, with 4 cycles of doxorubicin/cyclophosphamide given post-operatively to non-responders. No grade 4 toxicities were observed in the preoperative chemoradiation phase. In the postoperative phase, the only grade

4 toxicity was leucopenia (10%). Ninety percent of patients received the prescribed concurrent treatment. Three patients (7%) experienced grade 3 skin toxicity; other grade 3 toxicities noted in the preoperative treatment were hypersensitivity to the paclitaxel, fatigue, stomatitis, and dyspnea limited to one case each. Dose reductions in chemotherapy occurred in 20% of patients: 3 for grade 3 neuropathy, 3 for grade 3 neutropenia, one for grade 3 stomatitis, and 1 for grade 3 esophagitis. No cases of radiation pneumonitis were reported. Post-mastectomy complications occurred in 6 patients (14%). These included 4 infections with delayed healing, one tram flap necrosis requiring revision and one mastitis. A clinical response was achieved in 91% of patients, with 11% CR and 80% PR. Sixteen percent of patients achieved a pCR, with 18% a pPR. There was no association between total dose of preoperative chemotherapy and pCR. Overall survival with a median follow-up of 32 months is 93.9%.

A third phase I/II study in 33 inoperable LABC and metastatic patients evaluated infusional paclitaxel with or without vinorelbine and concurrent 60-70Gy radiation. A 50% rate of grade 3 dermatitis was reported, with a pCR in the LABC subset of 46% [111].

Two studies have also been published in abstract form only. One reported on 112 patients with stage IIB – IIIB breast cancer who received 4 cycles of neoadjuvant FAC or AC given every 3 weeks followed by chemoradiation with mitomycin C 5mg weekly, 5-FU weekly, or cisplatin 30mg, gemcitabine 100mg weekly; 60Gy was delivered, but the fractionation schedule was not reported. This was followed by surgery, and 2 additional courses of postoperative

chemotherapy [112]. The pCR rate was 42% in breast, and 29.5% in breast and axillary nodes. No relationship between pCR and RFS was found. The only grade 3 toxicity reported was skin toxicity in 22.4%. The second study reported on 23 patients receiving 50.4Gy over 6 weeks, with paclitaxel 175mg/m^2 day 1, and 5-FU 1000mg/m^2/day continuous infusion day 1-3 for 3 cycles every 3 weeks [113]. This was followed by 3 cycles of FEC every 3 weeks, then surgery. Grade 3 toxicities included 39% radiation dermatitis. The clinical complete response rate was 82.6%, with a pCR rate of 52.2% and an overall 2-year survival rate of 80.7%.

In addition to the perceived improved response to chemoradiation when delivered in the neoadjuvant setting, it may be that radiation planning with the disease *in vivo* may improve dose delivery, with minimization of unwanted dosage to critical structures, although that has not been studied.

1.7 THESIS RATIONALE

Locally advanced breast cancer remains a significant clinical challenge with inferior survival in spite of improved systemic treatments. The cumulative data across tumour sites demonstrates a clear association between pCR and improved survival. Neoadjuvant therapy, when used in LABC, does not yield the high response rates that are seen and frequently cited in patients with operable tumours. Although pCR rates may be impressive in triple negative and HER2-positive patients, for those who achieve less than a pCR, poor outcomes are likely. Taxanes have improved OS in the metastatic and adjuvant setting, as well

as pCR rates and survival in the neoadjuvant setting; however, the pCR rates still remain disappointingly low. Concurrent administration of taxanes and radiation have demonstrated improved outcomes including survival across different tumour sites. In LABC, the concurrent approach has been shown to be feasible with manageable toxicity. The current standard treatment for locally advanced breast cancer is preoperative chemotherapy with an anthracycline and a taxane. However, the ideal regimen and number of cycles remain under debate. Given the improvement in local control, pCR and survival in other tumour sites the current study is aiming to determine whether a concurrent approach will improve pCR in locally advanced breast cancer.

1.7.1 Hypothesis

Our hypothesis was that concurrent neoadjuvant chemotherapy with regional radiation would improve the pCR rate from the current provincial rate of 10-15%, when compared to matched LABC patients undergoing standard of care (neoadjuvant systemic chemotherapy followed by surgery and adjuvant regional radiation). The goal was to use current standard full regimen chemotherapy (FEC-D) in order to avoid compromising on systemic efficacy for distant relapse with dose reductions, avoiding sandwich techniques which were felt only to create dose delays in the chemotherapy delivery, while delivering standard adjuvant doses of regional radiation in the neoadjuvant setting for radiosensitivity, while maintaining locoregional control in these high risk patients. The regimen was modified to include weekly docetaxel instead of q3 weekly (for which funding

for and approval of this modified regimen was secured from Ontario's Ministry of Health, Health Canada and Cancer Care Ontario), in order to provide maximal overlap of radiosensitizing chemotherapy during radiation and to minimize potential toxicity from the heightened effects of docetaxel as a result of the concurrent radiation.

The secondary hypothesis was that response to concurrent chemotherapy and radiation could be predicted by biological markers, such as imaging changes seen on serial cross-sectional imaging, serial measurements of tissue RNA concentration and integrity, and potentially predicted *ex vivo* using a 3D *in vitro* invasion assay. The findings of the secondary hypothesis are described in Appendix IV; they remain preliminary and hypothesis-generating at present.

1.7.2 Outline of this Thesis

We undertook a prospective Phase II Clinical Trial to test the effectiveness of the new protocol, concurrent radiation during the weekly docetaxel portion of neoadjuvant FEC-wD chemotherapy. As demonstrated in Chapter 2, we found that the neoadjuvant chemoradiation therapy significantly improved the pCR rates when compared to standardly treated LABC patients, with a trend toward improved disease-free and overall survival.

This regimen was not without toxicity, and the increase in pneumonitis seen as a result of combining docetaxel with radiation is evaluated and reported in Chapter 3.

Finally, we undertook to evaluate how effective the plasma levels of biomarker osteopontin (OPN) would be, as a predictor of pCR or overall survival with our novel neoadjuvant chemoradiotherapy protocol. This study and its findings are described in Chapter 4.

Overall conclusions and recommendations for future directions are outlined in Chapter 5.

The appendix chapters contain the full clinical trial protocol, the current LABC guidelines (written by Muriel Brackstone for Cancer Care of Ontario), and the preliminary findings of the biological correlative trials (secondary hypotheses).

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CHAPTER 2

Clinical Trial Evaluation of Concurrent Neoadjuvant Chemotherapy and Radiotherapy in Locally Advanced Breast Cancer.

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CHAPTER 2: CLINICAL TRIAL EVALUATION OF CONCURRENT NEOADJUVANT CHEMOTHERAPY AND RADIOTHERAPY IN LOCALLY ADVANCED BREAST CANCER.

2.1 INTRODUCTION

Breast cancer is the most common non-cutaneous cancer diagnosis for women in Canada, with an anticipated 24,400 Canadian women diagnosed in 2014, and 5,000 women dying of the disease [1]. Although newer treatments have improved both overall survival and progression-free survival for early and metastatic cancer patients respectively [2], there remains a subgroup of women with Locally Advanced Breast Cancer (LABC) who do poorly.

LABC is most commonly defined as stage IIB (T3N0) and Stage IIIA/B/C from the TMN classification [3]. Clinically these tumours are greater than 5 cm in size and/or extend beyond the breast tissue into the surrounding skin or muscle, with/without matted axillary lymph nodes (N2), internal mammary nodes (N3) or ipsilateral supraclavicular lymph node involvement [3]. LABC represents approximately 10-15% of all breast cancer cases, and the overall survival has historically been estimated at 30-42% at 5 years [4] a significant portion of whom will be living with metastatic disease. However, a small subset of women receiving neoadjuvant chemotherapy who achieve a complete pathological response, or pCR, (defined as no residual invasive breast cancer following neo-adjuvant treatment) to treatment are projected to have a vastly improved 5 year disease free survival rate of 87% [4], with 5 year overall survival rates of 89% [4]

and 90% [5]. As such, pCR rates have become the surrogate measure for favorable long-term outcomes in trials involving neoadjuvant treatment [6], particularly since the efficacy of systemic therapy can only readily be evaluated with the tumour *in vivo*. Neoadjuvant, or pre-operative chemotherapy has become a standard of care for locally advanced inoperable breast cancer or operable LABC where breast-conserving surgery is being contemplated [7, 8].

A number of cytotoxic chemotherapy drugs have been shown in other disease sites to have radiosensitizing features, as assessed through improved clinical outcomes with increased locoregional toxicity, although the molecular mechanisms are not fully understood. Of these, the most notable are fluoropyrimidines [9, 10], mitoxantrones [11], taxanes (docetaxel and paclitaxel) [12, 13] and platinum [14] drugs. However, limited published data exists for the use of neoadjuvant chemo/radiotherapy in LABC. The most common reported use of radiotherapy concurrent with radiosensitizing chemotherapy is in the metastatic setting, locally advanced (inoperable) or inflammatory breast cancer patients who progress on first line anthracycline-based chemotherapy [14-17], where 5-fluorouracil (5-FU) or capecitabine were used as the radiosensitizing agent.

Our hypothesis was that concurrent neoadjuvant radiosensitizing chemotherapy with regional radiation would improve the pCR rate from the current provincial rate of 10-15%. The goal was to use current standard full regimen chemotherapy in order to avoid compromising on systemic efficacy for distant relapse with dose reductions, avoiding sandwich techniques which could

create dose delays in the chemotherapy delivery, while delivering standard adjuvant doses of regional radiation to provide optimal locoregional control in these high risk patients.

2.2 METHODS

2.2.1 Rationale

At the time that the clinical trial was created, the only Ontario health care funded chemotherapy regimen for breast cancer in the neoadjuvant setting was AC-T (doxorubicin 60mg/m^2 and cyclophosphamide 600mg/m^2 IV q3 weekly x 4 cycles, followed by paclitaxel 175mg/m^2 IV q3 weekly x 4 cycles). The choice of FEC (5-fluorouracil 500mg/m^2, epirubicin 100mg/m^2 and cyclophosphamide 500mg/m^2 IV q3 weekly x 3 cycles) and docetaxel was based on the superior survival in high risk patients in the PACS-01 study [18]. Furthermore, epirubicin is associated with a lower risk of cardiotoxicity than doxorubicin, which must be considered in light of the concurrent radiation [19, 20]. Weekly docetaxel is as effective as docetaxel given every 3 weeks in the metastatic setting [21], but is associated with less toxicity; in particular, less myelosuppression, which should reduce the chances of having to reduce or eliminate cycles of chemotherapy. Therefore, FEC-D (5-fluorouracil 500mg/m^2, epirubicin 100mg/m^2 and cyclophosphamide 500mg/m^2 IV q3 weekly x 3 cycles, then docetaxel 100mg/m^2 IV q3 weekly x 3 cycles) was selected.

The weekly docetaxel regimen (35mg/m^2) was selected in order to provide constant radiosensitizing potential during chemotherapy [21, 22]. Special

permission was obtained from Cancer Care Ontario to have this regimen funded through Ministry of Health for this trial only. Standard regional intensity-modulated radiotherapy (IMRT) (45Gy in 25 fractions plus or minus boost for gross residual disease) was selected in order to provide optimal regional therapy for these patients at high risk of locoregional and distant relapse. Permission was obtained from Health Canada for use of these chemotherapy and radiation regimens concurrently in the neoadjuvant setting. This study was approved by Western University's Health Subjects Research Ethics Board (Appendix I).

2.2.2 Patient Description

Thirty-two patients presenting to the London Regional Cancer Program with non-inflammatory LABC were offered participation in this single-arm prospective Phase II clinical trial. Patients were deemed eligible if they had biopsy-proven LABC (defined as any T3 or T4 tumour stage or any N2 or N3 nodal stage by American Joint Committee on Cancer [23] staging). Patients were all female, at least 18 years of age and able to give informed consent, with a negative serum pregnancy test, no prior history of invasive cancer and adequate renal, hepatic, pulmonary and cardiac function. The clinical trial schedule is outlined in the trial calendar (Table 2.1). Patients were staged using CT chest/abdomen/pelvis and bone scan to rule out metastases (protocol details in Appendix III).

Table 2.1. LABC clinical trial calendar.

WEEK	*0	1	4	7	9	10	11	12	13	14	15	16	17	18	19	22	23	25	27	36, 48,60, 84, 108, 132, 156, 180, 204, 228, 252 (q3 mo x 1yr, q6 mo x 4yrs)
Medical History	X																			
Physical exam	X	X	X	X		X	X	X	X	X	X	X	X°	X	X			X	X	X
Calipers/Ruler	X	X	X	X		X			X			X			X					
Toxicity Assessment	X	X	X	X		X	X	X	X	X	X	X	X	X	X			X	X	X
Diagnostic MRI	X																			
Mammogram																				X
Needle Core Biopsy◊	X				X												X◦			
CBC w diff	X	X	X	X		X	X	X	X	X	X	X	X	X						
Biochemistry**	X	X	X	X		X			X			X								
B-HCG	X																			
Wall Motion Study***	X					X												X		X
Chest x-ray/CT & Abdo. Pelvis CT/ultrasound♣	X																			
Pulmonary Function Test	X																			
Bone Scan	X																			
FEC (q3 week)		X	X	X																
Weekly DOC****						X	X	X	X	X	X	X	X	X						
Daily Radiation						X	X	X	X	X	X									
Sesta MIBI SPCT/CT	X				X											X				
Radical Mastectomy																	X			
Blood for plasma osteopontin		X	X	X		X			X			X				X				X

ECG = electrocardiogram, bHCG = beta human chorionic gonadotropin (pregnancy test), DOC = Docetaxel, FEC = 5-fluorouracil, Epirubicin, Cyclophosphamide, CBC= complete blood count, with diff = differential, Ab CT = abdominal computerized tomography, MRI = magnetic resonance imaging

◦ By radiation oncologist

*...Some investigations may have been performed up to 6 weeks before start of treatment as part of usual care.

◊ To be done after sestaMIBI

' If remaining tumour is < 5mm, by ultrasound, final biopsy will not be performed

**...Biochemistry (calculated creatinine clearance, AST, ALT, alk phos, Ca, Total Protein, electrolytes, Total Bilirubin, Albumin, Random Glucose, urea, Uric Acid, LD)

*** Wall motion for patient treated with trastuzumab after 3 cycles of FEC and q3 months while on trastuzumab.

♣ Accept chest x-ray/CT & abdo. pelvis CT/ultrasound but prefer Chest &abdo. Pelvis CT

****HER2/neu positive breast cancer will receive Trastuzumab with docetaxel at the start of chemoradiation Q 3 wks for 1 year.

2.2.3 Treatment Regimen

Patients were treated with 3 cycles of FEC (intravenous 5-fluororuracil 500mg/m^2, epirubicin 100mg/m^2 and cyclophosphamide 500mg/m^2) q3 weekly, followed by docetaxel weekly x 9 weeks (dose adjusted to 35mg/m^2) (Figure 2.1). On the evening prior to docetaxel chemotherapy, dexamethasone 8mg oral was taken by each patient. Concurrent radiation therapy was started during the first day of docetaxel. Radiation therapy consisted of external beam IMRT therapy for a total dose of 45Gy in 25 fractions over 5 weeks. A reduced volume boost of 5.4Gy in 3 fractions to 9Gy in 5 fractions was given to residual gross disease in the breast and/or regional lymph nodes. All treatment planning was performed on the Phillips Pinnacle workstation, and radiation treatment was delivered on megavoltage machines using 6MV energy or greater. Chemotherapy with radiation was followed by modified radical mastectomy (including standard level I and II axillary node dissection) 5 weeks after the last dose of docetaxel, allowing 8 weeks of radiation recovery preoperatively.

Adverse events from chemotherapy and radiation therapy as well as grading of any developed toxicity were assessed by the oncologist as per the National Cancer Institute [24]. Patient tolerability was assessed every 3 patients, and any grade 4 or higher toxicities or any treatment delays were reviewed by an independent data safety monitoring committee (IDSMC). Mid-study, the protocol was modified to include a normal pulmonary function test and non-smoker status after the first three patients with pneumonitis were reviewed by the IDSMC.

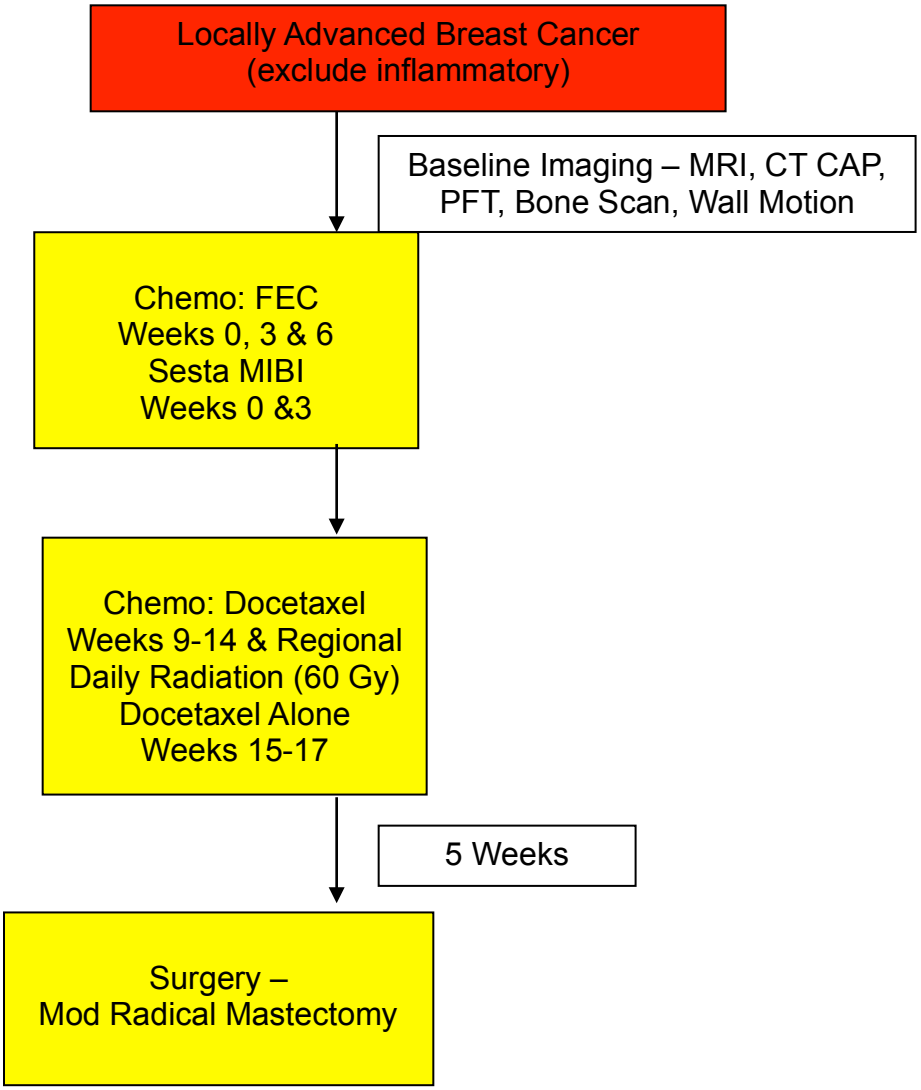

Figure 2.1. Schema for LABC clinical trial.

Women with HER2/neu positive breast cancer received one year of trastuzumab, initiated in the neoadjuvant setting concurrently with docetaxel as per standard of care, given the absence of evidence to support increased cardiotoxicity even when administered concurrently with radiation or taxanes [25, 26]. Monitoring for cardiac toxicity from trastuzumab included a wall motion study performed every 3 months while on therapy. Dose modification was made as per international and institutional guidelines for trastuzumab-associated cardiac dysfunction [27]. Women with estrogen receptor positive breast cancer received postoperative endocrine therapy according to their menopausal status.

2.2.4 Assessment of Pathological Response

Pathological response was subcategorized as follows [28]: pCR – pathological complete response (no residual invasive breast cancer in the breast tissue or axila); pSPR – pathological significant partial response (<10 foci of microscopic invasive tumour within breast); pPR – pathological partial response (<30% of original invasive breast tumour volume remaining); SD – stable disease (30-80% of original invasive breast tumour volume remaining); NR – no response (81-120% of original invasive breast tumour volume remaining).

Molecular subtype was categorized using tumour phenotype as a surrogate for genotypic classification as follows:

- Luminal A: Estrogen receptor (ER) and/or Progesterone receptor (PR) positive, epidermal growth factor receptor erb2 (HER2) negative, NOT high grade

- Luminal B: ER and/or PR positive, high grade only (HER2 positive or negative)
- HER2+: ER and PR negative, HER2 positive
- Basal: ER and PR negative, HER2 negative

The proliferation marker Ki67 is not measured at our institution.

2.2.5 Statistical Analysis

This study was designed to accrue 52 patients, based on a sample size calculation powered to detect a doubling of pCR rate (26% to 52%) from published clinical trials of neoadjuvant chemotherapy [8], but was closed prematurely after a treatment-related death, with 32 patients accrued, at the recommendation of the IDSMC. The treatment cohort (n=32) was compared to a concurrent control cohort of LABC patients off-study treated at the same institution, all of whom received neoadjuvant chemotherapy (FEC-D or AC-D), modified radical mastectomy and equivalent locoregional radiation delivered in the adjuvant setting (50Gy in 25 fractions using IMRT). Patients were matched 1:N by stage, age, and molecular subtype using greedy matching by propensity score +/- 0.1 to minimize selection bias. Cox regression analysis was employed using a robust sandwich estimator for paired comparison of pCR rates between the concurrent chemoradiation cohort and the control cohort. Cox proportional hazards analysis was used to compare disease-free and overall survival between the two cohorts. SAS 9.3 was used for all statistical analyses (SAS Institute Inc., Cary NC).

2.3 RESULTS

Of the 32 patients accrued to the study, one patient progressed during the FEC portion of the treatment and was taken off study in favor of second line chemotherapy. Another patient with inoperable bilateral LABC received bilateral regional radiotherapy during the docetaxel/radiation portion of the study and developed pneumonitis-induced acute respiratory distress syndrome shortly after completion of radiation. This patient did not go on to surgery and died shortly thereafter. From 30 remaining patients who all completed neoadjuvant therapy and surgery, 27 were successfully matched to 81 concurrent control patients using propensity score greedy matching to minimize selection bias, since statistical power was optimized with a 1:3 matching.

No statistically significant difference in patient age, pre-treatment tumour size, pre-treatment nodal status or molecular subtype was found using Cox regression analysis (Table 2.2). A statistically significant difference in post-chemotherapy tumour size was seen (mean residual tumour size in concurrent chemoradiation cohort was 13.16mm versus 31.12mm in control cohort, $p<0.001$) (Table 2.3).

The overall pCR rate was significantly higher in the concurrent chemoradiation cohort (22.6% versus 14.9% in control cohort, $p=0.019$) (Table 2.3). The number of patients in each molecular subtype group was too small to permit statistical comparisons of pCR rates by molecular subtype. None of the concurrent chemoradiation cohort patients who achieved a pCR have had a

Table 2.2. Patient demographics comparing LABC neoadjuvant concurrent chemotherapy and radiotherapy study patients to matched control cohort.

Variable	LABC Chemotherapy Matched Cohort (3:1) (n=81)	Chemoradiation LABC Study (n=27)
Mean age at registration (years)	51.2	49.3
Baseline mean tumour size (mm) – pre-treatment (baseline ultrasound)	42.0	43.2
Baseline clinical node (%)		
N0	10.1%	28.1%
N1-N3c	65.9%	68.8%
NX	24.0%	3.1%
Luminal subgroup – N (%)		
Luminal A	29 (34.9)	9 (33.3)
Luminal B	33 (39.8)	10 (37.0)
HER2+	8 (9.6)	3 (11.1)
Basal	13 (15.7)	5 (18.5)

Table 2.3. Clinical response to neoadjuvant therapy (primary chemotherapy for LABC chemotherapy matched cohort versus concurrent chemotherapy with radiotherapy for LABC study patients). *p<0.05

Variable	LABC Chemotherapy Matched Cohort (3:1) (n=81)	Chemoradiation LABC Study (n=27)
Mean tumour size (mm) – post-treatment (pathology)	31.12	13.16
Lymph nodes positive (%) post-treatment	60.9	53.3
Luminal subgroup with pCR (%)		
Luminal A	6.0	0 (33.3)
Luminal B	13.9	10 (37.0)
HER2+	30.3	3 (11.1)
Basal	18.0	5 (18.5)
TOTAL with pCR (%)	14.9	22.6*
Follow-up – distant recurrence (%)	18.9	20.0

Table 2.4. Treatment related toxicity rates between LABC neoadjuvant concurrent chemotherapy with radiotherapy and matched control cohort.

Toxicity *	LABC Chemotherapy Matched Cohort (3:1) (n=81)	Chemoradiation LABC Study (n=27)
Dermatitis	0 (0%)	6 (22%)
Pneumonitis	1 (1%)	5 (19%)
Cardiomyopathy	2 (2%)	0 (0%)
Neuropathy/Arthralgia	3 (4%)	0 (0%)
Febrile Neutropenia	8 (10%)	0 (0%)

The numbers were too small for statistical comparison between groups.

* Only class 3 or higher toxicities causing treatment delays or interruptions were used; as most radiation pneumonitis occurs at the end of treatment (hence it rarely interrupts treatment), it is, therefore, likely that the effect is under-represented in the adjuvant treatment control cohort.

recurrence, while 36% of patients who did not achieve a pCR recurred and died of their disease within 36 months of treatment.

Although there was no significant difference in disease-free (DFS) or overall survival (OS) between the treatment groups due to premature study termination and resultant smaller sample size, there was a trend at 36 months in DFS for the concurrent chemoradiation cohort of 79% versus 64% for the control cohort (Figure 2.2). The Hazard Ratio (HR) for DFS in the concurrent chemoradiation cohort was 0.51 (95% CI=0.16-1.4; p=0.185). A similar trend was also seen for OS, where the OS for the concurrent chemoradiation cohort was 84% versus 69% in the matched control cohort (Figure 2.3). The HR for OS was 0.46 in favor of the concurrent chemoradiation cohort (95% CI=0.16-1.4; p=0.161).

2.4 DISCUSSION

This study demonstrated that the addition of neoadjuvant radiation to anthracycline and taxane-based chemotherapy significantly improved the pCR rate in LABC patients, with a trend of 15% higher overall survival at 3 years as well as disease-free survival that failed to reach statistical significance based on sample size.

Since the trial was initiated, other trials using concurrent neoadjuvant chemotherapy with radiation for breast cancer have been published. Follow-up data from Formenti's 2003 trial [12] was subsequently published [29],

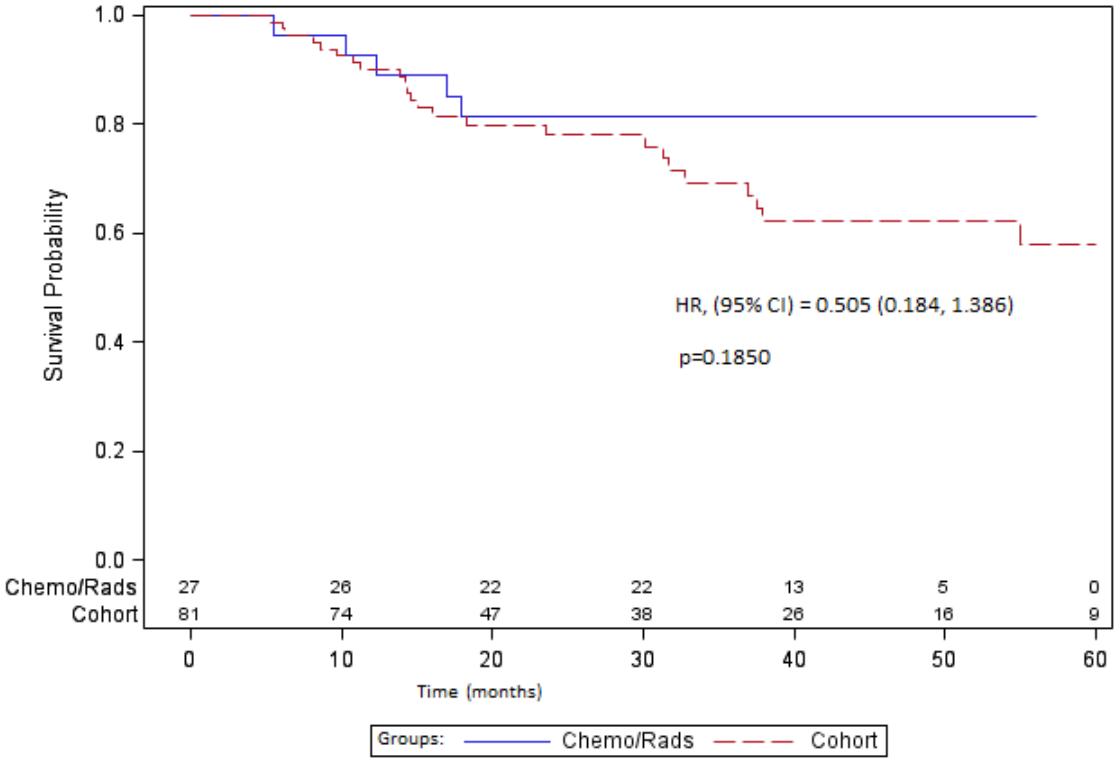

Figure 2.2. Disease free survival (DFS) comparing concurrent chemoradiation cohort to matched control cohort.

HR, hazard ratio; *Chemo/Rads*, chemoradiation.

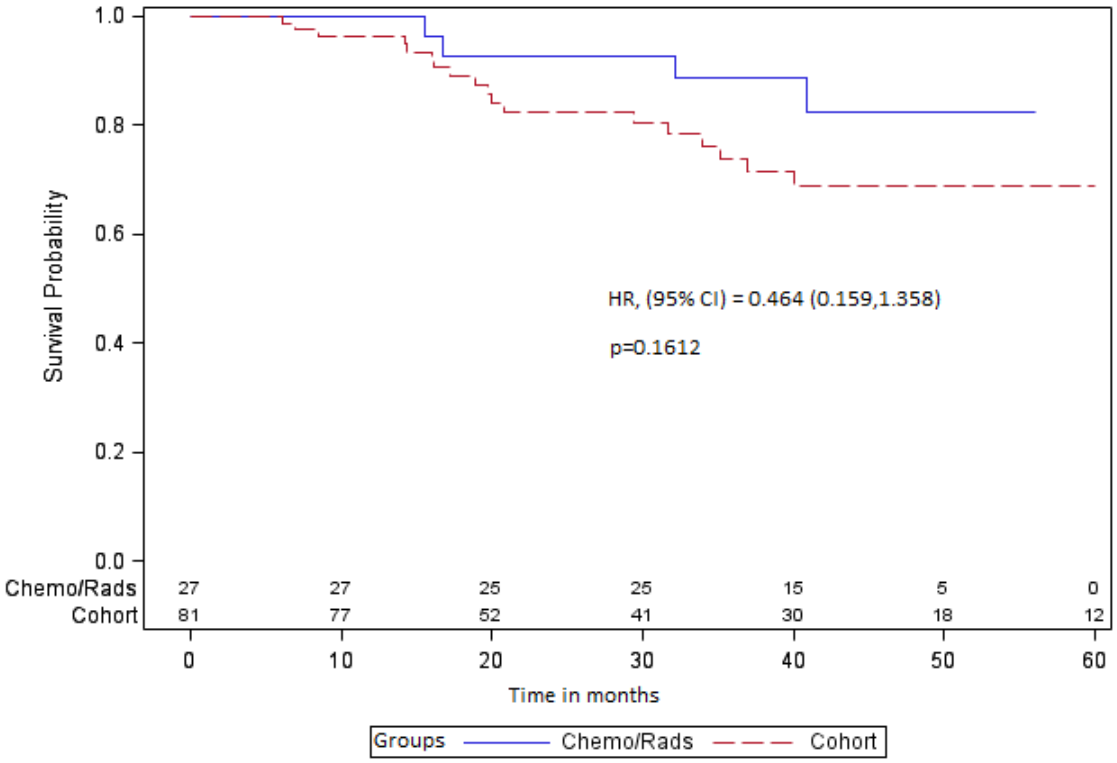

Figure 2.3. Overall survival (OS) in concurrent chemoradiation cohort compared to matched control cohort.

HR, hazard ratio; *Chemo/Rads*, chemoradiation.

demonstrating a combined pCR and pPR rate of 34% resulting in a significant association with better DFS and OS (hazard ratio (HR)=0.35 for recurrence and HR=4.27 for overall survival, both with $p<0.01$) when compared to non-responders within the same treatment cohort. In that study, only the taxane was given neoadjuvantly, with the remainder of the chemotherapy regimen being given adjuvantly following a perioperative delay. Formenti's study confirmed that patients able to achieve a pCR with concurrent chemoradiation obtain a significantly improved DFS and OS over non-responders treated the same way. It does not compare concurrent versus sequential chemotherapy and radiation as this trial does.

Trials of neoadjuvant chemoradiotherapy evaluating older regimens of chemotherapy without taxane have since also been published [30-33], most using 5-FU as the radiosensitizing agent, demonstrating feasibility and reasonable toxicity and pCR rates of 10-29% and an overall survival of 84%. These were mostly retrospective studies in highly selected patient cohorts.

Other trials evaluated neoadjuvant concurrent chemoradiotherapy as a rescue for LABC patients who progressed on first line neoadjuvant chemotherapy, using 5-FU as the radiosensitizer [34, 35] with reasonable pCR rates and resultant operability. Long-term outcomes were not reported.

Ours represents the first clinical trial evaluating full dose concurrent neoadjuvant chemotherapy including a radiosensitizing taxane as part of a modern chemotherapy regimen (FEC-D), delivered with locoregional radiation in LABC patients. Our findings support those of the Formenti group [29], where

pCR rates were significantly increased with concurrent delivery of radiation and taxane chemotherapy, and goes further to show a trend in improved DFS and OS at 3 years over standard sequential therapy.

This regimen was not without its toxicity, and while high rates (25%) of grade 3 dermatitis (moist desquamation of chest wall skin) might be considered clinically acceptable, the 25% rate of grade 3 or higher pneumonitis was concerning. All patients who presented with clinical pneumonitis had the diagnosis confirmed on CT scan and were treated with a 2-3 week tapering regimen of high dose corticosteroids. One patient suffered ARDS shortly after completion of preoperative therapy and died. None of the 30 patients proceeding to surgery required a delay in surgery due to pneumonitis. The pneumonitis experienced by the patients in this study behaved clinically like acute interstitial pneumonitis and not radiation pneumonitis in that the symptoms resolved acutely within weeks and did not lead to long-term impairment, however it is felt that the radiation likely exacerbated its presentation [36].

Capillary leak and interstitial pneumonitis from taxane chemotherapy is well known, although pre-treatment with 8mg of dexamethasone, as was used in this trial, is felt to reduce this risk. The typical rate of pneumonitis (1-5% for q3 weekly docetaxel) [37] increases when administered q-weekly, reportedly to 27%, which is comparable to our study [38]. Rates of pneumonitis are also elevated in patients with pre-existing lung disease [39]. On the other hand, with the q-weekly regimen, none of the patients suffered from other toxicity commonly associated with docetaxel, such as febrile neutropenia or peripheral neuropathy. None of the

study patients developed a postoperative wound infection or dehiscence, although one patient had a protracted seroma that required multiple aspirations.

This study was not without its limitations. As a single arm phase II trial, there was no randomization to a control arm to correct for unanticipated bias, and a matched design to a control cohort may have inadvertently introduced selection bias. Every effort was made to minimize this risk by having an independent statistician do the matching to our comprehensive patient population database by all variables thought to affect the outcomes of interest. It remains possible, however, that unanticipated confounders could have influenced the results.
Although we found a difference of 15% in overall survival at 3 years, the study failed to reach statistical significance. This lack of statistical power is due to premature termination of the trial due to an unexpectedly high rate of pneumonitis, with one death from ARDS.

The use of concurrent neoadjuvant chemoradiotherapy in LABC appears to significantly improve the pCR rate and result in a trend to improved overall survival. Given the poor outcome for LABC patients, any potential treatment regimen that could result in a 15% increase in overall survival should be aggressively pursued. Use of docetaxel appears to be associated with too high a rate of pneumonitis, therefore a future phase III multicentered randomized trial should be undertaken where the radiosensitizing benefit of taxanes can be exploited, using for example paclitaxel [29] concurrently with locoregional radiation as part of a full neoadjuvant chemotherapy regimen.

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CHAPTER 3

Role of Plasma Osteopontin as a Biomarker in Locally Advanced Breast Cancer.

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CHAPTER 3: ROLE OF OSTEOPONTIN AS A BIOMARKER IN LOCALLY ADVANCED BREAST CANCER.

3.1 INTRODUCTION

Locally advanced breast cancer (LABC) is considered an aggressive and advanced stage of non-metastatic breast cancer, accounting for approximately 5-15% of all breast cancer cases [1-3], with a five year overall survival rate of 30-42% [3-6]. Current treatment for this form of cancer is multimodal, involving neoadjuvant chemotherapy, surgery and radiotherapy [7, 8]. Approximately 10%-20% of patients achieve a clinical complete response (CR) and 50%-60% will achieve a partial response (PR) to neoadjuvant therapy. However, only one half to two-thirds of clinical CRs will be confirmed pathologically (pathological complete response, pCR, defined as no residual invasive breast cancer in the surgical specimen [1]). Response to neoadjuvant chemotherapy allows clinicians to identify patients who may have a good outcome, as pCR remains the best predictor for long-term survival [6, 9].

Osteopontin (OPN) is a secreted, integrin-binding phosphoprotein that is expressed by several normal tissues and cell types [10, 11]. OPN plays an important role in various aspects of malignancy, particularly those involved in tissue invasion and metastasis [10, 12-18], and OPN levels have been associated with aggressiveness in several cancer types, including breast cancer.

We, as well as other groups, have shown by immunohistochemistry that elevated levels of OPN found in primary tumours may be correlated with a poor

patient prognosis and tumour stage [19-21]. OPN can also be detected in the blood of patients with various forms of cancer, including breast, prostate, colon, lung, liver and stomach cancer and can be measured using an ELISA (enzyme-linked immunosorbent assay) [22-27]. Plasma OPN levels are found to be elevated in the majority of metastatic breast cancer patients and increased baseline levels of plasma OPN in metastatic breast cancer patients are associated with a worse prognosis and increased tumour burden [23, 25]. Additionally, in metastatic breast cancer patients monitored by serial OPN blood levels, survival decreases (despite treatment) as plasma OPN levels increase over time [23, 25]. Plasma OPN may thus have both a prognostic and a predictive role in metastatic breast cancer, making monitoring plasma OPN levels in metastatic breast cancer patients throughout treatment and over their disease course potentially useful to predict aggressive tumour behavior. In contrast, we recently reported data obtained for early breast cancer patients that failed to show prognostic value for baseline plasma levels in those patients, although we did find elevated plasma OPN in post-baseline samples from a subset of patients [26].

This study is, to our knowledge, the first study to measure plasma OPN serially in LABC patients. Serial measurement of plasma OPN levels over treatment may potentially provide information with respect to patient response to neoadjuvant therapy and long-term survival. The ability to more accurately monitor response to neoadjuvant therapy may lead to better management of these patients.

3.2 MATERIALS AND METHODS

3.2.1 Patient Enrollment and Treatment Course

Fifty-two female patients and one male patient diagnosed with LABC, being treated at the London Regional Cancer Program in London, Ontario, Canada, were enrolled during 2007-2011 into this study, which was approved by the Western University Health Sciences Research Ethics Board. All patients had a histologically confirmed clinical stage III breast cancer and were eligible for neoadjuvant therapy, excluding inflammatory breast cancer or patients with distant metastases. Patients with any prior history of invasive cancer or prior chemotherapy or radiotherapy were excluded. All patients provided written informed consent to participate in this study. No eligible patients declined participation. Standard patient treatment included neoadjuvant chemotherapy, modified radical mastectomy and adjuvant regional radiation. Patients received one of two standard neoadjuvant chemotherapy regimens: AC-T (Doxorubicin 60mg/m^2 and cyclophosphamide 500mg/m^2 IV q3 weekly x 4 cycles followed by docetaxel 100mg/m^2 IV q3 weekly x 4 cycles) or FEC-D (5-fluorouracil 500mg/m^2, epirubicin 100mg/m^2 and cyclophosphamide 500mg/m^2 IV q3 weekly x 3 cycles followed by docetaxel 35 mg/m^2 qweekly x 9 cycles). The patients receiving FEC-D received their regional external beam conformal radiotherapy (45 Gy in 25 fractions plus 5.4 Gy in 3 fractions or 9 Gy in 5 fractions depending on disease burden) concurrent with docetaxel therapy in the neoadjuvant setting versus identical radiotherapy in the adjuvant setting for AC-D patients. This was followed in all patients by modified radical mastectomy to remove the breast and axillary

lymph nodes, which were examined pathologically. All surviving patients were followed for at least 2.5 years. All patients, with the exception of the male patient who died unexpectedly of aspiration-induced respiratory arrest following chemotherapy cycle 2, completed the treatment course. Three female patients became metastatic during neoadjuvant therapy. Patient characteristics as well as tumour characteristics and subsequent occurrence of metastases during or after neoadjuvant therapy are listed in Table 3.1. All patients were followed prospectively and none were lost to follow-up.

3.2.2 Plasma Sample Collection and OPN Analysis

Blood samples for OPN measurement were collected in tubes with EDTA anticoagulant and processed as previously reported [22]. OPN was measured in plasma samples by ELISA (Human Osteopontin EIA Kit, catalogue #ADI-900-142, Enzo Life Sciences, Ann Arbor, MI) as previously described [27]. Samples were collected from all patients at baseline (just prior to the first cycle of chemotherapy) and again just prior to each subsequent chemotherapy cycle treatment or every three weeks throughout neoadjuvant treatment for patients receiving their docetaxel weekly. Plasma samples from a cohort of 90 healthy women without cancer were collected and measured for OPN as described previously [26].

Table 3.1. Patient and tumour characteristics, and site of metastasis.

OPN Number	Age at Baseline	Gender	Tstage	Nstage	ER	PR	HER2	Grade	Site of Metastasis
01	53	F	T3	N1	Pos	Pos	Pos	2	0
02	52	F	T4a	N0	Unk	Unk	Unk	1	0
03	68	F	T4b	N1	Pos	Pos	Neg	1	0
04	52	F	NA	NA	Pos	Pos	Neg	3	0
05	50	F	T4b	N1	Pos	Neg	Pos	3	Liver
06	64	F	T3	N1	Pos	Neg	Equiv	2	0
07	59	F	T4b	N1	Neg	Neg	Pos	3	Lung
08	47	F	T4b	N1	Pos	Pos	Neg	3	Liver
09	56	F	T2	N1	Neg	Neg	Pos	3	0
10	44	F	T3	N1	Neg	Neg	Pos	3	0
11	42	F	T4b	N1	Pos	Pos	Neg	3	Lung/Liver/Bone
12	76	M	T4b	NX	Unk	Unk	Unk	2	Unk
13	67	F	T3	N0	Pos	Pos	Neg	1	0
14	64	F	T4b	N1	Pos	Pos	Pos	1	Liver
15	39	F	T2	N1	Pos	Pos	Pos	2	Brain/Liver/Lung/Bone
16	46	F	T3	N0	Pos	Pos	Neg	2	0
17	42	F	T3	N1	Neg	Neg	Pos	3	Lung
18	48	F	T3	N1	Pos	Pos	Neg	1	0
19	53	F	T4b	N0	Neg	Neg	Pos	3	0
20	47	F	T4b	N1	Neg	Neg	Neg	3	0
21	44	F	T1c	N2	Neg	Neg	Pos	3	0
22	38	F	T2	N0	Neg	Neg	Pos	3	0
23	62	F	T2	N1	Neg	Neg	Pos	3	Brain
24	26	F	T2	N1	Neg	Neg	Neg	3	0
25	58	F	T3	N2	Pos	Pos	Pos	3	0
26	43	F	T2	N1	Pos	Pos	Pos	2	0
27	52	F	T2	N2	Pos	Pos	Pos	2	0
28	49	F	T3	N2a	Pos	Pos	Pos	2	0
29	63	F	T3	N1	Neg	Neg	Neg	3	0
30	48	F	T3	N1	Pos	Pos	Neg	2	0
31	61	F	T3	N1	Pos	Pos	Neg	3	0
32	39	F	T3	N1	Pos	Pos	Neg	2	Lung
33	43	F	T3	N0	Neg	Neg	Pos	2	0
34	47	F	T3	N0	Pos	Pos	Neg	2	0
35	49	F	T2	N0	Neg	Neg	Neg	3	Bone
36	64	F	T3	N2	Pos	Neg	Neg	2	Bone
37	34	F	T3	N3	Neg	Neg	Neg	3	0
38	40	F	T2	N1	Pos	Pos	Neg	1	0
39	58	F	T1	N3	Pos	Pos	Pos	3	0
40	42	F	T2	N0	Pos	Pos	Neg	2	0
41	53	F	T3	N0	Neg	Neg	Neg	3	0
42	44	F	T3	N1	Pos	Pos	Pos	2	0
43	45	F	T3	N0	Pos	Neg	Neg	2	0
44	57	F	T2	N0	Pos	Neg	Pos	3	0
45	60	F	T3	N0	Pos	Pos	Neg	2	Bone
46	50	F	T3	N2	Neg	Neg	Neg	3	Lung
47	44	F	T3	N1	Pos	Pos	Neg	1	0
48	45	F	T3	N1	Pos	Pos	Neg	2	0
49	62	F	T3	N2a	Pos	Neg	Pos	3	0
50	51	F	T3	NX	Pos	Pos	Pos	2	0
51	58	F	T2	N1	Pos	Pos	Pos	3	0
52	31	F	T2	N3	Pos	Pos	Pos	3	0
53	62	F	T2	N3	Pos	Pos	Neg	2	0

ER, estrogen receptor; *Equiv*, equivocal; *NA*, not available; *Neg*, negative; *Pos*, positive; *PR*, progesterone receptor; *Unk*, unknown.

3.2.3. Pathological Assessment

Surgical specimens were sent for final pathological assessment. Tumour response to neoadjuvant chemotherapy was sub-stratified as follows [28]:

i) Complete pathological response (pCR) (No evidence of residual invasive tumour in breast or axilla)

ii) Partial response (PR) (at least a 30% decrease in residual tumour volume)

iii) No evidence of response (stable disease) (NR)

iv) Progression of disease (PD) (at least a 20% increase in residual tumour volume).

3.2.4 Statistical Analysis

Repeated measures analysis of variance was used to compare OPN levels across cycles of treatment, with Tukey's multiple comparisons test used to make pair-wise comparisons between cycles. The co-variates used included age, gender, T stage, N stage, ER, PR, HER2 and grade. Student's t-test was used to compare data between healthy women and patients. The Kaplan-Meier technique was used to estimate survival, comparisons were made using the log-rank statistic, and the calculation of hazards ratios and evaluation of the effect of baseline OPN as a continuous variable were done using Cox regression. The relationship between OPN levels and response to chemotherapy was evaluated using Fisher's exact test. SAS 9.3 was used for all statistical analyses (SAS Institute Inc., Cary NC).

3.3 RESULTS

Plasma OPN levels in ng/ml for each patient over the course of neoadjuvant chemotherapy are shown in Figure 3.1. Patient 12, who was the only male in the study, died following the second cycle of chemotherapy from what appeared to be treatment-related toxicity. A baseline OPN level of 69.7ng/ml was measured and at cycle two, just prior to his death, OPN level was elevated to 141.3ng/ml. The two OPN values for this patient were not used for the following analyses. A second patient progressed during the first 2 cycles of chemotherapy and was, therefore, removed from the study and not included in the analysis; the third patient died from acute respiratory distress syndrome shortly after docetaxel/radiotherapy without surgery and was, therefore, also excluded from analysis. The remaining 50 patients were included for analysis.

OPN values at baseline/cycle 1 were obtained for 50 patients. Mean OPN value was 70.3ng/ml at baseline/cycle 1, while median value was 63.6ng/ml (range 33.3 – 189.8ng/ml). We have previously measured plasma OPN levels of 90 healthy women and found a mean value of 32.0ng/ml (median value 26.3ng/ml (range 11.8-108.6ng/ml) [26]. This differs significantly from the mean values of LABC patient plasma OPN at baseline/cycle 1 ($p<0.001$) (Figure 3.2).

OPN levels across 7 cycles of neoadjuvant treatment were compared for the 34 patients for whom we had a complete set (no missed blood draw at each chemotherapy cycle) using repeated measures analysis of variance. There was an increase in OPN levels seen during chemotherapy cycles that was significant overall ($p<0.001$). Mean patient plasma OPN levels between successive cycles

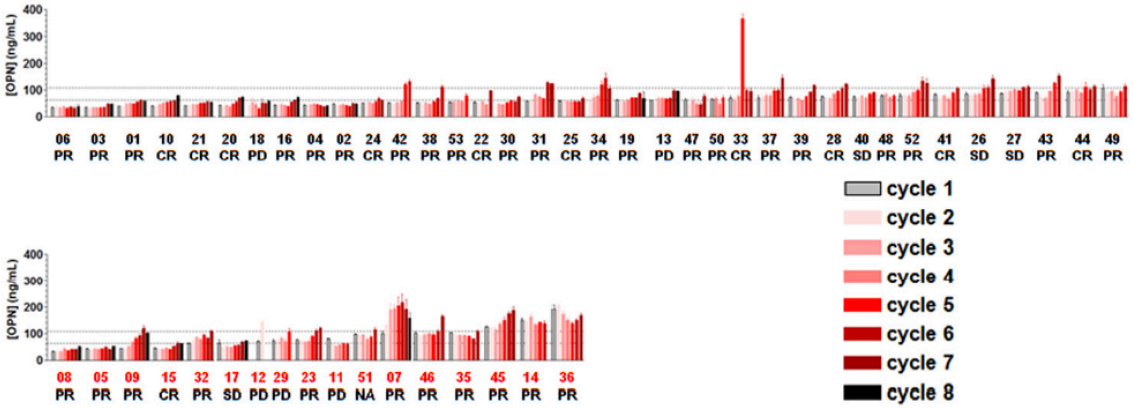

Figure 3.1. Plasma OPN levels during neoadjuvant therapy with final response to treatment and survival for all 53 LABC patients. At every treatment cycle plasma samples were obtained and OPN was measured in triplicate by ELISA. Average OPN levels are shown in ng/ml. Final patient response is denoted as complete response (CR), partial response (PR), no response (NR) or progressive disease (PD). Patient numbers in black indicate patients who were alive 2.5 years post-surgery and red numbers indicate patients who died within that period. Patients are grouped first according to status (alive vs dead) and then according to increasing baseline OPN, except patients for which no baseline OPN was obtained in which case OPN at cycle 2 was used. Patient 12 is a male patient who died following 2 neoadjuvant cycles. The dotted lines indicate upper limit of OPN levels in a cohort of 90 healthy women (108.6ng/mL) and median value at baseline/cycle 1 (63.6ng/mL), respectively.

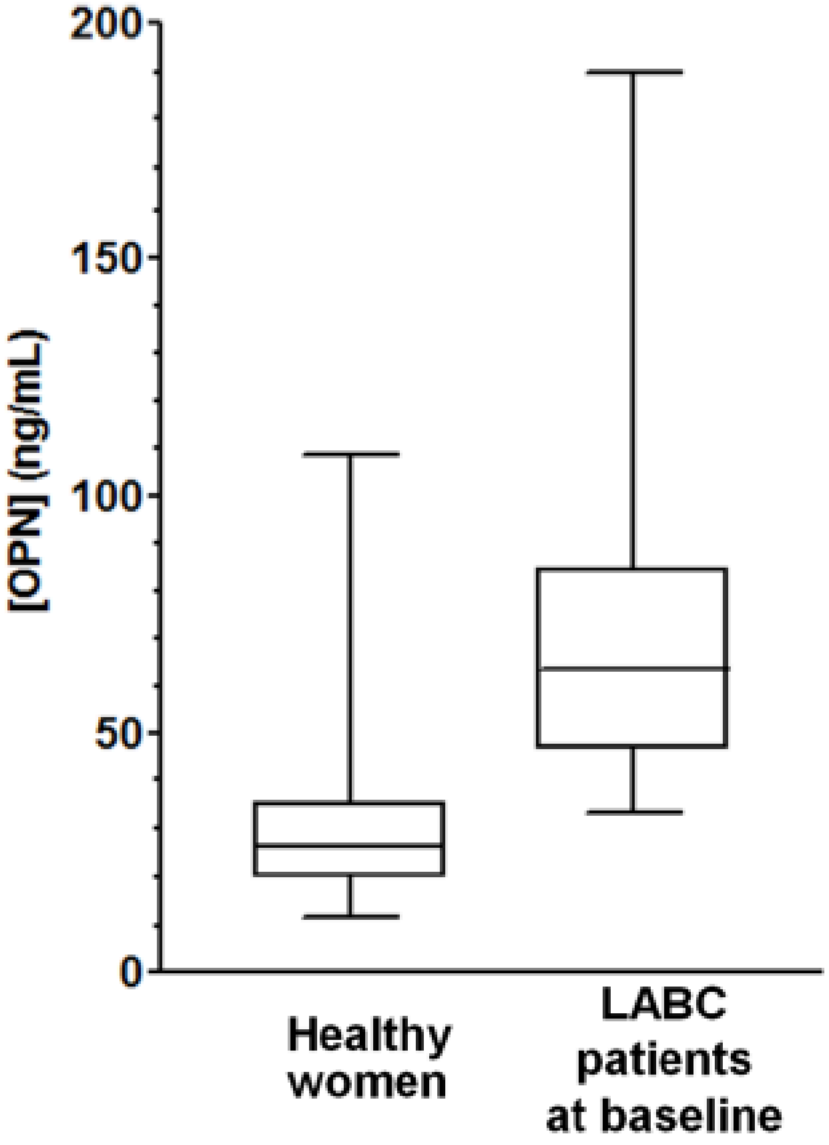

Figure 3.2. Plasma OPN levels at baseline/cycle 1 compared to OPN levels of 90 healthy women. The boxes show OPN value between the 25th and 75th percentiles, with whiskers showing ranges; the lines within the boxes mark the median values. OPN values from healthy women were as reported.

show no significant difference from baseline/cycle 1 through to cycle 5. Significant pairwise differences exist between the mean OPN levels of cycle 1 vs cycle 6 ($p<0.001$), and cycle 1 vs cycle 7 ($p<0.001$). Overall, all but three OPN values at baseline/cycle 1 as well as the majority of the LABC patient samples from cycles 2-8 have OPN values within the normal range (i.e. ≤108.6ng/ml) based on a prior study of 90 healthy women without cancer [26] (Figure 3.1).

3.3.1 Association of OPN Values with Overall Survival

To determine the association of OPN values with overall survival, patients were divided into 2 groups: OPN <63.6ng/ml versus OPN ≥63.6ng/ml, the median OPN value at baseline/cycle 1. By comparison, the majority (92%) of healthy women had OPN values <63.6ng/ml. The Kaplan-Meier survival curve indicates that patients with baseline/cycle 1 OPN <63.6ng/ml had a significantly improved overall survival than patients with baseline/cycle 1 OPN ≥63.6ng/ml (Figure 3.3). The log rank test showed that this difference was statistically significant (Chi-square=5.9; p=0.015; hazard ratio (HR) 3.3; 95% confidence interval (CI): 1.3 – 10.4). Cox regression analysis with OPN at baseline/cycle 1 as a continuous variable produced similar result (Chi-square=10.4; p=0.001).

3.3.2 Association of OPN Values with Response to Neoadjuvant Therapy

The majority of the female LABC patients (62% or 32 out of 52) had a PR to neoadjuvant therapy; eleven patients (21%) had a CR, four patients (6%) had PD and four individuals (8%) had NR to treatment. Plasma OPN levels at

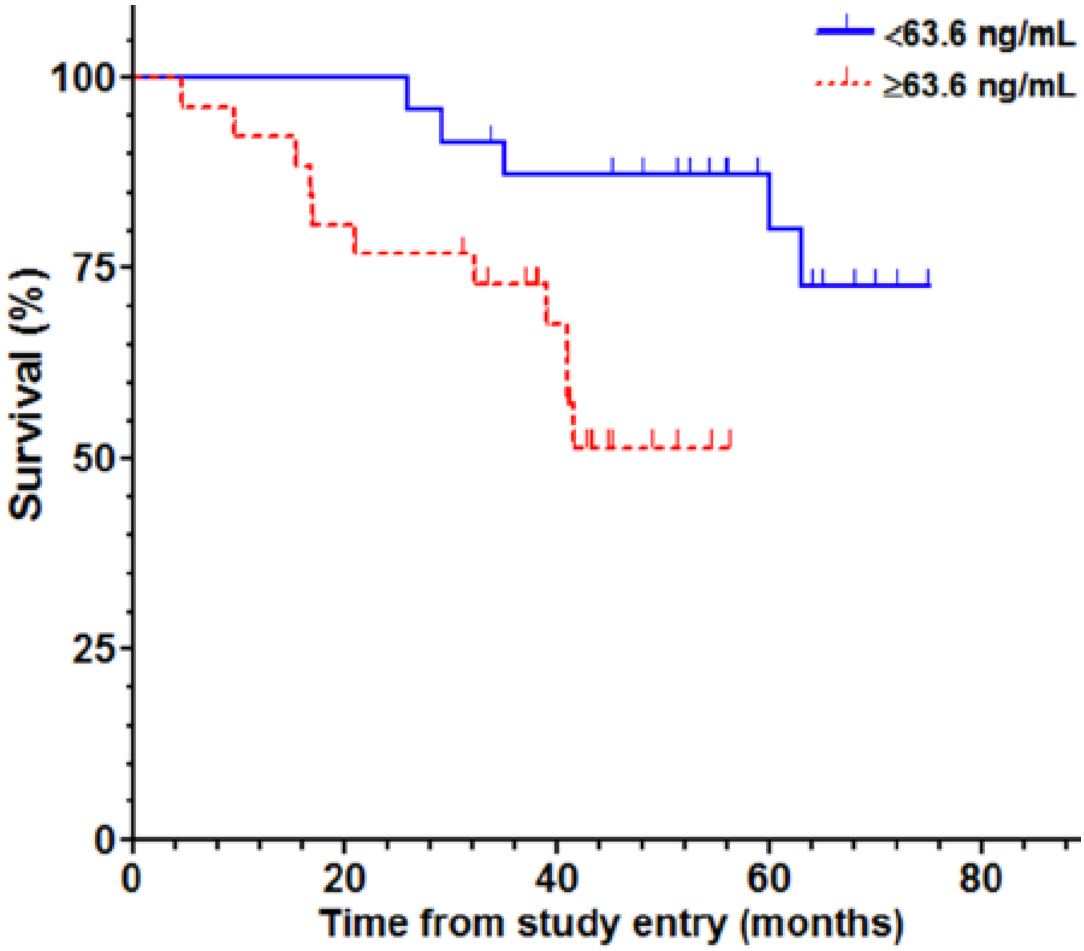

Figure 3.3. Association of plasma OPN levels at baseline with LABC patient survival. Kaplan-Meier survival curves were constructed after dividing the patients into two groups according to the median OPN value at baseline/cycle 1 (63.6ng/ml) (log-rank test, p=0.011).

baseline were compared with the final pathologic response to neoadjuvant chemotherapy. Mean OPN value at baseline/cycle 1 for patients with CR was 60.3ng/ml, while for patients with PD or NR, mean OPN value was higher at 75.1ng/ml (Figure 3.4) however this difference was not statistically significant (p=0.054; two-tailed). Six out of seven (86%) patients with PD or NR had OPN values ≥63.6ng/ml at baseline/cycle 1 (for patient 18 with PD, no baseline/cycle1 value was obtained), while 4 out of 11 (19%) patients with CR or PR had OPN values ≥63.6ng/ml at baseline/cycle 1 (p=0.066).

3.4 DISCUSSION

Tumour response to neoadjuvant treatment is an important predictor of prognosis and overall survival for the LABC patient population. In this current study, measuring serial plasma OPN levels was evaluated as a novel method for monitoring tumour response to neoadjuvant chemotherapy of LABC patients. The majority (83%) of the patients treated in this study had pCR or PR, of which pCR is believed to be the most important current prognostic marker for survival.

Our results show that during the course of neoadjuvant treatment, as the patients receive additional cycles of chemotherapy, a statistically significant increase is seen in OPN levels at later cycles (6, 7 and 8) compared to earlier cycles (1–5). Cytotoxic chemotherapies that are used for cancer treatment can stimulate the immune system. Up-regulation of OPN expression in immune cells allows for increased macrophage adhesion, migration, cytokine release and

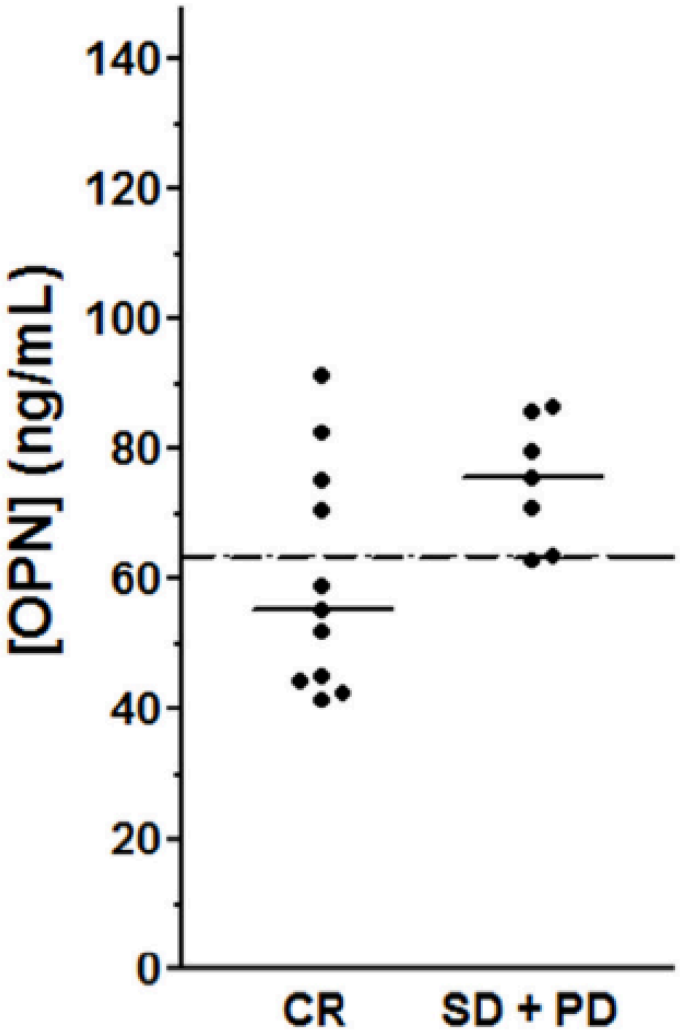

Figure 3.4. OPN levels at baseline for complete responders and non-responders to neoadjuvant treatment. Lines indicate median OPN value for eleven patients with pCR (55.5ng/ml) and median OPN value for seven patients with NR or PD (75.8ng/ml) (p=0.054). Dashed line indicates the median OPN value at baseline (63.6ng/ml) of the entire group of patients.

phagocytosis, all of which are important events of the immune and inflammatory response [29, 30]. Therefore, it is possible that the increased plasma OPN levels observed in patients over the course of neoadjuvant chemotherapy is from two different sources: OPN expressed by immune cells as a component of the inflammatory response, and that secreted by the primary tumour itself [14, 19, 26]. Further work is required in order to determine the exact source of plasma OPN detected during treatment.

We have recently reported that, in a group of 90 healthy women, plasma OPN levels ranged from 11.8-108.6ng/ml [26]. Based on this, a level of 108.6ng/ml has been used as the upper limit of normal in the present study and OPN levels above this value were considered elevated. We have previously shown that in a cohort of 70 patients with metastatic breast cancer, 70% had elevated levels of OPN [23] and patients with increased plasma OPN levels had significantly shorter survival times. In line with this, Bramwell et al. [25] reported that in a larger cohort of women with metastatic breast cancer, 66% had elevated baseline OPN levels that were inversely and significantly associated with survival. In addition, serial monitoring of OPN levels revealed that an increase of >250ng/ml at any time was the most prognostic variable for poor survival. This association of increasing OPN levels over time with poor prognosis supports the use of serial monitoring of OPN levels in order to help make treatment decisions by determining response. In contrast, in a cohort of postmenopausal women with early breast cancer, only 4 women out of 314 (1.2%) had elevated OPN (>108.6ng/ml) at baseline. That study found a mean baseline plasma OPN level

of 46ng/ml (range 22.6 – 290ng/ml), which did not differ statistically from normal levels [26] and found no evidence supporting a prognostic value of plasma OPN for that group of early breast cancer patients.

While plasma OPN levels have thus been reported to be elevated in women with metastatic disease but not with early breast cancer, levels for patients with locally advanced disease had not been previously studied. This current study showed that most LABC patients have plasma OPN levels that are not elevated above what had been previously considered the 'normal range' at baseline. However, mean OPN levels for LABC patients at baseline/cycle 1 were significantly higher than mean OPN level for the group of 90 healthy women.

When comparing baseline OPN levels to final pathologic response, patients who did not respond to treatment had a higher mean OPN level compared to the eleven patients who had pCR to treatment ($p=0.054$). The majority of non-responders also had OPN values ≥63.6ng/ml at baseline while the majority of responders had OPN values ≤63.6ng/ml at baseline ($p=0.066$). These differences, although not reaching statistical significance in this small number of patients, are clinically interesting as they may help identify which patients are likely to respond to neoadjuvant chemotherapy and are worthy of further study.

Baseline OPN levels were significantly associated with pathological response to treatment ($p=0.015$). Cox hazard ratio regression revealed that patients with OPN levels above 63.6ng/ml were significantly more likely to die of their disease (hazard ratio=0.3; 95% confidence interval 0.10-0.78; $p=0.01$), and

overall, baseline OPN level was significantly associated with survival (p=0.001). Therefore, OPN represents the first known predictive and prognostic plasma tumour biomarker for overall survival in both locally advanced and metastatic breast cancer patients.

In conclusion, LABC patients have a poor overall prognosis, due to their high risk of tumour recurrence and development of future metastases. The study reported here demonstrates a statistically significant difference in survival between patients using baseline plasma OPN level. Our results strongly support the need for prospective clinical trials to further validate the utility of measuring plasma OPN levels in LABC patients, and to determine its role in clinical decision-making regarding anticipated response to neoadjuvant chemotherapy.

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CHAPTER 4

Radiation-Induced Lung Injury after Concurrent Neoadjuvant Chemoradiotherapy for Locally Advanced Breast Cancer.

*A version of this chapter was published, with M Brackstone as a senior co-author: Chow TL, Louie AV, Palma DA, D'Souza DP, Perera F, Rodrigues GB, Warner A, Chambers AF**, Brackstone M**. Acta Oncologica 2014, vol. 53(5): 697–701. Reproduced with permission.*

CHAPTER 4: RADIATION-INDUCED LUNG INJURY AFTER CONCURRENT NEOADJUVANT CHEMORADIOTHERAPY FOR LOCALLY ADVANCED BREAST CANCER.

4.1 INTRODUCTION

Outcomes for women with locally advanced breast cancer (LABC) remain suboptimal, with five-year survival of approximately 50%, despite aggressive treatment with a combination of chemotherapy, radiotherapy and surgery, all delivered sequentially [1, 2]. Achieving a complete pathological response (pCR) to neoadjuvant therapy (defined as no residual cells in the breast or axilla) is a major prognostic factor, as women with a pCR have a significantly longer overall survival (OS) and disease free survival (DFS) than women without a pCR [3-6]. However, rates of pCR are low: a large meta-analysis demonstrated that 17% of women, on average, achieve a pCR after neoadjuvant treatment [4].

For other malignancies, such as head and neck cancers, lung cancer, and cervical cancer, concurrent chemoradiotherapy provides a survival advantage over sequential chemotherapy and radiation therapy [7-10]. Delivery of both modalities concurrently affords several potential advantages, including spatial co-operation, temporal co-operation, non-cross resistance, and radiosensitization [11]. In an attempt to improve the pCR rate for LABC, a phase II trial was launched, evaluating the efficacy of a regimen consisting neoadjuvant docetaxel concurrent with locoregional radiotherapy. At the recommendation of the data

safety monitoring committee, the trial closed early due to a higher-than-anticipated rate of symptomatic radiation pneumonitis (RP).

In breast cancer, rates of RP after radiotherapy are usually low, often <5%, with fatal RP being rare [12, 13]. In contrast, rates of RP after treatment of lung are higher, affecting up to 13-37% of patients [14, 15]. Although predictors of symptomatic RP and CT-based radiation-induced lung injury (RILI) (as measured by Hounsfield unit density changes) have been evaluated in lung cancer patients, and in breast cancer patients receiving radiotherapy alone [16-18], to our knowledge no similar data exists examining patients receiving concurrent chemoradiotherapy for breast cancer. Therefore, the goal of this study was to evaluate predictors of symptomatic RP and CT-based RILI in a unique cohort of breast cancer patients treated with concurrent neodajuvant chemoradiation therapy.

4.2 MATERIALS AND METHODS

4.2.1 Patient Description

From August 2009 to June 2011, thirty-two patients with biopsy-confirmed T3/T4 and/or N2/N3 LABC were accrued for this University of Western Ontario Research Ethics Board approved protocol. Patients with prior malignancies, systemic treatment within the last 5 years, or prior radiotherapy to the head, neck, breast or thorax, were excluded. Patients with the diagnosis of inflammatory breast cancer were also omitted.

4.2.2 Treatment Details

Neoadjuvant chemotherapy was a standard anthracycline-based regimen. It consisted of three cycles of intravenous 5-fluoruracil (500mg/m^2), epirubicin (100mg/m^2) and cyclophosphamide (500mg/m^2) administered every three weeks (FEC). This was followed by a period of concurrent chemoradiotherapy. Weekly IV docetaxel (35mg/m^2) was given over nine weeks, with daily external beam radiation therapy (intensity modulated radiation therapy or three-dimensional (3D)-conformal radiotherapy, calculated using a collapsed-cone algorithm for dose calculation) administered concurrently during the first six weeks. A dose of 45Gy in 25 fractions was given over 5 weeks, and a boost dose of either 5.4Gy in 3 fractions or 9Gy in 5 fractions was given during the sixth week if residual disease was present. Radiation treatment was delivered on megavoltage machines using 6MV energy or greater. Five weeks after the last dose of docetaxel, patients underwent a modified radical mastectomy (MRM).

4.2.3 Image Registration and Lung Density Measurements

This report examines symptomatic RP and CT-based RILI. Oncologic outcomes (pCR rates and survival) will be reported separately once the survival data matures. All trial patients were eligible for this sub-study of symptomatic RP and RILI. All 32 patients were scored for possible symptomatic RP using National Institute of Health Common Terminology Criteria for Adverse Events (CTCAE) v3.0 (CTCAE grade ≥2). The trial mandated that each patient received at least three CT scans. The first was done prior to FEC chemotherapy, the second

before the start of concurrent docetaxel-radiotherapy and the third was completed before surgery. Any additional CT scans were at the discretion of the treating oncologists. To assess CT-based RILI, 27 out of 32 patients had at least one follow-up CT scan available and were evaluable for that endpoint.

Radiotherapy treatment planning scans were overlaid onto their post-treatment CT scans in order to measure changes in lung density over time (Figure 4.1). The relationship between dose and lung density changes was assessed similarly to previous studies [17]. Briefly, isodose levels (5Gy, 10Gy, 20Gy, 30Gy, 40Gy) were converted into contours on the planning scan and were transferred from the planning CT scans onto follow up scans after coregistration of the scans (MIM Software 5.5, Ohio, USA). Deformable registration was attempted, but due to the substantial differences between pre-radiotherapy and post-radiotherapy scans which resulted in difficulty obtaining adequate registrations, non-deformable algorithms were used instead. Contours were then examined and manually adjusted if necessary. To assess changes in lung density over time, (HU) density changes in each 'dose band' (5-10Gy, 10-20Gy, 20-30Gy, 30-40Gy, >40Gy) were generated and compared among scans. Contralateral lung receiving <5Gy was considered unirradiated and used as a control to correct for baseline differences between scans.

4.2.4 Statistical Analysis

Descriptive statistics were generated for baseline patient, tumour and treatment characteristics. Univariable logistic regression analysis was performed

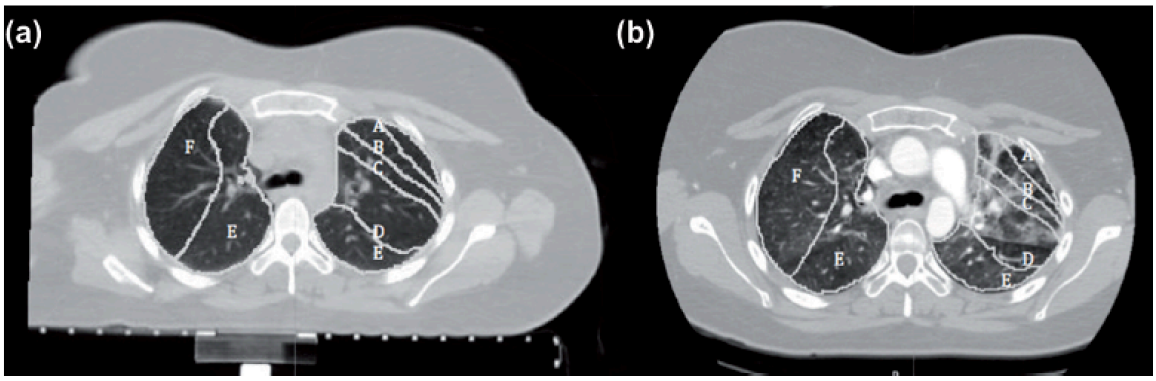

Figure 4.1. Representative example of image registration with overlaid isodose lines: (A) >40Gy, (B) 30-40Gy, (C) 20-30Gy, (D) 10-20Gy, (E) 5-10Gy, (F) <5Gy. (a) Planning CT scan with contoured isodose lines (b) Post-treatment follow-up CT scan with the contours from the planning scan overlaid via rigid registration.

for each available factor to identify predictors of symptomatic RP. T-tests and ANOVAs (Analysis of Variance) were used to identify significant differences in density change stratified by various combinations of: (a) RP grade (≥2 versus <2), (b) radiation dose (5-10, 10-20, 20-30, 30-40 and >40Gy), and (c) time (0-3, 3-6, 6-12 and >12 months). Linear mixed models were generated to examine relationships between radiological lung density changes (dependent variable), radiation dose (fixed effect), time (fixed effect), and other potential predictors (fixed effects). Statistical analysis was performed using SAS version 9.2 software (SAS Institute, Cary, NC, USA) with two-sided statistical testing at the 0.05 significance level.

4.3 RESULTS

Baseline characteristics for the 31 evaluable patients are reported in Table 4.1, and radiotherapy planning parameters are reported in Table 4.2. In total, 17 (53%) patients developed symptomatic RP (CTCAE v3.0 grade ≥2). Eight developed grade 3 pneumonitis requiring supportive oxygen, and one died of acute respiratory distress syndrome (ARDS) associated with RP (grade 5 toxicity). Univariable logistic regression of potential predictors of symptomatic RP is shown in Table 4.3. No treatment, patient, or tumour factors were significantly associated with symptomatic RP. Since all patients received concurrent chemotherapy, the effect of chemotherapy could not be assessed on logistic regression.

Table 4.1. Baseline tumour, patient and treatment characteristics of all patients (n=31).

Characteristic	All Patients (n=31)
Age – median (mean, max)	**49** (27, 64)
T stage – n (%)	
T1	**1** (3.2)
T2	**4** (12.9)
T3	**21** (67.7)
T4	**5** (16.1)
N stage – n (%)	
N0	**9** (29.0)
N1	**11** (35.5)
N2	**6** (19.4)
N3	**4** (12.9)
NX	**1** (3.2)
Smoking History – n (%)	**11** (35.5)
Left Ventricular Ejection Fraction (LVEF) (%) – median (min, max)	**64** (50, 77)
HER2/Neu status – n (%)	
Negative	**17** (54.8)
Positive	**8** (25.8)
Equivocal	**6** (19.4)
Total docetaxel dose received (mg) – median (min, max)	**522** (360, 666)
Received trastuzumab (Herceptin) – n (%)	**11** (35.5)
Radiation delivery – n (%)	
3D-CRT	**16** (51.6)
IMRT	**13** (41.9)
Tomotherapy	**2** (6.5)

Table 4.2. Dosimetric parameters.

Parameter	Median (mean, max)
Lung V5 (%)	31.2 (25.5, 97.0)
Lung V10 (%)	27.2 (20.9, 45.4)
Lung V13 (%)	23.7 (19.4, 34.8)
Lung V20 (%)	21.0 (10.5, 31.1)
Lung V30 (%)	17.7 (7.628.8)
Lung V40 (%)	11.7 (0.7, 24.1)
Mean Lung Dose (Gy)	10.3 (8.1, 15.7)
Heart V5 (%)	29.5 (3.6, 100)
Heart V10 (%)	22.5 (0.03, 91.8)
Heart V20 (%)	11.5 (0.0, 52.5)
Heart V30 (%)	6.0 (0.0, 49.2)
Heart V40 (%)	0.7 (0.0, 38.9)
Mean Heart Dose (Gy)	8.7 (1.6, 26.9)

Table 4.3. Univariable logistic regression models examining the relationship between individual predictors of pneumonitis grade ≥2 (n=31).

	Odds Ratio (95% CI)	p-Value	C-statistic
Age (per 10 unit increase)	1.00 (0.48, 2.08)	0.997	0.519
Age (ref: ≤45)			
>45–≤55	2.80 (0.46, 16.93)	0.262	0.609
>55	1.20 (0.22, 6.68)	0.835	
		**0.503	
T stage (ref: T1/T2)			
T3	0.61 (0.08, 4.41)	0.621	0.609
T4	2.67 (0.16, 45.14)	0.497	
		**0.446	
N stage (ref: N0)			
N1	0.96 (0.16, 5.64)	0.964	0.609
N2	1.60 (0.19, 13.70)	0.668	
N3	0.27 (0.02, 3.65)	0.322	
		**0.665	
Stage (ref: IIB)			
IIIA	1.25 (0.20, 7.96)	0.813	0.599
IIIB	2.00 (0.11, 35.81)	0.638	
IIIC	0.50 (0.03, 8.95)	0.638	
		**0.948	
Right (ref: Left)	1.60 (0.38, 6.82)	0.525	0.557
Multifocal disease (ref: No)	0.77 (0.15, 3.86)	0.750	0.525
Major ongoing medical problems (ref: No)	0.38 (0.03, 4.64)	0.445	0.542
Smoking history (ref: No)	0.98 (0.22, 4.31)	0.981	0.502
Left ventrical ejection fraction (LVEF) (per 10% increase)	0.30 (0.08, 1.20)	0.089	0.628
IMRT (ref: No)	1.60 (0.38, 6.82)	0.525	0.557
Her-2Neu Status (ref: Negative)			
Positive	0.89 (0.17, 4.78)	0.891	0.555
Equivocal	1.78 (0.25, 12.45)	0.562	
		**0.806	
Total docetaxel dose received (mg) (per 50 mg increase)	1.10 (0.64, 1.89)	0.731	0.496
Received trastuzumab (herceptin) (ref: No)	0.56 (0.13, 2.46)	0.438	0.567
Breast radiation dose (per 5 Gy increase)	0.84 (0.25, 2.80)	0.770	0.538
Bolus (ref: No)	0.48 (0.07, 3.40)	0.459	0.553
Boost (ref: No)	0.89 (0.22, 3.66)	0.871	0.515
Breast boost radiation dose (per 1 Gy increase)	1.23 (0.68, 2.22)	0.488	0.583
Lung V5 (per 10 unit increase)	0.77 (0.47, 1.26)	0.296	0.508
Lung V10 (per 10 unit increase)	0.75 (0.24, 2.34)	0.622	0.492
Lung V13 (per 10 unit increase)	0.97 (0.19, 4.99)	0.968	0.456
Lung V20 (per 10 unit increase)	2.55 (0.42, 15.33)	0.306	0.622
Lung V30 (per 10 unit increase)	2.10 (0.45, 9.74)	0.342	0.649
Lung V40 (per 10 unit increase)	2.15 (0.54, 8.54)	0.276	0.627
Mean lung dose (Gy) (per 5 Gy increase)	1.94 (0.21, 18.16)	0.560	0.544
Heart V5 (per 10 unit increase)	0.96 (0.74, 1.25)	0.764	0.569
Heart V10 (per 10 unit increase)	1.07 (0.76, 1.50)	0.701	0.506
Heart V20 (per 10 unit increase)	1.56 (0.80, 3.05)	0.195	0.570
Heart V30 (per 10 unit increase)	1.71 (0.72, 4.06)	0.227	0.595
Heart V40 (per 10 unit increase)	1.99 (0.54, 7.33)	0.303	0.582
Mean heart dose (per 5 Gy increase)	1.32 (0.68, 2.56)	0.416	0.502

** overall analysis of effects (applicable to categorical variables only)

In total, 79 follow-up CT scans for 27 patients were co-registered with baseline CT scans and analyzed for RILI, with a median of three follow-up CT scans per patient (range 1-6). Following analysis of the post-treatment scans, linear mixed modelling showed both radiation dose and time post-treatment to be highly predictive of CT RILI ($p<0.001$ and $p=0.021$, respectively). Overall density changes at low dose levels (<10Gy) were minor, but a marked increase evident with increasing dose, with regions receiving ≥20Gy exhibiting density increases of 100 HU or more (Figure 4.2). For both 6-12 months and >12 months post-treatment, significant differences in density change were observed across all dose bands (both $p<0.001$), with greater differences observed for higher dose bands compared to lower dose bands, respectively. This trend was also observed during the 3-6 month period, although was not found to be significant ($p=0.058$).

Patients with symptomatic RP were observed to have higher rates of density change across all dose levels (Figure 4.3), with significant differences observed in the low-dose (5-10Gy, $p=0.040$) and high-dose regions (>40Gy, $p=0.024$). Patients who developed RP also had significantly larger CT density changes than patients without RP at both 6-12 months ($p=0.002$) and >12 months ($p = 0.013$) post-treatments, suggesting a sustained effect transitioning to fibrosis.

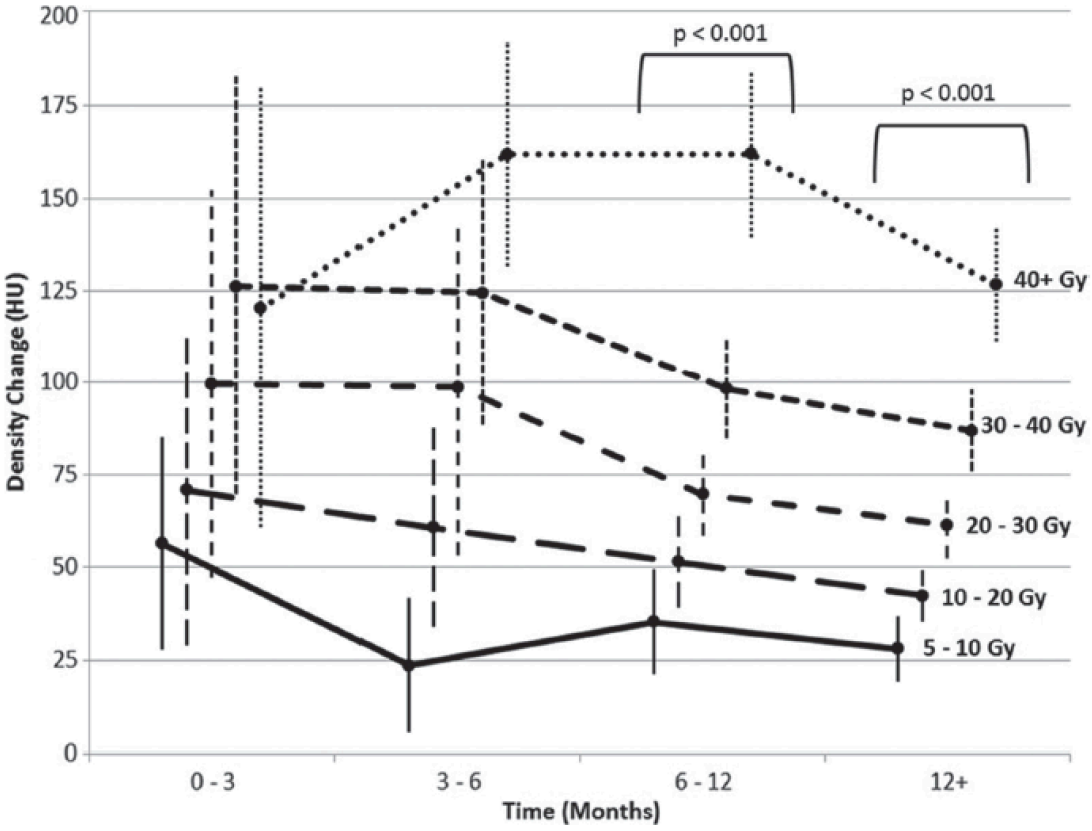

Figure 4.2. Estimated means (± standard error) for CT lung density changes (in Hounsfield Units (HU)) over time (months), stratified by radiation dose (Gy).

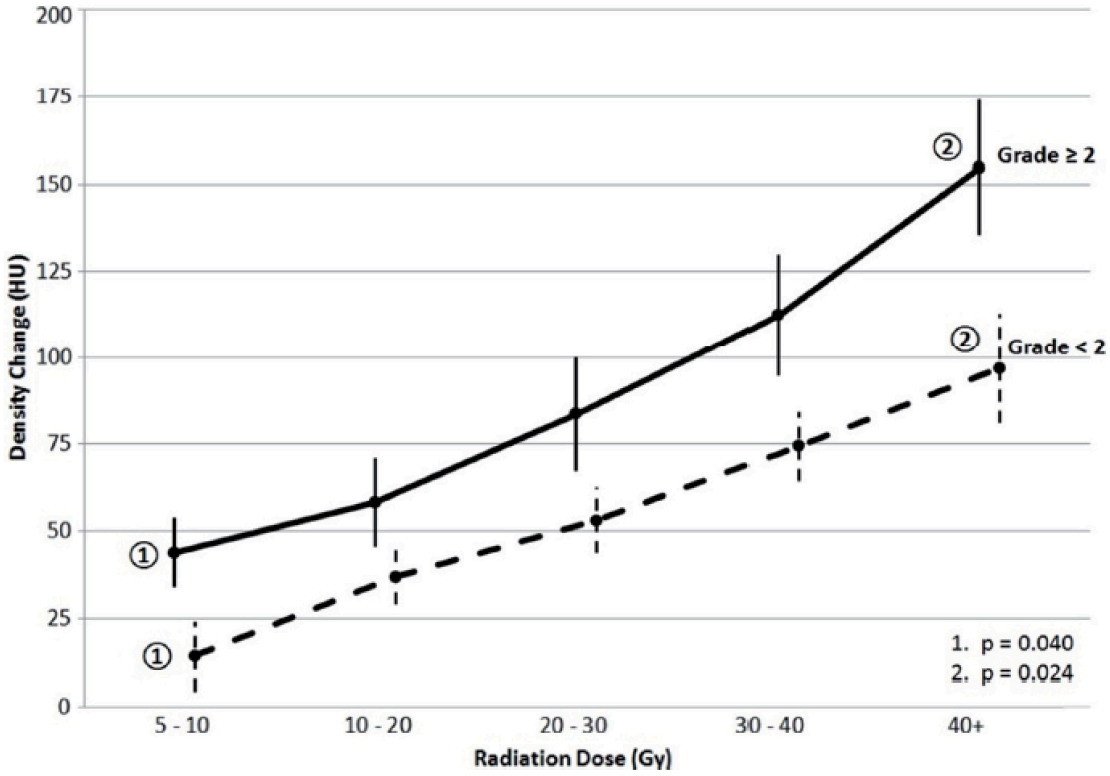

Figure 4.3. Estimated means (± standard error) for CT lung density changes (HU) relative to radiation dose (Gy), stratified by pneumonitis grade ≥2 versus <2.

4.4 DISCUSSION

In animal models, CT-based HU density changes after radiotherapy are strong surrogates of RILI, and correlate with histologic evidence of radiation injury, including the presence of inflammatory cells and infiltrative changes in the irradiated lung [19]. In this study of taxane-based concurrent chemoradiotherapy for LABC, more than half of patients developed symptomatic RP and one patient sustained a grade 5 toxicity. RILI was evident even in regions areas of lung receiving modest doses of radiotherapy, with a significant relationship evident between radiation dose, time post-treatment, and RILI.

The extent of CT-based RILI evident in this cohort appears to be higher than in patients who do not receive concurrent taxanes. In a cohort of 25 patients with stage III NSCLC where CT-based RILI was measured after cisplatin-based (non-taxane) chemotherapy with 60-66Gy of radiotherapy, there was no change in HU density within the first 3 months after treatment at any dose level. In that group, regions of lung receiving 40-50Gy did not show increases of more than 100 HU at any time in follow-up [18]. Similar results were seen in a mixed cohort of 118 patients, including breast cancer, lymphoma, and lung cancer patients treated with a variety of fractionations: relative to the lowest dose levels (0-5Gy), areas receiving modest doses of radiotherapy (<40Gy), showed very little RILI at 3-months, and relatively minor HU increases (<50 HU) with further follow-up. Although care must be taken in drawing conclusions from comparisons across studies, due to differences in baseline factors and data collection, these findings

suggest that the more profound, early HU increase seen herein (Figure 4.2) may be related to the radiosensitizing properties of concurrent taxanes.

When given concurrently with radiotherapy, taxanes are potent radiosensitizers [20-23], a property that this phase II study attempted to exploit to improve the pCR rate. Locoregional radiation for breast cancer is usually well tolerated, with only <5% of patients experiencing symptomatic RP, which is typically transient [12]. In a recent randomized trial of locoregional vs. local radiotherapy after lumpectomy for node-positive breast cancer, the rate of RP in the arm receiving locoregional radiotherapy was 2.3% [13]. Although the final data on oncologic outcomes, including pCR rate and survival, will be reported once the data matures, this interim analysis suggests that concurrent taxane-based chemoradiotherapy for breast cancer should be used with caution and only in the context of a controlled trial. The radiosensitizing properties of taxanes are recognized in the treatment of non-small cell lung cancer. For such patients, a recent individual patient data meta-analysis demonstrated that the use of taxane compared to non-taxane chemotherapy with radiotherapy was a significant predictor for developing pneumonitis ($p<0.001$) [14].

Since taxanes have become incorporated into routine oncologic use for node-positive breast cancer, the challenge of maximizing their benefits while minimizing toxicity, namely pneumonitis, has become complex. The synergistic interaction of taxanes and radiotherapy has shown promise with regards to disease-free and overall survival of patients with node positive breast cancer [24, 25], yet pneumonitis remains an important cause of morbidity. Limited studies

have been conducted for concurrent chemoradiotherapy to treat LABC, but a few small trials have shown promise and are the basis upon which we conducted our phase II trial [26, 27]. A retrospective review of 44 high-risk breast cancer patients demonstrated the feasibility of concurrent radiation delivered with either paclitaxel or docetaxel every three weeks [26]. Treatment was well tolerated with nine (20%) patients experiencing Grade 3 skin toxicity, with higher rates of toxicity associated with docetaxel, and no reports of pneumonitis. Another study was conducted on 44 LABC patients who received 30 mg/m^2 paclitaxel twice weekly with concurrent radiation prior to surgery [27]. An improved pCR was achieved, with acceptable toxicity and no cases of RP. These differences in RP may be related to choice of taxane (docetaxel vs. paclitaxel), their dosing, or the frequency of administration, and further research is needed to determine the optimal, safe parameters.

The findings of this study must be considered in the context of its strengths and limitations. The clinical data used herein was collected as part of a rigorous, phase II trial, but the analysis of CT-based RILI was an unplanned, retrospective analysis. The CT registration process is associated with some inherent imprecision [28], which we attempted to correct by manually inspecting and correcting isodose line contours. Some CT scans were done at the discretion of the treating oncologists, which may introduce unmeasured confounding factors. The small sample size resulted in limited power to detect predictors of RP, and the selected nature of the study population may affect the generalizability of our findings.

In conclusion, rates of RP after concurrent docetaxel-based chemoradiotherapy are higher than would be expected after breast radiotherapy. In this population, CT density changes of RILI occur earlier and appear to be more profound than in other studies measuring RILI after thoracic radiotherapy, suggesting a radiosensitizing effect of the docetaxel. Mature oncologic outcomes from this study are required to fully define the therapeutic ratio, but in the interim, concurrent taxane-based chemoradiotherapy should be used cautiously. Further study is needed to determine optimal, safe strategies for delivery of highly active chemotherapy with locoregional radiotherapy for patients with LABC.

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CHAPTER 5

General Discussion

CHAPTER 5: GENERAL DISCUSSION

5.1 OVERVIEW OF RESULTS

Locally advanced breast cancer (LABC) represents a challenging subgroup of advanced disease requiring more aggressive treatment than is currently being provided, given its low survival. Chapter 1 of this thesis represents an overall review of breast cancer and of the existing LABC treatment [1]. The current guidelines for LABC patients on for Cancer Care Ontario can be found in Appendix II [2]. Chapter 2 represents a clinical trial that was undertaken. Thirty-two LABC patients were treated with neoadjuvant FEC-weeklyD chemotherapy with concurrent locoregional radiation during docetaxel, reporting the clinical outcomes of this study. The full protocol for this clinical trial can be found in Appendix III. Chapter 3 represents a biological correlative study of the same clinical trial, evaluating plasma osteopontin (OPN) as a tumour biomarker and predictor of response to treatment or prognostic marker of survival [3]. Chapter 4 represents an evaluation of the radiation pneumonitis toxicity seen during this same clinical trial, which ultimately resulted in its premature termination [4]. In addition, other exploratory biological correlative substudies were undertaken, and the results are reported in Appendix IV. The findings of the studies reported in each chapter of this thesis are summarized separately below.

5.1.1 Neoadjuvant Chemoradiation Therapy in LABC

Thirty-two LABC patients were treated with neoadjuvant FEC q3 weekly x 3 cycles followed by weekly docetaxel x 9 cycles with locoregional radiation delivered during the first 6 of these weeks (45Gy in 25 fractions plus 5.4Gy in 3 or 9Gy in 5 fractions). All patients underwent a modified radical mastectomy five weeks after completing treatment. These patients were matched 1:3 to a concurrent control cohort of LABC patients treated with the standard treatment sequence (neoadjuvant chemotherapy regimen AC-D or FEC-D) using propensity matching, where 27 patients were successfully matched to 81 control patients for age, stage and molecular subtype.

Patients treated with the study regimen using concurrent chemoradiation were significantly more likely to have a pathological complete response to treatment (i.e. no residual invasive breast cancer in the breast or lymph nodes). There was a trend towards a 15% improvement in overall and disease-free survival that failed to reach statistical significance to due premature termination of the study following one treatment-related death.

Based on the results of the clinical trial reported in this thesis, and given the poor clinical outcomes in LABC, the improvement in treatment response with concurrent chemoradiation by exploiting the radiosensitization of taxanes should be further explored.

5.1.2 Osteopontin as a Tumour Marker

Osteopontin is a secreted, integrin-binding phosphoprotein found in several tissue types, including breast cancer. Plasma OPN levels have been demonstrated to be prognostic among metastatic breast cancer patients, where baseline levels at diagnosis were significantly elevated in comparison to normal healthy controls. There have been no studies to-date evaluating OPN levels among locally advanced, but not yet metastatic, breast cancer patients.

OPN levels were measured in 32 patients with LABC treated every three weeks during neoadjuvant q3 weekly FEC chemotherapy followed by weekly docetaxel concurrent with locoregional external beam radiation (see Chapter 3). These patients were added to 20 LABC patients treated with neoadjuvant anthracycline and cyclophosphamide q3 weekly x 4 cycles followed by docetaxel q3 weekly x 4 cycles, where OPN levels were also collected every 3 weeks. OPN levels were measured using ELISA. The serial OPN levels seemed to demonstrate a slight trend toward increasing over treatment, which did not appear to be related to clinical response to treatment. Baseline pre-treatment OPN levels were studied and contrasted to clinical outcomes, to evaluate whether plasma OPN levels predicted treatment response. Our study demonstrated that, when the patient population was dichotomized based on the median value of 63ng/mL, baseline elevated OPN level significantly predicted for overall survival, and showed a trend to predicting pathological response to treatment [3].

Thus, OPN appears to be a predictive marker for neoadjuvant treatment response in LABC, as well as a known prognostic marker for metastatic breast cancer. These findings should be validated in a larger prospective clinical trial.

5.1.3 Toxicity of the Neoadjuvant Chemoradiation Therapy

Although chemosensitization by docetaxel resulted in an increased pCR and a trend toward improved disease-free and overall survival, it was not without an increase in toxicity (see Chapter 4). Eight of the 32 patients experienced grade 3 pneumonitis following docetaxel and radiation, most commonly presenting as shortness of breath at 1-2 weeks after the completion of radiation. One patient, who was a long-time smoker, was the only patient to require transient oxygen therapy. All were treated with a tapering regimen of high dose steroids (see study protocol, Appendix III), and in all but one of these cases, patients were clinically resolved by the time surgery was done 5 weeks later. The prior smoker remained with grade 1 shortness of breath for several months after surgery. One patient experienced acute respiratory distress syndrome after having been treated bilaterally with radiation using intensity-modulated radiation therapy for bilateral locally advanced breast cancer. As a result of this death, the study's independent data safety monitoring committee recommended premature termination of the study.

Radiation dosage and time to radiation were found to be predictive of radiation-induced lung injury. Serial CT lung images of patients with pneumonitis were contrasted to the radiation planning CT images for the 27 of 32 patients

who had follow-up CT scans of the lungs after radiation. Lung tissue density was found to increase with increasing radiation dosage, time and degree of pneumonitis [4].

Radiation density was found to be higher than expected from radiation alone, and therefore it is possible that docetaxel itself resulted in chemical pneumonitis secondary to its known capillary leak syndrome risk. Prior studies evaluating the use of another taxane, paclitaxel, with concurrent radiation showed very low rates of clinical pneumonitis [5, 6]. If this concurrent radiosensitizing chemotherapy regimen is considered for future trials, it is recommended that paclitaxel, rather than docetaxel, be utilized in order to avoid this toxicity while retaining its beneficial effects on improvement of pCR.

5.2 LIMITATIONS AND FUTURE DIRECTIONS

The clinical trial reported in this thesis was a prospective single arm Phase II trial, and as such, it is prone to limitations common to all studies that are not randomized controlled. Despite every effort being made to independently match the patients to concurrently treated control patients by variables that could impact on pCR or survival, it remains possible that other patient, tumour or treatment factors were not adjusted between the two groups, and therefore impacted on the differences in pCR rates, disease-free and overall survival rates seen. This finding should therefore be validated in a prospective randomized controlled trial.

More needs to be understood about the relationship between regional radiation therapy and resultant improved overall survival. An older but eloquent

simple study done by Wallgren and colleagues in 1978 [7] randomized patients with breast cancer to preoperative versus postoperative radiation. These patients all received the same radiation modality, all had modified radical mastectomies and did not receive any hormonal therapy or chemotherapy. Consequently, any impact on overall survival can be attributed to the random assignment of radiation timing. Patients who received preoperative radiation had a significantly improved survival compared with postoperatively treated patients. More recent work in the exploding field of immuno-oncology has suggested that radiation may prime immunogenic cell death, likely by a number of mechanisms related to antigenic T-cell activation [8]. Clearly, more research needs to be done, but further work should focus on exploration of the tumour antigen priming effects of radiation when delivered preoperatively in breast cancer patients. Future trials involving preoperative radiation should all include the collection of serial tumour samples, in order to evaluate this potential complex immunogenic effect of radiation.

Given the toxicity seen with docetaxel, specifically resulting in pneumonitis rates higher than expected from radiation alone or from other taxanes, such as paclitaxel concurrent with radiation, future randomized trials should be considered with paclitaxel using the dosage and schedule evaluated by Dr. Formenti and colleagues [5]. It has only been studied in a sandwich regimen with neoadjuvant paclitaxel twice weekly with concurrent locoregional radiation followed by surgery followed by anthracycline-based chemotherapy. Therefore, a future trial should be undertaken to exploit chemosensitizing radiation impacts on

clinical response to treatment with a novel regimen using paclitaxel, such as anthracycline and cyclophosphamide q2-3 weekly x 4 cycles followed by twice weekly paclitaxel x 9 weeks with locoregional radiation during the first 6 of those weeks. Patients should be randomized to this novel regimen versus the same regimen delivered in the adjuvant setting. This could be followed by breast conserving surgery for patients experiencing a good response to treatment, or mastectomy for those with a less favourable response to treatment.

At the time that this clinical trial was undertaken, there were significant shortages in cancer funding to hospitals, and chemotherapy chair time was at a premium. It was, therefore, not feasible to propose a clinical trial where patients who were currently receiving chemotherapy once every 3 weeks would instead be coming for chemotherapy twice a week. Since that time, weekly paclitaxel and weekly docetaxel regimens have become much more common. As a result, it is much less of a stretch to propose a twice-weekly regimen to patients who would otherwise be treated weekly during the taxane component of chemotherapy.

5.3 CONCLUSION

A phase II clinical trial was undertaken in LABC patients treated with a novel neoadjuvant chemotherapy regimen, where docetaxel was given weekly and concurrently with daily locoregional radiation. When compared to standard treatment, chemoradiation significantly improved pCR and appeared to show a trend in improved disease-free and overall survival. This needs to be exploited in

future randomized clinical trials using paclitaxel, to avoid an elevated risk of pneumonitis. Plasma OPN as a prognostic tumour biomarker can then also be validated in this future proposed trial.

5.4 REFERENCES

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APPENDICES

APPENDIX I. RESEARCH ETHICS BOARD APPROVAL

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Full Board Initial Approval Notice

Principal Investigator: Dr. Muriel Brackstone
Department & Institution: Schulich School of Medicine and Dentistry\Oncology,St. Joseph's Health Care London

HSREB File Number: 105643
Study Title: A PHASE II TRIAL TO EVALUATE SINGLE DOSE STEREOTACTIC RADIATION THERAPY (SBRT) PRIOR TO SURGERY FOR EARLY STAGE BREAST CARCINOMA: SIGNAL (Stereotactic Image-Guided Neoadjuvant Ablative radiation then Lumpectomy) TRIAL
Sponsor:

HSREB Initial Approval Date: October 15, 2014
HSREB Expiry Date: December 31, 2023

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Instruments	Modified Harvard-Harris Cosmetic Scale	2014/08/06
Instruments	FACT-B questionnaire	2007/11/16
Sponsor Protocol	Received for information only.	2014/08/05
Sub-Study Letter of Information & Consent		2014/09/21
Revised Letter of Information & Consent		2014/09/21
Revised Western University Protocol		2014/09/23

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review. If an Updated Approval Notice is required prior to the HSREB Expiry Date, the Principal Investigator is responsible for completing and submitting an HSREB Updated Approval Form in a timely fashion.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

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APPENDIX II

Locoregional Therapy of Locally Advanced Breast Cancer: Guideline Recommendations

A version of this chapter was published, with M. Brackstone as the first author: Brackstone M, Fletcher GG, Dayes IS, Madarnas Y, SenGupta SK, Verma S and Members of Breast Cancer Disease Site Group. Curr Oncol 2015, vol. 22(Suppl1): S54 –66. Reproduced with permission.

APPENDIX II: LOCOREGIONAL THERAPY OF LOCALLY ADVANCED BREAST CANCER: GUIDELINE RECOMMENDATIONS

II.1 INTRODUCTION

This guideline addresses several questions related LABC as defined previously. In early breast cancer, breast-conserving surgery (BCS) with adjuvant radiotherapy (RT) has been found equivalent to mastectomy (in patients meeting BCS selection criteria) for long-term outcomes and it is preferred by many patients for cosmetic and psychological reasons. The applicability of BCS to LABC and the use and extent of RT after mastectomy is still a matter of debate. Historically, LABC has had poor outcomes. Although neoadjuvant (preoperative, induction) therapy was first introduced in an attempt to improve tumour resectability and overall survival (OS) rate with early adjuvant treatment, improved OS was not realized [1-5]. However, other clinically important outcomes were observed, including disease downstaging and feasibility of breast conservation in select cases, which form the basis for continued use of this approach. Furthermore, neoadjuvant chemotherapy (NACT) also allows an in vivo assessment of chemosensitivity, potentially allowing a regimen change that would not otherwise be made with traditional postoperative adjuvant treatment. Finally, NACT provides a platform for important biomarker and correlative studies to enhance our understanding of this disease.

Although BCS becomes technically feasible in some patients with LABC with good response to NACT, there is uncertainty as to whether mastectomy or BCS is most appropriate. Conversely, optimal treatment when LABC does not respond to initial NACT is unclear. Sentinel lymph node biopsy (SLNB) is used in early breast cancer as an alternative to full axillary lymph node dissection (ALND). The role of SLNB compared with ALND in patients with LABC receiving NACT has not been established.

NACT has expanded beyond classically unresectable LABC and it is being used more frequently for some smaller tumours, especially certain biologic subtypes (e.g., triple negative, HER2+). Although this document does not evaluate effectiveness of NACT, its expanded use means that clinical trials often cover a heterogeneous patient population (see Section III.1.2 Target Population).

II.2 METHODS

II.2.1 Guideline Development

The evidence-based guideline series developed by Cancer Care Ontario's (CCO's) Program in Evidence-Based Care (PEBC) use the methods of the practice guidelines development cycle. The core methodology used to develop the evidentiary base for the present project was the systematic review. The resulting evidence underpins the recommendations developed by the working group and the Breast Cancer Disease Site Group (DSG). The systematic review and companion recommendations are intended to promote evidence-based

practice in Ontario. The full three-part evidence series can be found on the Cancer Care Ontario (CCO) Web site.

II.2.2 Questions

1. In female patients with locally advanced breast cancer with good response to neoadjuvant therapy, what is the role of breast-conserving surgery (BCS) compared with mastectomy?
2. In female patients with LABC,
    a. is radiotherapy indicated for those who had mastectomy?
    b. does locoregional irradiation result in higher survival and lower recurrence rates compared with breast/chest wall irradiation alone?
    c. is RT indicated for those achieving pathological complete response (pCR) to NACT?
3. In female patients with locally advanced breast cancer who receive neoadjuvant chemotherapy is sentinel lymph node biopsy (SLNB) or axillary dissection the most appropriate axillary staging procedure? Is SLNB indicated before neoadjuvant chemotherapy rather than at the time of surgery?
4. How should female patients with locally advanced breast cancer who do not respond to initial neoadjuvant therapy be treated?

II.2.3 Target Population

This guideline is pertinent to female patients with locally advanced breast cancer (LABC). For purposes of this guideline, LABC includes Stages IIB and IIIABC and inflammatory cancer, as defined in *the AJCC Cancer Staging Manual, 6th edition* [6]. Most studies in the evidentiary base (see Section 2) included heterogeneous populations spanning Stages IIB – IIIC and sometimes included inflammatory breast cancer. Very few studies dealt only with Stage III or specific subgroups such as patients with T3N0 cancer. As most of the major studies did not report results separately for patients with Stage IIB and Stage III cancers, the evidence did not support recommendations based on a narrower definition of LABC or subdivided by stage. Although some people do not consider Stage IIB to be locally advanced, there is an increasing trend to treat less bulky disease (Stage IIB) in a similar manner, including neoadjuvant therapy; therefore, the recommendations may also be applicable to this group.

II.2.3.1 Intended Users

The intended users are **s**urgeons and medical and radiation oncologists specializing in breast cancer.

II.2.4 Literature Search

The full search strategy and inclusion criteria are presented in the systematic review (Brackstone et al, 2014); only a brief summary is provided here. The literature in the medline and embase databases (1996 to December 11,

2013) and the Cochrane Library was searched for relevant studies. Searches of the Web sites of Canadian and international health organizations were also conducted to identify existing clinical practice guidelines, systematic reviews, and health technology assessments relevant to the guideline questions. All studies identified through the literature search were assessed against the selection criteria by a health research methodologist from the working group (GGF), with Cindy Walker–Dilks screening results from preliminary searches. Studies of uncertain eligibility were discussed with the other authors.

The literature search was designed to retrieve systematic reviews, meta-analyses, randomized control trials (RCTS), cohort studies, and clinical practice guidelines concerning locoregional therapy for LABC. Studies had to include at least 50 patients (except for question 4), have a prospective design, and provide a statistical comparison of the interventions of interest. Systematic reviews and meta-analyses had to include a description of the review methods (literature search, study selection, data extraction).

Randomized controlled trials were included if they addressed stages IIB and IIIABC disease (including inflammatory breast cancer), as were RCTS that addressed stage II (unspecified) and stage IIA disease, provided that stage I plus stage IIA disease constituted fewer than half the cases or that subgroup results for either or both of stages IIB and III were available. Studies in which the title and abstract indicated only “early breast cancer” with no mention of stage or other indication that patients meeting our definition of LABC might form all or part of the population were excluded. An exception was made for RCTs located

based on another publication about LABC (review, guideline, or RCT): in such cases, the Methods and Results of the original RCT publication were reviewed to determine whether the study group actually met our definition of LABC despite a title and abstract indicating otherwise. Studies in which the cancer was described as metastatic were excluded unless metastasis only to regional lymph nodes was mentioned. Randomized controlled studies were the preferred publications. Cohort studies were considered in the initial screening, but were included only if the comparison groups were equivalent—for example, they had a similar tumour stage distribution. Cohort studies were excluded if the patients were assigned to treatment based on patient and disease factors instead of randomly, such that the prognoses in the groups (before treatment) were not equivalent.

For question 2(b) about the extent of RT (whole breast or chest wall, or locoregional), studies were excluded if they focused on partial compared with whole-breast irradiation (for example, accelerated partial breast irradiation, brachytherapy, intensity-modulated radiation therapy) or on intraoperative techniques (for example, targeted intraoperative radiotherapy or intraoperative radiotherapy with electrons), or if they compared RT techniques (dose-density, boost, hypofractionation) or focused on simulation or treatment planning.

II.2.5. Development of Recommendations

The working group drafted recommendations based on the systematic review. Where evidence from RCTs was limited, recommendations were based on the authors' professional experience, together with a consideration of current

practice and recommendations in other guidelines. Such limitations are clearly indicated in the key evidence and qualifying statements that follow each recommendation.

II.2.6. Internal and External Review Process

Before submission of the draft report for external review, the systematic review and practice guideline were reviewed by the members of the Breast Cancer DSG and the PEBC Report Approval Panel (RAP). The latter group consists of the PEBC director and two other members with expertise in clinical and methodology issues. The DSG and RAP members reviewed the draft systematic review and practice guideline and provided feedback, which was incorporated into the guideline. The revised draft document was then distributed for external review. External review included both targeted peer review (intended to obtain direct feedback from a small number of content experts) and professional consultation (intended to facilitate dissemination of the guideline to Ontario practitioners and to provide opportunity for additional feedback). Results of those two sources of feedback can be found in the full guideline report on the CCO website (Brackstone et al, 2014).

II.3 RESULTS

After removal of duplicate citations, the searches in Medline and embase resulted in 42,138 publications. After application of the inclusion and exclusion

criteria, 143 publications of trials, 18 clinical practice guidelines, and 27 systematic reviews or meta-analyses remained. Most studies included a mix of cancer stages. The full systematic review (Brackstone et al. 2014) provides details of the methodologic characteristics and clinical outcomes of the included trials.

No studies meeting the inclusion criteria were located for question 1 (BCS vs. mastectomy after good response to NACT). Several RCTs dealt with question 2(a) (RT after mastectomy), with some studies including patients receiving anthracycline-based chemotherapy, but not taxanes. For question 2(b) (extent of RT), one prospective nonrandomized study [7] met the inclusion criteria. Three RCTs were relevant (two published only as abstracts), but they included both early cancer and LABC and therefore did not meet the threshold of 50% or more of the patients having stage IIB–III cancer. A large number of studies compared the technical feasibility of SLNB and ALND, but they did not compare long-term survival outcomes. Data for question 4 were also very limited.

II.4. DOCUMENT REVIEW PROCESS

II.4.1. Internal Review

During the internal review by DSG members (other than those of the working group), 16 approved the document, 1 had strong concerns about the inclusion of stage IIB in the guideline and did not approve, and 1 abstained because the document was outside his area of expertise. Most of the comments

received were related to the definition of LABC. Although 1 reviewer preferred that stage IIB be removed from the definition of LABC, the working group decided that it was neither feasible nor desirable to redo the evidence summary, because most studies reported a heterogeneous patient group, and few dealt specifically with stage III cancers. As suggested by 1 reviewer, we incorporated a footnote describing the rationale and limitations of the LABC definition into the text describing the target population, because those aspects are essential to the document and address some of the other comments.

There was concern that, in recommendation 1, modified radical mastectomy was said to be the standard of care for LABC (that is, for all patients with LABC) and that such treatment did not really apply to patients with stage IIB breast cancer. Although the working group did not feel it appropriate to list all situations in which bcs might be considered, recommendation 1 was modified to clarify that mastectomy does not apply to everyone and that the judgment of the surgeon—and patient preference—is required. A qualifying statement was also revised to clarify that evidence for BCS in LABC is weak overall, but that exceptions exist.

As a result of 2 comments, we included a qualifying statement for recommendation 1 indicating that the type of surgery offered (for example, skin-sparing mastectomy with immediate reconstruction) continues to evolve, but that such advancements are beyond the scope of the guideline.

A comment about question 4 suggested that some patient groups (for example, estrogen receptor–positive, lobular histology) do not respond as well to

chemotherapy. The working group believes that recommendation 4(b) (consider second-line chemotherapy, hormonal therapy if appropriate, RT, or immediate surgery) is sufficient. A separate guideline on lobular cancer could be useful, but addressing that variant in the current guideline is not feasible.

The RAP members had several suggestions that were addressed in the revised document. The key evidence and qualifying statements were edited to be less narrative and more succinct; the reader should review the evidence summary[8] (literature review) for more details. The description of the study selection criteria was reworded to be clearer to the reader. The Recommendations and Key Evidence and Literature Search sections were both revised to ensure that studies for question 2(b) are clearly understood to have been conducted in a broad group of patients with stages I–III cancer and not specifically LABC. Those studies do not meet the inclusion criteria of approximately 50% or more LABC cases in either the full study or a reported subgroup analysis; however, two studies were reported only as abstracts and might include subgroup data relevant to LABC when fully reported. Adverse effects had been included in the recommendations during the development process; additional details for some questions were added to the Discussion section of the systematic review.

II.4.2. External Review

Responses were received from 7 targeted peer reviewers (2 surgical oncologists, 3 radiation oncologists, 2 medical oncologists) considered to be

clinical experts on the topic of the guideline. The documents and a brief questionnaire were also distributed to professions in our database with an interest in breast cancer. During the latter professional consultation, 28 responses were received: 10 from medical oncologists, 4 from pathologists, 6 from radiation oncologists, 5 from surgeons, and 3 from surgical oncologists. Most reviewers considered the guideline to be of high quality and said that they would make use of it in their practice. Most comments were related to choice of wording or unclear phrasing, and revisions were made accordingly. Some reviewers wanted further or more specific recommendations, but available RCT data would not allow for that. Other queries related to items outside the scope of the questions and the literature review. Detailed comments and responses from the authors are reported in the full evidence document[8]

II.5 RECOMMENDATIONS

II.5.1 Preamble

Communication between physicians, surgeons, and pathologists is essential. A multidisciplinary case conference is the recommended forum for discussion of cases.

Any prior use of neoadjuvant therapy should be indicated when specimens are submitted for pathologic examination. Clinical details often affect the pathologic examination and interpretation, whereas details of pathology reports will determine appropriate treatment. Prior therapy (including neoadjuvant

therapy) can change the nature of the specimen and what should be reported. The experience of the authors is that use of neoadjuvant treatment is frequently not indicated when submitting specimens.

It is recommended that surgical clips marking the original (pretreatment) tumour location be inserted before administration of neoadjuvant therapy. Neoadjuvant therapy may result in change in the extent or distribution of tumour, or complete disappearance (clinically or pathologically complete response). The consensus reached at the Canadian Consortium for Locally Advanced Breast Cancer (COLAB) in 2011 [8] was that clips should be inserted at the time of diagnosis to mark tumour location and this should be considered the standard of care. Use of clips allows more accurate identification of the original tumour site (especially if there is complete response), resection of all (previously) cancerous tissue with adequate margins, pathologic diagnosis of the most appropriate area of specimens, and better accuracy of molecular analyses.

II.5.2 Recommendation 1

For most patients with LABC, modified radical mastectomy should be considered to be the standard of care.

BCS may be considered for some patients with non-inflammatory LABC on a case-by-case basis when the surgeon deems the disease can be fully resected and there is strong patient preference for breast preservation.

II.5.2.1 Key Evidence

No randomized controlled trials (RCTs) that directly compared BCS with mastectomy in patients with LABC were found in the literature review.

Evidence in early breast cancer is that BCS plus radiation is equivalent to mastectomy alone [9, 10]. There is a continuum in breast cancer stage, as opposed to a sharp cut-off between early and locally advanced (see Target Population). The Cancer Care Ontario/Program in Evidence-Based Care (CCO/PEBC) guideline [10] included all of Stage I and II, although the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) defined *early* as "breast cancer in which all clinically apparent disease can be removed surgically" [11]. Therefore, at least some cancers defined as LABC in the current guideline (e.g., Stage IIB) are covered in the recommendations of these other guidelines.

Guidelines by the American College of Radiology (ACR) [12], National Comprehensive Cancer Network (NCCN) [13], and the Consensus Conference on Neoadjuvant Chemotherapy in Carcinoma of the Breast [14] indicate BCS is appropriate for some patients with LABC after NACT. This may include small N2/N3 tumours with nodal response, or large (T3N0 or T3N1) tumours with good response. NCCN recommends patients initially Stage IIIABC (except T3N1) with good response be treated with mastectomy or consider lumpectomy (plus ALND plus radiotherapy). We endorse the criteria for BCS as outlined in the ACR [12] and Consensus Conference guidelines [14] and The International Expert Panel on Inflammatory Breast Cancer [15].

II.5.2.2 Qualifying Statements

Patients should be informed that for LABC as a whole the data are insufficient to recommend BCS as a rule; however, there may be some exceptions that can be considered on a case-by-case basis.

The extent of surgery, including BCS, should be determined after full discussion between the patient and the treating oncologist, taking into consideration the patient's values and the lack of direct evidence regarding the relative benefit of BCS versus mastectomy in this particular situation.

When considering between mastectomy and BCS (for those meeting selection criteria), benefits and harms must be weighed. BCS is considered to have generally better cosmetic effects, and for some female patients may have less impact on body image, self-esteem and sexuality than complete breast removal by mastectomy. With BCS there is usually no need for additional reconstructive surgery and the operation may be less complex. In some cases of BCS, there may be positive margins requiring re-excision. In cases of recurrence after BCS, further surgery may be needed, and some patients may wish to eliminate this possibility by having mastectomy as initial treatment.

Wide excision of the remaining tumour in the region of the original pre-neoadjuvant treatment tumour bed plus RT is recommended for patients with LABC who strongly desire BCS.

BCS is not advised in inflammatory breast cancer because the extent of tumour involvement cannot be reliably ascertained.

There is continuing evolution in the type of surgery offered (e.g., skin-sparing mastectomy with immediate reconstruction), but these are beyond the scope of this guideline.

II.5.3 Recommendation 2(a)

Radiotherapy following mastectomy is recommended for patients with LABC.

II.5.3.1 Key Evidence

The EBCTCG meta-analyses [16] found radiotherapy (RT) reduced recurrence rates and increased survival rates in high-risk patients (15-year breast cancer mortality rate 44.6% vs 49.5%, $p<0.00001$; overall mortality rate 51.4% vs 55.2%, $p=0.0002$).

In patients with node-positive breast cancer who had mastectomy plus axillary clearance (ALND) there was improvement in 5-year local recurrence risk (5.8% vs 22.8%, $p<0.00001$), 15-year breast cancer mortality risk (54.7% vs 60.1%, $p=0.0002$), and 15-year overall mortality rate (59.8% vs 64.2%, $p=0.0009$). There were significantly increased survival rates in patients with 1-3 positive nodes or ≥4 positive nodes, for all T groups, and for patients receiving systemic therapy.

The benefit of RT in reducing breast cancer recurrence and mortality rates appears to be offset by adverse effects in older trials (primarily cardiovascular and lung adverse effects) especially in female patients with low risk of recurrence.

The ratio of breast cancer mortality rate to other mortality rates was strongly affected by nodal status, age, and decade of follow-up. The absolute benefit still favoured RT overall, but not necessarily in subgroups with particularly low risk of recurrence. More recent reviews found that the effectiveness of RT is increased and cardiopulmonary adverse effects are greatly reduced with modern RT planning and technique; therefore, the non-cancer mortality rate data in the EBCTCG meta-analyses may not be relevant to current practice.

II.5.3.2 Qualifying Statements

The use of three-dimensional (3D) treatment planning is important to minimize the dose to the lung and heart to ensure improvements in breast-cancer-specific survival rates are not offset by non-breast cancer mortality rates. Treatments provided should conform to accepted standards with respect to tissue coverage and dose. Techniques such as gated RT or active breath-hold are used in some centres to reduce cardiotoxicity, although these were not evaluated in this guideline series.

Radiotherapy after BCS was not part of this review, however guidelines for early breast cancer recommend radiation following BCS [9, 10] and this is the current standard of care. In the absence of RCTs to the contrary, it is logical that radiation be used following BCS for LABC as well. Radiotherapy following BCS for LABC is the current standard of care.

The EBCTCG meta-analysis found RT improved recurrence and survival rates in the subgroup of patients with systemic treatment, and improved

recurrence rates (but without significant improvement in survival rate) in patients without systemic treatment. RT significantly improved the local recurrence rate in patients receiving anthracycline-based chemotherapy but there was no effect on survival rate. Several of the studies used older regimens such as cyclophosphamide + methotrexate + fluorouracil (CMF). Whelan et al [17] also found RT reduced mortality in patients with node-positive breast cancer who received systemic treatment. No studies were included in the systematic review using taxane-based chemotherapy. Newer chemotherapies and targeted therapies may reduce the absolute benefit of RT for some patients, although in the absence of RCTs, RT is still recommended.

Patients should be informed that improvements in recurrence and disease-specific survival rates have not necessarily translated into advantages in OS, possibly related to radiation-induced adverse effects in older studies. This may be especially relevant to patients with low risk of recurrence. RT reduced the recurrence rates in all groups reported, but the absolute benefit in patients with very low risk of recurrence due to disease characteristics and systemic therapy may be small, and some may consider the incremental benefit of RT, although statistically significant, to be clinically unimportant.

Lymphedema is more likely when surgery includes ALND or/and when RT includes the nodal areas. Decreased shoulder mobility, decreased strength, arm weakness, and paresthesia/hypesthesia have also been reported. The Bundesministrium für Forshung und Technologie (BMFT; German Breast-Cancer Study Group) 03 study [18] found that 25% of RT patients had acute skin

reactions, and 28% had long-term skin alterations (1-2 years after RT). Radiation pneumonitis has been reported in approximately 4% of patients [19, 20], although this increased to 23% (p=0.008) when RT and anthracycline chemotherapy were both used. In some older RT regimens there was a significant increase in contralateral breast cancer and non-cancer mortality rates, primarily from heart disease and lung cancer [16, 21]. Careful treatment planning is likely to reduce (but not eliminate) risks other than lymphedema and skin effects.

The benefit of post-mastectomy radiotherapy (PMRT) in patients with node-negative LABC (T3-4, N0) is less clear because they have not been reported in isolation. The fourth cycle of EBCTCG [16] revealed that patients with T3-4 cancer experienced a 5.7% reduction in mortality rate (70.1% vs 75.8%, p=0.20), whereas patients with node-negative cancer (primarily early cancer) had a 4.2% increase in mortality rate (42.4% vs 38.2%, p=0.0002). Patients with T3N0 cancer remain a group with limited data and should be discussed individually with regards to risks and benefits.

II.5.4 Recommendation 2(b)

It is recommended that patients with LABC receive locoregional radiation encompassing the breast/chest wall and local node-bearing areas following breast-conserving surgery or mastectomy.

II.5.4.1 Key Evidence

The recommendation for breast/chest wall irradiation is based on several RCTs as summarized in the EBCTCG meta-analyses [11, 16, 22-25] and is discussed in Question 2a.

A prospective nonrandomized study [26] in high-risk patients with Stage II-III breast cancer found improved disease-free survival (DFS) rates at median 77 months follow-up (73% with internal mammary (IM) node RT vs 52% without, p=0.02), whereas OS was 78% vs 64%, p=0.08. Subgroups at higher risk of recurrence may have greater benefit, as has been reported for patients with positive nodes.

A meta-analysis of the role of RT to regional nodes included three trials (two abstracts and one full publication) in patients with early/LABC [27] and concluded that regional RT to IM and medial supraclavicular (MS) nodes improves DFS, OS, and distant metastasis-free survival (DMFS) in Stage I-III breast cancer. This analysis did not meet our inclusion criteria because only approximately 36% of patients had LABC; therefore, the results need to be confirmed when the trials are fully published including subgroup data.

The recommendation to include local node-bearing areas is consistent with current practice and other clinical practice guidelines. The NCCN guideline [13] recommends that if IM lymph nodes are clinically or pathologically positive, RT should be administered to the IM nodes; otherwise, treatment to the IM nodes should be strongly considered in patients with node-positive and T3N0 cancer. NCCN also states that RT to the infraclavicular region and supraclavicular area is

recommended for patients with ≥4 positive nodes and should be strongly considered if 1-3 nodes are positive, and considered for patients with T3N0 cancer (especially if inadequate axillary evaluation or extensive lymphovascular invasion).

The American College of Radiology [28] recommends PMRT for T1-2N2+ and T3-4N+, usually including ipsilateral supraclavicular fossa for patients with positive nodes. There is more variation for IM nodes, but IM RT is considered for patients at risk of IM involvement such as those with medial or centrally located tumours and positive axillary lymph nodes. PMRT treatment of T1-2N1 and T3NO is controversial and should be individualized.

II.5.4.2 Qualifying Statements

Locoregional treatment (compared with breast/chest wall alone) increases the risk for cardiovascular/pulmonary adverse effects. The additional fields are more technically complex to administer. The use of 3D treatment planning is important to minimize the dose to the lung and heart to ensure improvements in breast-cancer-specific survival are not offset by non-breast cancer mortality.

The risk of long-term adverse effects from locoregional radiation should be weighed against the potential benefits in patients with lower-risk disease, particularly those with left-sided tumours. Ideally, such patients should be discussed in a multidisciplinary setting.

In light of the incomplete data, any recommendations regarding the role of extended radiation in LABC are significantly limited. Although some studies

attempted to isolate the role of irradiation to the IM nodes [29, 30], others included additional radiation to the MS nodes [31-33] or all locoregional nodes [34, 35].

The additional benefit of regional nodal RT is small, but significant for the overall patient groups studied in RCTs (early cancers plus LABC combined).

The incidence and/or severity of lymphedema is higher with locoregional RT. Especially in patients with lower-risk disease, the risk of long-term adverse effects from locoregional radiation should be weighed against the potential benefit of reduced recurrence rates and increased survival rates.

Patients with T3N0 cancer (verified to be N0 pre- and post-neoadjuvant therapy) remain a group with limited data and should be discussed individually with regards to risks and benefits. An updated EBCTCG analysis on mastectomy patients [36] was published in March 2014 (after the literature review). A comparison of the effect of RT in female patients with node-negative cancer who had axillary sampling or ALND found RT significantly reduced recurrence only in those with axillary sampling. Patients with ALND had significantly worse overall mortality with RT than without (RR=1.23, p=0.03), whereas in patients with axillary sampling RT had no significant effect (RR=1.00, p>0.1). Although this does not separate the effect of locoregional from chest wall RT, it suggests that RT to the axilla is necessary when there is not full ALND.

II.5.5 Recommendation 2(c)

It is recommended that postoperative radiotherapy remains the standard of care for patients with LABC who have pathologically complete response to neoadjuvant therapy.

II.5.5.1 Qualifying Statements

No prospective randomized studies were found in the literature review (see Section 2) that compared treatment with vs without RT in female patients with pathologically complete response (pCR) to neoadjuvant therapy. The consensus of the authors is that postoperative RT should therefore remain the standard of care.

When examining the evidence, it is important for the clinician to be aware of the various definitions for pCR that have been used in clinical studies. These range from no microscopic evidence of viable tumour cells, only residual necrotic or nonviable tumour cells, or only residual intraductal tumour cells in the resected specimen. The MD Anderson Cancer Center requires the added disappearance of axillary lymph node metastasis for a pCR.

Randomized trials such as those planned by the Athena Breast Cancer Network [37, 38] and the NSABP B51/RTOG 1304 trial may provide data to re-evaluate the recommendation for specific subgroups in the future.

II.5.6 Recommendation 3(a)

It is recommended that axillary dissection remain the standard of care for axillary staging in LABC, with the judicious use of SLNB in patients who are advised of the limitations of current data.

II.5.6.1 Key Evidence

The median sentinel lymph node (SLN) identification rate (SLN ID rate) was 93% in patients with cN0 cancer and 85% in patients with clinically positive nodes. SLN ID rates depend on the experience of surgeons and the techniques used.

The ACOSOG Z1071 trial [39, 40] conducted with patients with positive nodes (>85% LABC) is one of the largest and most recent studies. It found a 93% SLN ID rate for cN1 cancer and 89% for cN2 cancer. The false negative (FN) rate is not dissimilar to the recommended FN rates for early breast cancer surgery [41].

Although the studies indicate that SLNB is technically feasible in both early and locally advanced breast cancer, a small percentage of patients will be understaged using SLNB alone. This risk needs to be weighed against the increased adverse effects of ALND.

This recommendation is based on the authors' valuing potentially increased survival rates with use of ALND over increased postoperative complications. Given the results of the Z0011 and EBCTCG studies for early or

operable cancers, some patients may decide that for less advanced LABC (e.g.. Stages 2b-3a) the adverse effects of ALND are greater than the benefits.

II.5.6.2 Qualifying Statements

Although the SLNB technique in patients (mostly with LABC) receiving NACT is comparable to that in early breast cancer, the clinical implications of a FN SLNB is not known in these patients.

The benefit of ALND is that more nodes are removed and examined, giving more accurate staging for some patients. Provided that locoregional RT is to be administered in all patients, as recommended in Questions 2a and 2b, the staging may have no impact on treatment. However, some patients may value the additional prognostic information. If a patient is not going to receive locoregional RT, then ALND is recommended.

There may be a secondary treatment benefit of ALND in that involved nodes are removed and, therefore, will not metastasize further.

More than 80% of female patients undergoing ALND have at least one postoperative complication in the arm and psychological distress is common [42]. In the Z0011 trial [43, 44] ALND added to SLNB resulted in more wound infections, axillary seromas, paresthesias, and subjective reports of lymphedema than SLNB alone.

The NCCN guideline [13] (not specifically on NACT) indicates “in the absence of definitive data demonstrating superior survival [with axillary lymph node staging], the performance of ALND may be considered optional in patients

who have particularly favourable tumours, patients for whom the selection of adjuvant systemic therapy is unlikely to be affected, for the elderly, or those with serious comorbid conditions". They recommend that cN0 plus SLN negative (including T3N0) need no further ALND. However, the authors of the current guideline note that most patients with LABC are pathologically node positive before neoadjuvant therapy, even those considered clinically negative; therefore, a high portion may still be pathologically node positive after neoadjuvant therapy.

None of the studies included inflammatory breast cancer; therefore, these findings cannot be extrapolated to that cohort of patients.

II.5.7. Recommendation 3(b)

Although SLNB before or after NACT is technically feasible, there is insufficient data to make any recommendation regarding the optimal timing of SLNB with respect to NACT. Limited data suggests higher SLN ID rates and lower FN rates when SLNB is conducted before NACT; however, this must be balanced against the requirement for two operations if SLNB is not performed at the time of resection of the main tumour.

II.5.7.1 Key Evidence

Only three of the studies in Table 6 of the evidence summary [45-47] compared timing of SLNB (before or after NACT) and one additional study (abstract only) performed SLNB before neoadjuvant therapy [48]. The rest of the studies performed SLNB and ALND after completion of NACT. Before NACT the

SLN ID rate was 98-99%, whereas after NACT it was a median of 93% in patients with clinically node-negative cancer and 88% overall. The studies also suggest FN rates are lower when SLNB is conducted before NACT.

The SENTINA study [45] did not conduct ALND if the SLNB before NACT was negative so FN rates could not be determined for this subgroup. Arm B of the SENTINA trial included patients initially cN0 with a positive SLN ($pN1_{SN}$) before NACT and conducted a second SLNB plus ALND after NACT. SLN ID rate was 76% in the second SLNB and the FN rate based on the second SLNB was 61% compared with a SLN ID rate of 99% in patients with cN0 cancer when SLNB was performed before NACT. This suggests that SLNB should not be performed both before and after NACT.

II.5.7.2 Qualifying Statements

It is often considered that adjuvant treatment should be based on the initial stage as determined before any treatment, although the extent of surgery depends on the size/extent of the tumour immediately before surgery (i.e., after any neoadjuvant treatment). Some studies suggest NACT often eliminates cancer from the SLN but not all the other nodes. For these reasons, there is theoretical justification for performing SLN biopsy before NACT. The very limited data would support this, but is considered insufficient at this time to make a strong recommendation due to the trade-off required in risk and inconvenience of needing to perform two separate operations (one for SLNB and one to remove

the main tumour) compared with the normal procedure of removing the tumour and SLN (or ALND) in one operation.

II.5.8 Recommendations 4(a) and 4(b)

II.5.8.1 Recommendation 4(a)

It is recommended that patients receiving neoadjuvant anthracycline-based therapy whose tumours do not respond or where there is disease progression be expedited to the taxane portion of the anthracycline-taxane regimen.

II.5.8.2 Recommendation 4(b)

For patients who fail to respond or who progress on first-line NACT, there are several therapeutic options to consider including second-line chemotherapy, hormonal therapy (if appropriate), radiotherapy, or immediate surgery (if technically feasible). Treatment should be individualized considering tumour characteristics, patient factors and preferences, and risk of adverse effects. Management of patients who do not respond to initial neoadjuvant therapy should be individualized through discussion at a multidisciplinary case conference.

II.5.8.3 Key Evidence (Recommendations 4(a) and 4(b))

Anthracycline-taxane is a standard therapy, with the taxane administered either concurrently or consecutively. The NSABP B-27 trial [49-51] found AC

followed by docetaxel gave significantly improved clinical and pathological response and lower rates of local recurrence compared with neoadjuvant AC alone. Because most patients were not LABC and patients were not randomized based on response, the trial is not included in the evidence review of Section 2.

The GeparTrio study [52] and a trial by Qi et al [53] evaluated early switching to second-line chemotherapy after nonresponse to two cycles of first-line chemotherapy and demonstrated conflicting findings: the first demonstrated no improved response to treatment but better tolerability and the second demonstrated some improved response but worse adverse effects and treatment delays. There is therefore insufficient evidence to switch chemotherapy mid-treatment.

The recommendations are based on current practice and are consistent with the guidelines by NCCN [13], Health Canada [54], and the Consensus Panel for Neoadjuvant Chemotherapy [14].

II.5.8.4 Qualifying Statements (Recommendation 4(b))

There is a body of literature including patients with locally advanced and metastatic disease (mostly single-arm case series, small pilot studies, or retrospective studies) that supports a variety of second-line single agent and multi-agent NACT and/or RT regimens to improve response (including pathologically complete response) and, thus, operability or survival. Although the data are limited and not within the rigorous inclusion criteria of the literature review, Table 8 of Section 2 lists some of these studies as examples of regimens

in the medical literature that have been tried in this clinical scenario. These data are not systematically reviewed nor of quality sufficient to make a recommendation as to preferred regimens. It is advised that oncologists individualize the choice of therapy based on the patient and risk of adverse effects.

II.6 FUTURE RESEARCH

Prospective RCTs designed for patients with LABC who fail to respond to NACT are needed so that more definitive treatment recommendations can be developed.

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APPENDIX III

LABC Clinical Trial Protocol

APPENDIX III. LABC CLINICAL TRIAL PROTOCOL

III.1 PATIENT ELIGIBILITY

III.1.1 Eligibility Criteria

- Biopsy proven LABC, Locally advanced breast cancer. (operable or non-operable)
- Any T3/T4 or N2, N3 Clinical TNM stage breast cancer without metastases
- Adequate renal function, as evidenced by a measured or calculated creatinine clearance ≥50 ml/minute. If calculated, the following formula must be used:

$$\text{Calculated creatinine clearance (ml/min)} = \frac{(140\text{-age}) \times \text{weight (kg)} \times 1.04}{\text{Cr } (\mu\text{mol/l})}$$

- Adequate hematologic reserves, as evidenced by an absolute neutrophil count ≥1.5 x 10^9/L, platelets ≥100 x 10^9/L
- Adequate hepatic function as evidenced by a total bilirubin ≤1.5 x the upper limit of normal (ULN), and AST ≤2.5 x ULN.
- ECOG Performance Status of 0, 1 or 2.
- Patients should be able to comprehend the Letter of Information and be capable of giving informed consent.
- Female age ≥18 years old
- Negative serum pregnancy test
- Adequate wall motion study results (LVEF ≥50%)

- Patients with other prior malignancies will be considered eligible if they are felt to be beyond risk of recurrence of the previous malignancy (generally >5 years after diagnosis, with no evidence of recurrence). There are no restrictions on time from a basal cell or squamous cell carcinoma of the skin or a carcinoma in situ of the cervix
- Adequate baseline pulmonary function studies must be confirmed prior to consent for radiation or radiation treatment planning. FEV1 should be equal to or greater than 1.0 litre.

III.1.2 Ineligibility Criteria

- Inflammatory cancer (as defined by clinical evidence of dermal-lymphatic tumour involvement.)
- Patient refuses modified radical mastectomy
- No patient may have received prior systemic treatment for disease within last 5 years, no prior radiotherapy given to head and neck, breast, or thoracic site.
- Previous ipsilateral breast cancer diagnosis.
- Pregnant or lactating females are ineligible.
- Female patients of reproductive potential who decline to employ an adequate contraceptive method are ineligible.
- Participation in any concomitant trials.

III.2 REGISTRATION PROCEDURES

All eligible patients enrolled on the study will be entered into a patient registration log located at London Regional Cancer Program (LRCP). This will provide a serial number for that patient which should be used on all documentations and correspondence.

All registration will be carried out by LRCP and will be obtained by calling the LRCP Clinical Research Unit at 519-685-8623. At the time of calling, a completed eligibility and signed consent must be available. There will be no exceptions to the eligibility/ineligibility criteria.

III.3 OVERAL TREATMENT PLAN

III.3.1 Concurrent Neoadjuvant Chemotherapy and Radiation (CNCR)

III.3.1.1 Initial Chemotherapy

CNCR treatment will begin within 6-8 weeks of their diagnosis of LABC (study enrollment) and will consist of 3 cycles of intravenous FEC chemotherapy (5-fluoruracil (500mg/m^2), epirubicin (100mg/m^2) and cyclophosphamide (500mg/m^2)) q3 weekly. The FEC chemotherapy will be followed by additional chemotherapy using docetaxel, concurrent with radiation. Adverse events from chemotherapy and radiation therapy as well as grading of any developed toxicity will be assessed by the oncologist as per National Cancer Institute. Any dose delays or dose reductions will be reported to the principal investigator, but dose reductions to 80% prescribed dose or one week dose delay will be acceptable for

this study. Patient tolerability will be assessed every 3 patients, and any grade 4 toxicities or treatment delays will be reviewed by an independent clinical review board and will be reported to the Health Sciences Research Ethics Board at the University of Western Ontario.

III.3.1.2 Radiation Concurrent with Chemotherapy

Docetaxel (35mg/m^2) will be given IV weekly with radiation treatment daily during the first 6 weeks of docetaxel. Concurrent radiation therapy will start during the first day of week one of docetaxel chemotherapy. Radiation therapy will consist of external beam therapy for a total dose of 45Gy in 25 fractions over 5 weeks. A reduced volume boost of 5.4Gy in 3 fractions to 9Gy in 5 fractions will then be given to residual gross disease in the breast or regional lymph nodes during the sixth week. The patient is to be placed in the supine position on an angle board with straight spine and the ipsilateral arm raised and supported by an armrest and the chin extended with appropriate headrest. All treatment planning will be performed on the Phillips Pinnacle workstation. All radiation treatment will be delivered on megavoltage machines using 6MV energy or greater with the following procedures/variables:

Treatment Interruption: any treatment delay of less than one week, radiation should be completed to prescribed dose. Any treatment delay of greater than one week, radiation should be completed to prescribed dose at the discretion of the treating radiation oncologist. All treatment delay causes and the length of the delay shall be reported.

Dose and Fractionation: Phase I – the dose will be 45Gy in 25 daily fractions over 5 weeks. 95% of the PTVs should receive 95% of this dose. Dose variation within the breast should be no more than plus 7% and no less than minus 5 percent.

Phase II – the boost dose will be 5.4Gy in 3 daily fractions to 9Gy in 5 daily fractions over 1 week. If there is concern that the residual gross tumor volume is too extensive, the boost will be limited to 5.4Gy in 3 fractions over 1 week. Any gross residual in the supraclavicular area will be limited to a boost of 5.4Gy in 3 fractions.

Prescription Point: For the tangents, this will usually be the point at a depth of two-thirds of the distance from the overlying skin contour to the posterior tangents at mid-separation. The normalization point is placed away from the underlying lung. For the supraclavicular and axillary fields, the prescription point is at midplane.

III.3.1.3 Surgery

Chemotherapy with radiation will be followed by modified radical mastectomy 5 weeks after the last dose of docetaxel, which would give 8 weeks of radiation recovery preoperatively. The patient will receive a single dose of preoperative antibiotic 30 minutes prior to commencement of the surgery. The modified radical mastectomy will be performed in the standard fashion, resecting the breast parenchyma through an elliptical incision in order to allow for primary skin closure. Through the same wound, a complete level I and II axillary

dissection will be performed. A 19 Blake drain will be place beneath the skin flaps and secured through a stab incision with Prolene suturing, and the skin flaps will be reapproximated with buried subdermal 3-0 monocryl sutures and the epidermis closed with a running subcuticular 4-0 monocryl suture and the wound covered with steri-strip dressings. Homecare nursing will be arranged for daily wound assessment and drain care, which will be removed when the serous drainage falls below 30ml per day.

III.3.1.4 Translational Research Components

Plasma OPN – blood will be drawn for plasma osteopontin at the same time blood is drawn for CBC or biochemistry. The blood will be labelled with the patient ID # and sent to the 4th floor laboratory at the LRCP for storage and analysis.

Sesta MIBI SPECT/CT – each patient will have 3 CTs done. The first CT will be performed just prior to the start of FEC chemotherapy. Second CT will be done just prior to the start of the Docetaxel chemotherapy. The third CT will be done just prior to surgery. CT scans will be performed by the nuclear medicine department at LHSC.

Core Needle Biopsy – at the time of your diagnostic biopsy procedure, you may have agreed to participate in a biopsy evaluation study and have signed a separate consent and letter of information. If you have participated in that study, the additional samples taken will also be used as the first set of tumour samples for this current study. If you have refused to participate in the biopsy evaluation

study, it does not affect your participation in this study. If you were NOT invited to participate in a research study at the time of your diagnostic biopsy procedure, you will be asked to undergo another ultrasound-guided biopsy procedure, where three extra pieces of tumour tissue will be taken. Taking extra pieces will not change the ability of doctors to diagnose or treat your cancer, and will not change the outcome of the treatment. It is important to be able to test whether the ability of the cancer cells to grow and spread changes over the duration of the treatment. This study involves having 3 sets of biopsies IN TOTAL to be used for the research study only – at the baseline as described above, at the half-way point of chemotherapy (after 9 weeks), and when you are having your surgery (after completing chemotherapy and radiation) (when you are already asleep for your surgery, to avoid any discomfort to you). Each biopsy will take approximately 3-5 minutes. These biopsies will be performed at St. Joseph's Health Centre.

Ex Vivo Tumour Invasion Model – the serial tumour biopsy samples will be collected fresh in phosphate-buffered saline and delivered to Dr. Costello's laboratory, where these samples will be dissected into 1mm tumour plugs. One of these will be immediately stored in RNA Later and kept frozen at -80^{o}C for later analysis. The remaining samples will be placed in individual culture wells and incubated in fresh bovine Type I collagen at 37^{o}C for five days after each row of wells has been treated with the chemotherapies used in the trial according to CPS maximum allowable IV dosage (FEC and D). Half of the wells will be radiated at 0.8Gy once. At the completion of 5 days, optical spectroscopy will be

used to determine the maximal diameter of tumour cell invasion into the matrigel. Half of the tumour plugs in these wells will be formalin fixed and paraffin-embedded, and the other half will be flash frozen at -80^{o}C. This will be performed by Dr. Costello's company titled Oncoscreen®. The laboratory will be completely blinded to patient identifiers or treatment response of any patient.

RNALater Samples – the samples frozen in *RNALater* will be shipped frozen to Sudbury Ontario to be processed by RNA Diagnostics Inc. under the supervision of Dr. Amadeo Parissenti. The RNA integrity will be assessed and quantified. Samples will be de-identified so that the analysis will be completed prior to any information regarding patient identifiers or individual treatment response. If sufficient RNA quality is identified in a given tumour sample, its DNA and RNA will be extracted. DNA copy counts of proteins felt to be involved in treatment resistance will be measured and full genomic RNA array analysis will be performed using micro-array technology. The remaining sample products will be kept for potential micro-proteomic analysis if required to quantify tumour proteins felt to be involved in treatment resistance.

Immunohistochemical Protein Expression Analysis: Samples which are paraffin-embedded will be analyzed using immunohistochemical (IHC) staining for the proteins involved in drug or treatment resistance or apoptosis.

Laser Cytometric Analysis of Cancer Stem Cells – samples which are flash-frozen will be analyzed for cancer stem cell population counts per 0.4µm slide using immunofluorescence for markers of breast cancer stem cells including CD24, CD44 and ALDH and measured by computerized cell count analysis.

III.3.2 Chemotherapy Treatment

The planned regimen is 3 cycles of FEC (5-fluorouracil 500mg/m^2, epirubicin 100mg/m^2 and cyclophosphamide 500mg/m^2 intravenously) every 21 days to be followed by concurrent daily radiation with once weekly docetaxel 35mg/m^2 IV for 6 weeks. Upon completion of the concurrent treatment, subjects will continue on with weekly docetaxel for an additional 3 weeks. In the study of concurrent radiation with bi-weekly paclitaxel, although all women were able to complete the 6-week course of chemoradiation, over 90% of subjects required more than the planned 2 weeks for skin recovery from completion of treatment to surgery. The additional chemotherapy will allow time for tissue healing preoperatively, as well as give a complete course of systemic treatment. This will be followed by primary surgery 5 weeks after completion of chemotherapy.

Women with Her2/neu positive breast cancer will receive neoadjuvant trastuzumab as per standard of care. The trastuzumab will be initiated with docetaxel at the start of chemoradiation. Although trastuzumab is associated with a small risk of cardiotoxicity, the updated results of adjuvant studies do not demonstrate any increased risk, even when trastuzumab is administered concurrently with radiation or taxanes. Monitoring for cardiac toxicity of trastuzumab will be done as per institutional standard at the London Regional Cancer Program with wall motion study performed every 3 months while on therapy. Dose modification will be made as per international and institutional guidelines for Trastuzumab-associated cardiac dysfunction. Trastuzumab will be continued for a total duration of 1 year as per standard of care.

Women with estrogen receptor positive breast cancer will receive endocrine therapy according to their menopausal status. This will be initiated after completion of their systemic chemotherapy.

III.3.2.1 Administration of Chemotherapy

G-CSF not routinely given, but could be given as per LRCP standard. Timetable of administration of FEC is as follows:

- standard prophylaxis with antinausea and antiemetics
- 0min – start hydration using 500ml/hr 0.9% NaCl for a total of 500ml
- 15min – Fluorouracil 500,mg/m^2 IV push then Epirubicin 100mg/m^2 IV push by chemo suite nurse over 5-10min
- 20min – Cyclophosphamide 500mg/m^2 IV infusion, full dose over 40min
- 60min – flush with 0.9% NaCl and disconnect the patient

Docetaxel will be given weekly for 9 weeks. Her2/neu positive breast cancer will receive trastuzumab with docetaxel at the start of chemoradiation and the protocol is as follows:

- week 1 – start trastuzumab, over 90min, 1hr observation
- week 4 – start trastuzumab, over 60min, 30min observation
- week 7 – start trastuzumab, over 30min, 30min observation
- week 11 – start trastuzumab, over 30min, no observation

On the evening prior to chemotherapy, dexamethasone (8mg tablet) will be taken by the patient. Docetaxel administration during week 1 and week 2 will be as follows:

- 0min – start hydration using 500ml/hr 0.9% NaCl for a total of 500ml
- patient receives another 8mg dexamethasone from chemo suite nurse
- 15min – docetaxel 35mg/m^2 in 250 ml of 0.9% NaCl infused as:
    - ¼ rate for first 15 minutes, then BP check by nurse
    - ½ rate for next 15 minutes, then BP check by nurse
    - ¾ rate for next 15 minutes, then BP check by nurse
    - full rate for remaining 60 minutes, then BP check by nurse

Patient is then taken to radiation suite to receive daily regional breast radiation (IMRT) for first 2 weeks. On the evening of chemotherapy, dexamethasone (8 mg tablet) is taken by the patient.

During week 3 to 9 (if patient tolerates the docetaxel without significant hypotension), dexamethasone (8mg tablet) is taken by the patient on the evening prior to chemotherapy; docetaxel administration will be as follows:

- 0min – start hydration using 500ml/hr 0.9% NaCl for a total of 500ml
- patient receives another 8mg dexamethasone from chemo suite nurse
- 15min – docetaxel 35mg/m^2 in 250 ml of 0.9% NaCl infused at full rate for 60 minutes, then BP checked by nurse.

Patient is then taken to radiation suite to receive daily regional breast radiation (IMRT) for first 2 weeks. On the evening of chemotherapy, dexamethasone (8mg tablet) is taken by the patient. During all infusions of

docetaxel, patient is wearing ice mitts and ice slippers to minimize toxicity to nail beds.

In the event of toxicity, the doses of FEC and docetaxel will be adjusted according to the guidelines shown in the dose delays/modifications table (Table III.1). If an adverse event is not covered in this table, doses may be reduced or held at the discretion of the investigator for the subject's safety. Dose adjustments for hematologic toxicity are based on the blood counts obtained in preparation for the day of treatment.

No dose reductions will be made for any hypersensitivity reactions. All patients receiving docetaxel will also receive dexamethasone (8mg PO) night prior, 1hour prior and immediately prior to docetaxel administration. If, despite pre-treatment, the patient experiences a hypersensitivity reaction, treatment should be as indicated in Table III.2.

III.3.2.2 Side Effects – FEC Chemotherapy

These side effects, occur in **25%-50%** of patients taking the chemotherapy used in this study:

- Nausea, vomiting, fatigue
- Lowered white blood cell count (may lead to infection), lowered red blood cell count (may lead to anemia, tiredness, shortness of breath)
- Irregular or permanent stoppage of menstrual cycles, inability to get pregnant
- Complete hair loss

Table III.1 Rules for dose and schedule adjustments in LABC patients.

FEC x 3 cycles (wk 1-9)		
	Grade	**Treatment Modification**
Myelosuppression:		
Asymptomatic NP	<1.5 x 10^9/L	Defer 1 week If defer >1wk:dose-reduce all agents of FEC by 20% subsequently
Febrile NP	<1.5 x 10^9/L Temp ≥ 38.5ºCor 38.3ºC at least 1hr apart	Dose reduce all agents of FEC by 20% subsequently
Asymptomatic TCP	Platelets ≤75x 10^9/L	Hold FEC until platelets >75x 10^9/L.
All other toxicities (except alopecia)	Grade 3/4	Hold until resolve to ≤ Grade 1
Weekly docetaxel with concurrent radiation (wk 10-15)		
Myelosuppression:		
Asymptomatic NP	<1.5 x 10^9/L	Defer 1 week If defer >1wk:dose-reduce by 20% subsequently
Febrile NP	<1.5 x 10^9/L Temp ≥ 38.5ºCor 38.3ºC at least 1hr apart	Dose reduce by 20% subsequently
Asymptomatic TCP	Platelets ≤75x 10^9/L	Hold FEC until platelets >75x 10^9/L.
Fluid Retention:	Grade 1-2	No adjustment mandated; diuretics discretionary.
	Grade 3	Diuretics mandatory. If life-threatening despite optimal medical management: OFF PROTOCOL
Hypersensitivity Reactions:		No dose adjustment. Anaphylaxis: OFF PROTOCOL
Hepatic Dysfunction:	Total Bili AST AlkPhos Normal >1.5xULN >2.5xULN Normal 2.5-5xULN --	Dose Reduce 25% 25%

	Normal >5xULN --	50%
	26-43µmol/L -- --	50%
	>43µmol/L -- --	75%
Skin Reactions:	Acute Gr 1-2	No modifications
	Gr ≥3	See section on Radiation Toxicity
All other toxicities (except alopecia)	Grade 3/4	Hold until resolve to ≤Grade 1
Weekly docetaxel (wks 16-18)		
Myelosuppression:		
Asymptomatic NP	<1.5 x 10^9/L	Defer 1 week begin G-CSF (if assessable) if deferred >1wk then dose reduce by 20% subsequently
Febrile NP	<1.5 x 10^9/L Temp ≥ 38.5ºCor 38.3ºC at least 1hr apart	Defer 1 week begin G-CSF (if assessable) if deferred >1wk then dose reduce 20% subsequently
Asymptomatic TCP	Platelets ≤75x 10^9/L	Hold until platelets >75x 10^9/L
Fluid Retention:	Grade 1-2	No adjustment mandated; diuretics discretionary.
	Grade 3	Diuretics mandatory. If life-threatening despite optimal medical management: OFF PROTOCOL
Hypersensitivity Reactions:		No dose adjustment. Anaphylaxis: OFF PROTOCOL
Hepatic Dysfunction:	Total Bili AST AlkPhos Normal >1.5xULN >2.5xULN	Dose Reduce 25%
	Normal 2.5-5xULN --	25%
	Normal >5xULN --	50%
	26-43µmol/L -- --	50%
	>43µmol/L -- --	75%
Skin Reactions:	Acute Gr 1-2	No modifications
	Gr ≥3	See section on Radiation Toxicity
All other toxicities (except alopecia)	Grade 3/4	Hold until resolve to ≤Grade 1

Table III.2 Management of hypersensitivity reaction in LABC patients enrolled in the clinical trial.

Mild symptoms: Localized cutaneous reactions such as mild pruritus, flushing, rash	Consider decreasing the rate of infusion until recovery from symptoms, stay at bedside and monitor patient. Then, complete docetaxel infusion in the initial planned rate.
Moderate symptoms: Any symptom that is not listed above (mild symptoms) or below (severe symptoms) such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic blood pressure >80mmHg.	1. Stop docetaxel infusion 2. Given diphenhydramine 50mg iv with or without Hydrocortisone 100mg IV; monitor patient until resolution of symptoms 3. Resume docetaxel infusion after recovery of symptoms; depending on the physician's assessment of the patient, docetaxel infusion should be resumed at a slower rate, then increased incrementally to the initial planned rate, (e.g. Infusion at an 8hr rate for 5min, then at a 4hr rate for 5min, then at a 2hr rate for 5min, then finally, resume at the 1hr infusion rate) 4. Depending on the intensity of the reaction observed, additional oral or iv premedication with an antihistamine should also be given for the **next cycle** of treatment, and the rate of infusion should be decreased initially and then increased back to the recommended 1hr infusion, (e.g. infuse at an 8hr rate for 5min, then at a 4hr rate for 5min, then at a 2hr rate for 5 min, and finally, administer at the 1hr infusion rate).
Severe symptoms: Any reaction such as bronchospasm, generalized urticaria, systolic blood pressure ≤80mmHg, angioedema	Stop docetaxel infusion. Give diphenhydramine 50mg iv with or without hydrocortisone 100mg iv and/or epinephrine as needed with physician order: monitor patient until resolution of symptoms. The same treatment guidelines outlined under moderate symptoms (ie. 3rd and 4th point) should be followed. If severe reaction recurs despite additional premedication, the patient will go off protocol treatment.
Anaphylaxis (Grade 4 reaction)	OFF PROTOCOL TREATMENT

- Temporary red-coloured urine following chemotherapy (not blood)
- Time away from work
- Hot flashes (in premenopausal women)

These side effects occur in **10-24%** of patients taking the chemotherapy in this study:

- Sores in mouth and/or throat, infection
- Taste changes
- Skin and nail changes, including discolouration and peeling
- Pain at the site where chemotherapy is administered

These side effects occur in **3-9%** of patients taking the chemotherapy in this study:

- Diarrhea, constipation, loss of appetite
- Low platelet count, leading to increased bruising or bleeding
- Headache, abdominal pain, skin rash/itching, muscle pain, eye irritation
- Darkening of the soles of the feet or palms of hands
- Thickening of the walls of the veins used for chemotherapy
- Blood in the urine
- Fever
- Fever with a low white blood cell count

Rare but serious side effects that occur in **less than 3%** of patients taking the chemotherapy used in this study include:

- Decreased ability of the heart to pump blood. If severe, you could have shortness of breath and other symptoms of heart failure. (If mild, you may not have any symptoms.)
- Skin damage (due to leakage of the drug)
- Acute leukemia (cancer of the blood cells)
- Lung damage
- Lowered red blood cell count severe enough to require red blood cell transfusion; lowered platelet count severe enough to require a platelet transfusion
- Severe infection
- Blood clots
- Changes in blood test results that indicate possible liver injury
- Allergic reaction including itching, hives,rash, flushing, hypersensitivity, shortness of breath, wheezing, chest tightness, fever, chills, muscle stiffening, severe breathing problems

III.3.2.3 Side Effects – Docetaxel (Taxotere)

These side effects occur in **25% -50%** of patients receiving docetaxel:

- Hair loss, nausea, vomiting, taste changes
- Weakness/loss of strength, fatigue

• Hot flashes (in premenopausal women), Irregular or permanent stoppage of menstrual cycles (periods), inability to become pregnant

• Skin and nail changes, including discoloration and peeling

• Lowered white blood cell count (may lead to infection), lowered red blood cell count (may lead to anemia, tiredness, shortness of breath)

• Time away from work

These side effects occur in **10-24%** of patients receiving docetaxel:

• Diarrhea, constipation, loss of appetite

• Mouth sores, infection

• Pain in muscles, bones, or joints

• Headache

• Fluid retention (bloating or swelling)

• Numbness, tingling, prickling, and burning in the hands and feet

These side effects occur in **3-9%** of patients receiving docetaxel:

• Ulcers in the stomach or bowels

• Darkening of the soles of the feet or palms of the hands

• Peeling of the skin (including hands and feet)

• Lowered number of platelets (which may lead to increased bruising or bleeding)

• Eye irritation, blurred vision

• Dizziness

• Changes (high or low) in blood pressure

• Hardening of the walls of the veins used for chemotherapy

• Reversible changes in blood test results that show possible liver injury

Symptomatic lung damage generally occurs in fewer than 3 percent of patients secondary to docetaxel alone. Symptomatic lung damage from locoregional radiation alone for breast cancer occurs in 5 percent or fewer patients, with severe shortness of breath restricting activities of daily living (grade 3 or greater) occurring in a subset of patients. The combination of docetaxel and locoregional radiation for breast cancer may increase the frequency and severity of symptomatic lung damage.

These side effects occur in **less than 3%** of patients receiving docetaxel:

• Liver failure

• Gastrointestinal problems (such as bleeding, blockage, or perforation [opening of a hole] in the stomach or bowel)

• Lowered red blood cell count severe enough to require red blood cell transfusion

• Skin and tissue damage in the area surrounding the catheter where the chemotherapy drugs are injected

• Acute leukemia (cancer of the blood cells)

• Blood clots that may be life-threatening

• Heart damage, lung damage

• Severe infection

• Inflammation of the pancreas causing abdominal pain

• Allergic reaction including itching, hives, skin rash, flushing, shortness of breath, wheezing, chest tightness, fever, chills, severe shivering, sinus congestion, or swelling of face, especially eyelids

• A group of symptoms which may include a blister-like rash that may be severe; fever; inflamed eyes; redness, swelling and painful sores on lips and in mouth (If this occurs, you may need to be hospitalized and have IV fluids and medicines.)

III.3.3 Radiation Therapy

Concurrent radiation therapy will start during the first day of docetaxel. Radiation therapy will consist of external beam therapy for a total dose of 45Gy in 25 fractions over 5 weeks. A reduced volume boost of 5.4Gy in 3 fractions to 9Gy in 5 fractions will be given to residual gross disease in the breast and/or regional lymph nodes. Gross disease in the high axilla or supraclavicular area will be limited to 5.4Gy in 3 fractions maximum boost. If treatment is delivered using IMRT, gross disease will be limited to 5.4Gy concomitant boost (total dose 50.4Gy in 28 fractions to gross disease and 45Gy in 28 fractions to uninvolved breast, axilla, IMC, and supraclavicular volumes).

III.3.3.1 Patient Position and Immobilization

Patient is to be placed in the supine position on an angle board with straight spine and the ipsilateral arm raised and supported by an armrest and the chin

extended with appropriate head rest. Patient is to be instructed to breathe quietly in order to minimize respiratory motion during scanning and treatment. A radiopaque breast wire will be placed around the ipsilateral clinical breast mound.

III.3.3.2 Scanning Protocol

Serial CT is to be collected utilizing Philips Brilliance large bore CT scanner (or equivalent technology). Three-millimetre thick slices at 3 mm intervals will be scanned from the level of the lower jaw to L1 (in order to encompass the whole lung volume). For the single isocenter technique, the junction between the breast portals and the regional nodal portals is placed at the level of the inferior and medial ipsilateral clavicle. The junction line is marked by tattoos. Intravenous contrast is optional but may help with the delineation of gross residual tumor and the regional blood vessels.

III.3.3.3 Treatment Planning

All treatment planning will be performed on the Phillips Pinnacle workstation. In general one of the following three treatment techniques will be used:

a) Single isocenter technique: The single isocenter is located at the level of inferior border of the medial head of clavicle. The affected breast and ipsilateral internal mammary chain are treated using medial and lateral deep tangents with half beam blocking of the superior borders to create a non-divergent match with the supraclavicular and axillary fields. The tangents can be

non-opposed, if necessary, to create a non-divergent deep/ posterior border which can reduce underlying lung in the high dose volume. Both medial and lateral shielding should be checked to verify maximum sparing of normal tissues and also adequate coverage of any gross disease. The ipsilateral axillary and supraclavicular nodes are treated with anterior and posterior oblique fields with half beam blocking of the inferior borders to create a non-divergent match with the deep tangents. The medial field borders should fall along the medial border of the ipsilateral sternomastoid muscle with gantry rotation to avoid the spinal cord (usually 8 to 12 degrees gantry rotation to the contra lateral side for the anterior supraclavicular - axillary field). The gantry angle for the posterior supraclavicular-axillary field can be rotated such that the medial border creates a non-divergent match with the medial border of the anterior supraclavicular-axillary field. The superior border of the supraclavicular fields is usually at the level of the upper thyroid cartilage. The ipsilateral larynx, acromio-clavicular joint, and the upper one half to two-thirds of the humeral head should be shielded. If the ipsilateral lung volume receiving 20Gy exceeds 40 percent and /or if total lung volume receiving 20Gy exceeds 25 percent, the following alternative treatment techniques should be considered.

b) Intensity modulated radiation (IMRT) for the involved breast with half beam blocking of the superior borders to match the supraclavicular-axillary fields described above. This option is especially useful when the supraclavicular-axillary fields do not encompass much lung but the deep tangents would include too much lung. Usually, four or five field step and shot IMRT will reduce dose to

lung compared to the preceding technique but more detailed contouring will be necessary. Guidelines for treatment planning are in the treatment planning module. For this approach, the supraclavicular-axillary fields will receive 45Gy in 25 fractions over five weeks. The uninvolved breast and lower axilla within the IMRT volume will receive 45Gy in 28 fractions (no BED correction) over five and a half weeks while gross disease will receive 50.4Gy in 28 fractions over five and a half weeks (concomitant boost). If feasible, the uninvolved breast and lower axilla and gross disease will be treated, using IMRT, to a dose of 45Gy in 25 fractions. A reduced volume boost of 5.4Gy in 3 fractions using 3-D conformal radiation or IMRT boost is allowed.

c) IMRT for the entire volume, including the ipsilateral breast, axilla, supraclavicualr area, and internal mammary chain. This approach is useful when both deep tangents and supraclavicular-axillary fields encompass too much lung. Treatment planning requires detailed contouring of normal structures as well as target volumes. The ipsilateral breast, the right and left lungs, the heart, the larynx and trachea as an organ at risk (OAR) should all be contoured. The target volumes will also be contoured as defined below.

The uninvolved breast, IMC, axilla, and supraclavicular area will receive 45Gy in 28 fractions. Gross disease will be treated to 50.4Gy in 28 fractions

III.3.3.4 Treatment Delivery

All radiation treatment will be delivered on megavoltage machines using 6MV energy or greater. Calibration of all radiation treatment machines will be

under the supervision of the Department of Radiation Physics at the London Regional Cancer Program.

III.3.3.5 Critical Structure Dose Constraints

Maximum spinal cord dose shall be 45Gy. In the lung, maximum 30% of total normal lung volume is to receive less than or equal to 20Gy. While V_{20Gy} to total lung under 30 percent is acceptable, V_{20Gy} under 20 to 25 percent is preferred. In the heart, maximum dose to 25% of the heart volume will be less than or equal to 25Gy. For the ipsilateral humeral head, maximum dose to 50% will be less than or equal to 30Gy.

III.3.3.6 Treatment Interruption

Any treatment delay of less than one week, radiation should be completed to prescribed dose. Any treatment delay of greater than one week, radiation should be completed to prescribed dose at the discretion of the treating radiation oncologist. All treatment delay causes and the length of the delay shall be reported. Any treatment delay of greater than two weeks will result in the patient being discontinued from protocol.

III.3.3.7 Deviations in Radiation Protocol

Prescription Dose: Minor – between 6-10 % difference between protocol and prescription dose; major – greater than 10 % difference between protocol and prescription dose.

Dose Uniformity: Minor – either (-10% to -5%) or (+7% to +10%) variation in dose target volume homogeneity; major – dose variation to target greater than ± 10%.

Volume: Minor – margins are less than specified or fields excessively large; major – transecting tumour of lymph node bearing areas.

III.3.3.8 Radiation Planning

Target volumes are as follows:

- Ipsilateral **Breast**: This is defined as the clinical breast volume harbouring malignancy plus any gross clinical tumor extension beyond the ipsilateral breast. During treatment planning, it should be marked with a radiopaque wire around its periphery. The breast will be the volume bounded by the radio-opaque wire, excluding the chest wall and the overlying 5mm of skin.
- **PTVBREAST**: This is the expansion of the **BREAST** plus 7mm.
- **PTVEVALBREAST**: This is the **PTVBREAST** as defined above minus overlying 5mm of skin.
- **GTVPRIMARY**: –This is the volume of gross disease in the breast at defined at the time of CT simulation and any other available diagnostic information including clinical exam.
- **CTVprimary:** - **GTVPRIMARY** plus 1.5cm margin in all directions but limited to the anterior aspect of the pectoralis major muscle (if no muscle invasion) and to 5 mm or more deep to the skin contour of the breast (if no direct skin extension). The deep border of the CTV primary should include

the muscle to rib interface if there is pectoralis muscle involvement; the superficial border should include the skin surface if there is skin involvement.

- **PTVprimary** – **CTVprimary** plus 1cm.
- **PTVevalprimary** – **PTVprimary** constrained to within 5 mm of overlying skin, if no skin involvement, or to the skin surface if skin involvement. The deep boundary will be the rib to lung interface.
- **GTVNODESBOOST** – these are nodes considered to be grossly involved in the ipsilateral supraclavicular and axillary area and measuring 7mm or more in short axis at the time of planning CT scan. Pretreatment CT scan showing the same nodes to be larger can be used to identify significant nodes.
- **CTVnodesBOOST** – This is the **GTVNODESBOOST** plus 1cm in all directions but limited to the nearest surface of the adjacent muscles (pectoralis major or minor, serratus, lattisimus, sternomastoid) and to 5mm or more deep to the overlying skin. It should also lie within the **CTVAXSC**. The superficial border should include the skin surface if there is skin involvement.
- **PTVNODESBOOST** – this is **CTVnodesBOOST** expanded by 7 to 10mm (use 7mm except in large patients).
- **PTVEVALNODESBOOST** – this is **PTVNODESBOOST** minus the overlying 5mm of skin and minus any underlying lung.

- **CTVIMC** – this is the contour of the ipsilateral IMC vessels expanded medially to touch the ipsilateral border of the sternum at the level of the first, second, and third intercostal spaces. Any visible lymph nodes at these 3 levels should be contoured and expanded by 7mm. The **CTVIMC** should include the visible nodes plus the 7mm expansion.
- **PTVIMC** – this is the **CTVIMC** expanded by 7mm.
- **PTVEVALIMC –** this is the **PTVIMC** minus underlying lung and heart.
- **CTVAXSC:** This volume encompasses the axillary and supraclavicular lymph nodes at risk. The inferior level is usually at the level of the fifth rib in the mid-axillary line or at least 1cm below any grossly visible lower axillary lymph nodes. The lateral border is at least 1cm lateral to any visible lymph nodes in the low to mid axilla (level 1 and 2 nodes) and usually lies within a line drawn from the lateral edge of pectoralis major and the lateral edge of the latissimus muscle. At the level of the high axilla (level 3 nodes), the lateral border is medial to the coracoid. At the level of the supraclavicular area, the lateral border is at the mid-clavicular line. The medial border, anterior, and posterior borders of the axilla are formed by the nearest surfaces of the adjacent muscles (pectoralis, serratus, lattissimus). The supraclavicular fossa is bounded medially by the lateral margin of the sternomastiod muscle. Inferiorly, contouring may continue deep to the medial clavicle where the subclavian crosses the lung apex at the level of the first rib posteriorly to join the internal jugular. Care should

be taken to avoid extending the volume medially towards the thyroid and the larynx, unless there is gross supraclavicular adenopathy.

- **PTVNODES** – this is **CTVAXSC** and **CTVIMC** expanded by 7mm.
- **PTVEVALNODES** – this is **PTVNODES** minus 5mm of overlying skin and minus lung and heart.

Beam arrangement will be as follows: Phase I – the target volume is the ipsilateral breast plus tumour extension, the ipsilateral supraclavicular and axillary regions, the PTV primary, and the PTV nodal. Phase II – the target volume is any gross residual disease remaining at the time of CT simulation. Repeat CT simulation may be required if there has been more than a 2cm shrinkage in the surface contour secondary to tumor shrinkage. If feasible, gross residual tumor in the breast and or regional nodes can be boosted with direct electrons and clinical setup. For gross tumor more than 5cm deep to overlying skin, reduced volume boosts using multiple photon fields may be necessary (parallel pair in axilla, reduced tangents in breast, or 3 or 4 field techniques could be used).

Dose and Fractionation will be as follows: Phase I – the dose will be 45Gy in 25 daily fractions over 5 weeks. Ninety-five percent of the PTVEVALs should receive 95% of this dose. Dose variation within the breast should be no more than +7% and no less than -5%. Phase II – the boost dose will be 9Gy in 5 daily fractions over 1 week. If there is concern that the residual gross tumor volume is too extensive, the boost will be limited to 5.4Gy in 3 fractions over 1 week. Any

gross residual in the supraclavicular or high axillary area will be limited to a boost of 5.4Gy in 3 fractions Patients receiving concomitant boost with IMRT will receive 45Gy in 28 fractions to uninvolved areas and 50.4Gy in 28 fractions to grossly involved primary tumor and nodes.

Beam modifiers, such as shielding, multi-leaf collimators, wedges and compensators are allowed.

Prescription point: for the tangents, this will usually be the point at a depth of two-thirds of the distance from the overlying skin contour to the posterior tangents at mid-separation. The normalization point is placed away from the underlying lung. For the supraclavicular and axillary fields, the prescription point is at midplane.

Bolus (0.5cm thickness) will be placed to cover and gross skin extension of tumor or skin ulceration as well as any inflammatory skin involvement (note: patients with inflammatory breast cancer are not part of this protocol). Bolus should extend 1 to 2cm beyond the visible skin involvement.

III.3.3.9 Radiation Toxicities

These include the following:

- Radiation pneumonitis: symptomatic pneumonitis from radiation alone occurs in 1 to 5 percent of patients and can range in severity from a dry cough to severe shortness of breath requiring medical management (including admission to hospital, use of oxygen, steroids, and inhalers) but is almost always self-limited. The combination of chemotherapy and

radiation for breast cancer may increase the frequency and severity of symptomatic lung injury from treatment. The clinical course of such lung injury has not been well documented and there is the potential risk of permanent shortness breath secondary to treatment. Treatment for symptomatic acute pneumonitis is typically oral corticosteroids. Initiate prednisone at 50mg orally for one to two weeks. Reduce the dosage by fifty percent every 3 to 5 days based on patient symptomatic improvement. A more gradual taper of steroids may be appropriate for some patients. The majority of patients with pneumonitis recover. Progressive symptoms requiring oxygen or hospitalization are uncommon.

- Brachial plexopathy: this complication occurs in one percent or less of patients at doses of 50 to 54Gy in 2Gy or 1.8Gy fractions, respectively. Transient plexopathy can occur within the first few months post radiation but later plexopathy can be permanent.
- Rib fractures: this occurs in 2 percent or less of patients
- Lymphedema: upper limb edema of any degree can occur in 10 to 20 percent of patients but moderate to severe lymphedema occurs in 5 percent or less of patients. Lymphedema can be permanent.
- Cardiac toxicity: the risk of fatal MI from radiotherapy is estimated at 1 percent or less. Acute pericarditis is also uncommon (less than 1 percent).
- Second malignancies: there are reports of increase in lung cancer post radiotherapy for breast cancer. Skin cancers and rare sarcomas rarely occur post radiotherapy.

- Wound dehiscence requiring surgery: the rate of this complication is expected to be less than 5%.

Any of the above toxicities graded by NCIC/CTC v3.0 at grade 4 will be assessed by the PI, radiation oncologist and medical oncologist together to determine whether to stop radiation or docetaxel. There is concern that acute skin toxicity will be increased by the combination of chemotherapy and radiation but it is expected that most patients will complete the full regimen. It is common with locoregional radiation alone for patients to develop patches of moist desquamation in areas of skin folds like the axilla, infra-mammary crease, and medial neck. These acute skin reactions alone will not require treatment modification. General acute radiation skin toxicity management guidelines and treatment modifications during radiation are as follows:

(a) Grade 3 or greater (moist desquamation other than skin folds) acute skin reaction affecting more than 25 percent of the breast surface (excluding areas of direct skin involvement by tumor) or more than 25 percent of the supraclavicular skin surface: Hold both radiation and docetaxel up to one week until healing visible and then re-start docetaxel and radiation with no dose modifications.

(b) Grade 3 or greater acute skin reaction of onset earlier than fraction 16 of radiation (start of week 4): Hold both radiation and docetaxel up to one week until healing visible and then re-start docetaxel and radiation with no

dose modifications. Consider the possibility of acute cellulitis and manage with antibiotics as well, if index of suspicion high.

(c) Grade 3 or greater acute skin reaction as in 1 or 2 above but requiring more than one week but less than 2 weeks to show signs of healing: Re-start radiation alone and discontinue docetaxel.

(d) Grade 3 or greater acute skin reaction as in 1 or 2 above but with no healing within 2 weeks treatment break: Discontinue both docetaxel and radiation.

Management of grade 3 or greater acute skin reaction includes flamazine applied topically 2 to 3 times a day (polysporin triple could be considered in patients allergic to flamazine). If superimposed acute cellulitis is suspected, antibiotic management for cellulitis can be initiated as well. Grade 4 radiation skin toxicity will be treated symptomatically in keeping with best clinical practice as already decided by the breast multi-disciplinary team regarding this protocol.

Early experiences with the first ten patients going through this treatment protocol have raised concern about increased incidence and severity of radiation pneumonitis. Toxicity on this protocol is being monitored by an independent data safety monitoring committee. Clinical shortness of breath secondary to radiation typical develops after radiation treatment is completed and usually one to four months post radiation. Among the first ten patients, clinical shortness of breath developed within the first few weeks after radiation and docetaxel. Shortness of breath was grade 3 in 3 of ten 10 patients and, in 2 of the 3 patients, was felt to

be definitely treatment related. In all 3 patients shortness of breath responded quickly to steroids and all 3 went on to definitive surgery without any delay. Only 1 of the 3 women has residual shortness of breath grade 2. This patient had a significant history of smoking and continued to smoke throughout treatment. Complete pathologic response occurred in 5 of the first 10 patients, well above the 20% reported in the literature for chemotherapy alone. The breast multidisciplinary team decided that an acceptable incidence of grade 3 pneumonitis that does not improve to grade 1 or less within 8 weeks from the end of radiation or prior to surgery date as per protocol is five percent or lower. As well grade 3 or greater shortness of breath attributed to treatment that does not improve to grade 1 or less within 8 weeks from the end of radiation or prior to surgery date as per protocol is five percent or lower.

Pneumonitis assessment and management are as follows:

(a) all patients should have baseline pulmonary function studies;

(b) clinical considerations in evaluating a patient with shortness of breath during or after docetaxel and radiation include pulmonary, emboli, infection, cardiac dysfunction, and treatment related pneumonitis;

(c) in addition to clinical assessment, patients reporting worsening shortness of breath at any time during radiation should have a complete blood count with differential and a chest x-ray. Respirology referral is appropriate if the patient has fever, neutropenia less than 1.0, or is not responding to medical treatment of pneumonitis;

(d) patients reporting grade 3 shortness of breath during or after completing radiation should have a CT scan of the thorax. Repeat CT scans of the thorax will be done as clinically indicated in patients who initially presented with grade 3 or greater shortness of breath felt to represent treatment related pneumonitis. It is recommended that CT thorax be obtained at 3 to 6 months post docetaxel and radiation in patients who initially present with grade 3 or greater pneumonitis, especially if there is residual shortness of breath;

(e) medical management of docetaxel and radiation related pneumonitis:

- Medical management is individualized.
- Grade less than or equal to 2 shortness of breath: follow up only is appropriate.
- Grade 3 or greater shortness of breath: Initiate prednisone at 50mg orally daily for one to two weeks. Reduce the dosage by fifty percent every 3 to 5 days based on patient symptomatic improvement. A more gradual taper of steroids may be appropriate for some patients. If the patient is not responding to treatment, respirology consultation is appropriate. Pentoxifylline 400mg orally three times a day could be considered in patients who are still quite symptomatic despite steroids for more than two weeks. Pentoxifylline has been tested in a double blind, randomized clinical trial versus placebo but was given throughout radiation.

(f) stopping rules for treatment related pneumonitis: a data safety monitoring committee review will be held if the number of patients with incidence of grade 3

pneumonitis (attributed to treatment) does not improve to grade 1 or less within 8 weeks from the end of radiation or prior to surgery date as per protocol; exceeds 2 of the first ten patients, 3 of the first twenty patients, 4 of the first 30 patients, or 5 of the first 40 patients (Table III.3). Toxicity profiles, risks and benefits, and the study protocol will be reviewed by the data safety monitoring committee. Protocol modifications will be discussed and reviewed by the breast multidisciplinary team. The revised study protocol will be approved by the data safety monitoring committee and the ethics review board before continuing.

III.4 MEASUREMENT OF EFFECT

For the purposes of this study, patients should be evaluated every 3 weeks during active treatment by caliper or ruler measurement. Where feasible, the caliper measurement is to be done by the same investigator.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term “evaluable” in reference to measurability will not be used because it does not provide additional meaning or accuracy.

Table III.3. Stopping rules for treatment-related pneumonitis in the experimental treatment protocol in LABC patients.

N	Largest acceptable value		First non-acceptable value	
	# Cases (%)	Exact 95% CI	# Cases (%)	Exact 95% CI
10	2 (20%)	2.5% - 55.6%	3 (30.0%)	6.7% - 65.3%
20	3 (15.0%)	3.2% - 37.9%	4 (20.0%)	5.7% - 43.7%
30	4 (13.3%)	3.8% - 30.7%	5 (16.7%)	5.6% - 34.7%
40	5 (12.5%)	4.2% - 26.8%	6 (15.0%)	5.7% - 29.8%

III.4.1 Definitions

III.4.1.1 Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20mm with conventional techniques (CT, MRI, x-ray) or as ≥10mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

III.4.1.2 Non-Measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter <20mm with conventional techniques or <10mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

III.4.1.3 Target Lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as *target lesions* and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be

calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

III.4.1.4 Non-Target Lesions

All other lesions (or sites of disease) should be identified as *non-target lesions* and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

III.4.2 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment. Tumor lesions that are situated in a previously irradiated area must not be the only site of measurable disease.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

III.4.2.1 Clinical Lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by colour photography, including a ruler to estimate the size of the lesion, is recommended.

III.4.2.2 Chest X-ray

Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

III.4.2.3 Conventional CT and MRI

These techniques should be performed with cuts of 10mm or less in slice thickness contiguously. Spiral CT should be performed using a 5mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

III.4.2.4 Ultrasound (US)

When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm

the complete disappearance of superficial lesions usually assessed by clinical examination.

III.4.2.5 Endoscopy and Laparoscopy

The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

III.4.2.6 Tumour Markers

Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

III.4.2.7 Cytology and Histology

These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor

types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

III.4.3 Response Criteria

III.4.3.1 Evaluation of Target Lesions

The definitions are as follows:

- Complete Response (CR): Disappearance of all target lesions;
- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD;
- Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions;
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

III.4.3.2 Evaluation of Non-Target Lesions

The definitions are as follows:

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level;
- Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits;
- Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

Although a clear progression of “non-target” lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the review panel (or study chair). Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

III.4.3.3 Evaluation of Best Overall Clinical Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started) (Table III.4). Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration.” Every effort should be made to document the objective progression, even after discontinuation of treatment.

Table III.4. Evaluation of best clinical response in LABC patients undergoing clinical trial experimental protocol.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

III.4.4 Confirmatory Measurement of Pathological Response

The final assessment of tumour response will be made by one and the same independent pathologist (confirmed by an unbiased secondary assessment by a second pathologist) based on the pathological assessment of the entire surgical specimen, according to current standards accepted by the Canadian Association of Pathologists, examining the entire specimen and taking representative blocks of tissue for analysis. Pathological response will be subcategorized as follows:

pCR – pathological complete response (no residual invasive breast cancer in the breast tissue);

pSPR – pathological significant partial response (<10 foci of microscopic invasive tumour within breast);

pPR – pathological partial response (<30% of original invasive breast tumour volume remaining);

SD – stable disease (30-80% of original invasive breast tumour volume remaining);

NR – no response (81-120% of original invasive breast tumour volume remaining);

DP – disease progression (>120% of original invasive breast tumour volume remaining).

The baseline diagnostic breast MRI will be used to calculate the pre-treatment tumour volume, as a surrogate measure for true pathological in vivo

tumour volume, given that it remains our most sensitive means of estimating in vivo breast tumour volume.

III.4.4.1 Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

III.4.4.2 Duration of Stable Disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

III.4.4.3 Progression-Free Survival

Progression free survival is defined as the duration of time from start of treatment to progression (as defined above), death or last contact, or last tumor assessment before the start of further antitumor therapy.

III.4.5 Adverse Event Reporting

The safety committee will consist of, at a minimum, the principal investigator, a statistician, the data manager, and one independent physician. They will meet annually and as required.

Toxicities occurring as a result of treatment should be reported to the principal investigator and the Data Collection Centre at the Clinical Research Unit of the London Regional Cancer Program in the manner described below. In addition, the IRB/REB will be notified in keeping with good clinical practice guidelines.

The investigator is responsible for the detection and documentation of events meeting the definition of an adverse event (AE) or serious adverse event (SAE) as provided in this section of the protocol. In order to fulfill international safety reporting obligations, the investigator should include in his or her assessment any SAEs resulting from study participation (e.g., complications resulting from the taking of a blood sample).

III.4.5.1 Definition of an AE

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can, therefore, be any unfavourable and unintended sign (that could include a clinically significant abnormal laboratory finding), symptom, or disease temporarily

associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE does include a/an:

- exacerbation of a pre-existing illness.
- increase in frequency or intensity of a pre-existing episodic event or condition.
- condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.
- continuous persistent disease or symptoms present at baseline (including cancer signs and symptoms if more severe than expected) that worsen following the start of the study.

An AE does not include a/an:

- medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an AE.
- pre-existing disease or conditions present or detected at the start of the study that do not worsen.
- situations where an untoward medical occurrence has not occurred (e.g. hospitalizations for cosmetic elective surgery, social and/or convenience admissions).
- the disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the subject's condition (e.g. subjects with advanced stages of cancer are expected to

experience progression of disease including increased tumor size, new sites of disease, malignant pleural effusion, malignant ascites, and death due to cancer).

- overdose of either study drug or concurrent medication without any signs or symptoms.

III.4.5.2 Definition of an SAE

An SAE is any adverse event occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse event.
- inpatient hospitalization or prolongation of existing hospitalization.
- a disability/incapacity.
- a congenital anomaly in the offspring of a subject who received study drug.
- important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Clarifications:

- "Occurring at any dose" does not imply that the subject is receiving study drug.
- Life-threatening means that the subject was, in the view of the investigator, at immediate risk of death from the event as it occurred. This definition does not include an event that, had it occurred in a more severe form, might have caused death.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen during the study is not considered an SAE.
- Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization, the event is an SAE. "Inpatient" hospitalization means the subject has been formally admitted to a hospital for medical reasons. This may or may not be overnight. It does not include presentation a casualty or emergency room.
- With regard to criteria above, medical and scientific judgment should be used when deciding whether prompt reporting is appropriate in this situation.

Events or Outcomes Not Qualifying as SAEs: Any sign, symptom, diagnosis, illness, and/or clinical laboratory abnormality that can be linked to the disease under study or disease progression and is not possibly attributable to study drug, are not reported as SAEs even though such event or outcome may meet the definition of SAE.

- Events that are exempt from reporting as serious adverse events include:

- Events emerging during the study that are part of the natural progression of the underlying cancer (including disease-related deaths) unless more severe than expected or not possibly attributable to study drug. For example, hospitalization for the evaluation or treatment of signs and symptoms of disease progression that are not possibly attributable to study drug will not be reported as an SAE.
- SAE that occur more than 28 days after the final dose of study drug that are judged by the investigator to be unrelated to prior treatment with study drug.

III.4.5.3 Lack of Efficacy as an AE or SAE

"Lack of efficacy" (e.g., disease progression as documented by increased tumor size, increased number of lesions, new sites of disease, malignant pleural effusions, malignant ascites and death due to cancer) per se will not be reported as an AE. The signs and symptoms or clinical sequelae resulting from lack of efficacy should be reported if they fulfill the AE or SAE definition (including clarifications).

III.4.5.4 Clinical Laboratory Abnormalities and Other Abnormal Assessments as AEs and SAEs

A laboratory abnormality per se will not be recorded as an AE or SAE unless it is serious (See definition of an SAE), represents the primary reason for treatment or study discontinuation, or is associated with a clinical diagnosis.

Sequelae of laboratory abnormalities (e.g., sepsis or fever in subjects with neutropenia) will be recorded on the Serious Adverse Event page.

Findings from disease assessments (e.g., CT scans, MRI scans, X-rays, bone scans, physical examinations or medical photographs) will not be recorded as AEs or SAEs. Clinically significant abnormal findings or assessments (e.g., vital signs, electrocardiograms, physical examinations excluding disease assessments) that are detected after study drug administration or that are present at baseline and worsen following the start of the study are included as AEs and SAEs.

The investigator should exercise his or her medical and scientific judgment in deciding whether an abnormal finding or assessment is clinically significant.

III.4.5.5 Method, Frequency, and Time Period for Detecting AEs and SAEs

All adverse events and serious adverse events (except as noted above), regardless of causality, that may occur anytime from the time of administration of the first dose of any study drug until mastectomy will be recorded on the CRF. Any delayed, continuing or New Toxicities related to study treatment must be recorded until 6 months after mastectomy.

III.4.5.6 Documenting SAEs

A separate set of SAE Report form pages should be used for each SAE. However, if at the time of initial reporting, multiple SAEs are present that are temporally and/or clinically related, they may be reported on the same SAE form.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not the individual signs/symptoms. If a clinically significant abnormal laboratory finding or other abnormal assessment meets the definition of an SAE, then an SAE form must be completed. A diagnosis, if known, or clinical signs and symptoms if diagnosis is unknown, rather than the clinically significant abnormal laboratory finding, should be completed on SAE form. If no diagnosis is known and clinical signs and symptoms are not present, then the abnormal finding should be recorded. The laboratory data should either be recorded on the SAE form with the reference range and baseline value(s) or copies of the laboratory reports and reference ranges should be sent with the SAE form pages. The SAE form should be completed as thoroughly as possible and signed by the investigator or his/her designee before transmittal to the Data Collection Centre. It is very important that the investigator provide his/her assessment of causality to study drug at the time of the initial SAE report.

III.4.5.7 Follow-Up of SAEs

All SAEs must be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or the subject is lost to follow-up. The investigator is responsible to ensure that follow-up includes any supplemental investigations as may be indicated to elucidate as completely as practical the nature and/or causality of the SAE. This may include additional laboratory tests

or investigations, histopathological examinations, or consultation with other health care professionals. New or updated information should be recorded on the originally completed SAE form with all changes signed and dated by the investigator.

III.4.5.8 Prompt Reporting of SAEs

SAEs must be reported promptly as described Table III.5, once the investigator determines that the event meets the protocol definition of an SAE.

III.4.5.9 Regulatory Reporting Requirements

The investigator must promptly report all SAEs to the Data Collection Centre. We have a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a drug under clinical investigation. Prompt notification of SAEs by the investigator to the appropriate project contact for SAE receipt is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

The investigator, or responsible person according to local requirements, must comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the IRB/IEC.

If new safety information (e.g., revised Clinical Investigator's Brochure) becomes available, the principal investigator is required to promptly notify her local IRB or IEC.

Table III.5. Time frames for submitting significant adverse event (SAE) reports.

Type of SAE	Initial SAE Reports		Additional Information on a Previously Reported SAE	
	Time Frame	Documents	Time Frame	Documents
Death, result of an AE or reasonable possibility	24/48 hrs[a]	SAE form	48 hrs	Updated SAE form
Death, not result of an AE and not a reasonable possibility	48 hrs	SAE form	48 hrs	Updated SAE form
Life-threatening event, regardless of relationship to study drug	24/48 hrs[b]	SAE form	48 hrs	Updated SAE form
Other SAEs	48 hrs	SAE form	48 hrs	Updated SAE form

[a] Initial notification should be sent within 24 hours of the investigator learning of the death. Fully completed documents (SAE form) should be sent within 48 hours.

[b] Initial notification should be sent within 24 hours of the investigator learning of the life-threatening event. Fully completed documents (SAE CRF pages) should be sent to within 48 hours.

III.4.5.10 Post-Study AEs and SAEs

Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE at any time after a subject has been discharged from the study, and such event(s) is(are) reasonably related to the study drug, the investigator should promptly notify the Data Collection Centre.

III.4.6 Subject Discontinuation/Withdrawal

Patients may discontinue protocol treatment for one or more of the following reasons/criteria:

- Unacceptable toxicity as defined in Section III.3.2.2 and III.3.2.3.
- Intercurrent illness which, in the opinion of the investigator, would seriously impair the successful completion of the protocol regimen. (If protocol treatment is stopped for his reason, every effort should be made to offer standard therapy or similar if and when it is possible to resume treatment).
- Tumour progression as defined using RECIST criteria in section III.4.
- Request by the patient (In this case the patient must be informed that he/she may be forfeiting substantial clinical benefit and even, potentially cure, and a standard alternative should be offered).
- If, in the judgement of the responsible investigator, the protocol is no longer in the best interests of the patient. A suitable alternative should be discussed with the patient.
- Other reasons, which should be explicitly recorded.

Every effort should be made to follow up all patients. Patients whom are prematurely discontinue from protocol therapy, should have blood for plasma osteopontin and survival completed according to study calendar. If protocol therapy is stopped prematurely, treatment is at the discretion of the investigator.

III.4.7. Statistical Considerations

Sample size: a 95% confidence interval about a proportion p is calculated using the formula:

$$p \pm 1.96\sqrt{p(1-p)/n}$$

For 52 patients and an anticipated response rate of 52% a 95% confidence interval will have bounds no greater than ±14%.

Statistical analysis: the pathological complete response rate will be calculated and a 95% confidence interval will be constructed. The response rate will be compared to patients who received the same regimen but without radiation using a chi-square test.

Kaplan-Meier estimates of overall survival and the disease free interval will be made and comparisons made with subjects on the same regimen but without radiation using log-rank tests.

The relationship between 99Tm sestaMIBI imaging at each of the three time points prior to surgery and response will be evaluated using logistic regression. ROC curves may be used to further evaluate the usefulness of this imaging technique as a marker for tumour response. Similarly, cellular

response/sensitivity to CNCR using the ex vivo 3D human tumour will be evaluated as a marker for tumour response.

Toxicities and adverse events will be described. Ninety-five percent confidence intervals about the percentages experiencing toxicities and adverse events will be calculated.

III.4.8 Ethical, Regulatory and Administrative Issues

III.4.8.1 Retention of Patient Records and Study Files

An Investigator shall retain records defined as essential under GCP guidelines for a period of 2 years following the date of marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued.

III.4.8.2 REB Approval

It is necessary to obtain local ethics approval of the protocol, letter of information and consent form. Annual re-approval is required as long as patients continue to be followed on the trial.

III.4.8.3 Amendments

An amendment to a protocol is a change significant enough to require review/approval by local REBs (and, if applicable, by the TTD of the HPB).

Protocol amendments will be circulated in standard format with clear instructions regarding REB review.

III.4.8.4 Informed Consent

The REB of an institution must approve the consent form document which will be used at that centre prior to its local activation; changes to the consent form in the course of the study will also required REB notification/approval.

The following elements must appear in the consent form: a description of the purpose of the study (indicating, if appropriate, that the drug is investigational); potential side effects; potential benefits; study design; voluntary participation; and confidentiality. It is essential that the consent form contain a clear statement which gives permission for information to be sent to and source medical records to be reviewed by the other agencies as necessary.

III.4.8.5 Consent Process/Patient Eligibility

Patients who cannot give informed consent (i.e. mentally incompetent patients, or those physically incapacitated such as comatose patients) are not to be recruited into the study. Patients competent but physically unable to sign the consent form may have the document signed by their nearest relative or legal guardian. Each patient will be provided with a full explanation of the study before consent is requested.

III.4.9 Publications

Results of this trial will be submitted for publication in a peer review journal.

III.5 REFERENCES

1. Adelstein DJ et al. An Intergroup phase III comparison of standard radiation therapy and two schedules of concurrent chemoradiotherapy in patients with unresectable squamous cell head and neck cancer. JCO 2003; 21: 92-98.

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APPENDIX IV

Evaluation of The Response Of Locally Advanced Breast Cancer To a Novel Neoadjuvant Chemoradiation Therapy Protocol: Biological Studies.

APPENDIX IV. EVALUATION OF THE RESPONSE OF LOCALLY ADVANCED BREAST CANCER TO A NOVEL NEOADJUVANT CHEMORADIATION THERAPY PROTOCOL: BIOLOGICAL STUDIES.

IV.1 INTRODUCTION

Locally advanced breast cancer (LABC) represents 10-15% of all new breast cancers, with a 5-year survival of 50% using standard treatment that includes neoadjuvant chemotherapy, surgery and adjuvant radiation [1]. LABC is traditionally defined as stage IIB (T3N0) and Stage IIIA/B from the TNM classification [2]. Clinically, these tumours are greater than 5 cm in size and/or extend beyond the breast tissue into the surrounding skin or muscle, with/without matted axillary lymph nodes (N2), internal mammary nodes (N2) or ipsilateral supraclavicular lymph node involvement (N3).

However, a small subset of women who receive neoadjuvant chemotherapy and achieve a pathologic complete response (pCR), (defined as no microscopic residual invasive breast cancer following neo-adjuvant treatment) have a vastly improved 5 year disease free survival rate of 87% [3] and 5-year overall survival rates of 89% [3] and 90% [4]. As such, pCR rates have become a surrogate measure for favourable long-term outcomes in trials involving neoadjuvant treatment [5], particularly since *in vivo* assessment is the only method by which a response can be measured.

Pathological complete response (pCR) at surgery is the best current surrogate for overall survival [5], therefore this was the primary end-point of this single-arm prospective Phase II trial. RNA Integrity Number (RIN), previously demonstrated to predict treatment response to neoadjuvant chemotherapy in NCIC MA-22 trial, has been validated as a predictive marker of pCR [6].

In order to improve survival from breast cancer, novel cytotoxic agents have been tested following, or concurrently with, anthracycline chemotherapy, notably taxanes [7, 8]. Docetaxel is a microtubule-stabilizing agent that induces cell-cycle arrest at mitosis and apoptosis [9, 10]. Based on its activity in the metastatic setting, docetaxel has been tested in randomized trials in early stage breast cancer, and demonstrated superior survival when added to anthracycline-based regimens compared to these regimens alone. FEC-D (fluorouracil 500mg/m^2 IV, epirubicin 100mg/m^2, cyclophosphamide 500mg/m^2 IV every 3 weeks x 3 cycles, followed by docetaxel 100mg/m^2 IV every 3 weeks x3) remains a commonly employed regimen in the adjuvant post-operative setting [11-15]. In spite of the improved outcomes associated with the addition of taxanes to neoadjuvant chemotherapy regimens, the gains in achieving pCR have been modest. The most striking pCR rates have been in Her2+ breast cancers with the use of neoadjuvant trastuzumab (Herceptin), where pCR rates may exceed 50-60%, however this is only true for a minority of patients [16].

It is well known that no two cancers are alike in their response to chemotherapy [6, 17]. Cancers of the same subtype, grade, stage and immunohistochemistry often respond quite differently to the same chemotherapy

regimen, very likely due to differences in tumour phenotype and genotype [18]. Despite this well-documented heterogeneity in response to chemotherapy, chemotherapy selection decisions continue to be based on large adjuvant randomized clinical trials, which have a “one for all” approach to chemotherapy drug selection. Since chemotherapy for breast cancer is usually delivered in the adjuvant setting, there is no clinical opportunity to assess *in vivo* response (or resistance) to the selected regimen. The tumour is deemed to have been resistant only when disease recurs, usually as distant metastases, which are no longer curable. Therefore it would be a significant clinical asset to develop early measures of chemotherapy sensitivity for any individual proposed regimen, allowing clinicians to tailor therapies effective for each individual patient (‘individualized medicine’). RNA integrity (RIN) has been demonstrated to be a good predictor of response to neoadjuvant chemotherapy as evidenced through the NCIC MA-20 clinical trial (Parissenti, Guo et al, 2015), and is therefore felt to be a potential individualized method of testing a tumour’s likelihood of responding well to chemotherapy early in treatment, rather than waiting until after the completion of a potentially ineffective cytotoxic regimen. RIN represents a quantification score of the degree of fragmentation of ribosomal RNA (rRNA), a process that occurs during degradation. Quantifying the rRNA integrity allows for a measure of how much degradation has occurred, and is a useful tool for scientists to gauge the reliability of the data they have obtained in RNA studies. The scale is from 1 (complete degradation) to 10 (completely intact RNA) [19].

Anatomic imaging tools, such as ultrasound and MRI, are capable of measuring size of the tumour; however, these modalities may not be as able to detect changes in amount of viable tumour a result of response to treatment (viable tumour in the specimen being replaced by stromal or fibrotic tissue). SPECT-CT imaging using ^{99m}Tc-bound to MIBI substrate is a functional nuclear medicine test that can show functional changes in the tumour as a response to treatment [20-22]. This substrate, injected intravenously, is avidly taken into tumour cells, showing a bright tumour on the initial 10min image. The substrate, however, actively effluxes through drug efflux transmembrane protein pumps, such as ABC transmembrane glycoprotein pumps (i.e. P-glycoprotein (Pgp), breast cancer resistance protein (BCRP) or multidrug resistance protein (MDP)) [23, 24]. These are the same drug efflux proteins felt to contribute, in part, to resistance to chemotherapy-induced cytotoxicity by actively effluxing the chemotherapeutic agent. Therefore, cancer cells able to efflux MIBI efficiently should be also able to efflux chemotherapy. As a result, if efflux pumps are working, rapid washout of MIBI substrate should be apparent, specifically in tumours with presumed chemotherapy resistance. It has been demonstrated that SPECT-CT imaging may be a useful test to predict sensitivity to chemotherapy in LABC [25, 26], but its sensitivity has not been evaluated in response to clinical outcomes among LABC patients, and has not been studied in the setting of serial evaluations during active treatment. We sought to evaluate whether SPECT-CT imaging could be used at baseline to predict sensitivity to chemotherapy when compared to pCR, the surrogate for survival. Secondarily, we sought to explore

whether there might be any interesting relationship between pre- mid- and post-treatment MIBI SPECT-CT imaging in terms of whether chemo-resistance could be seen to be developing over treatment in patients who then were found to be clinically resistant to treatment.

The OncoScreen® chemosensitivity assay is based on a 3D gel assay that was developed to examine the effects of radiation on the invasiveness of brain cancer cells (Dr. Penny Costello, personal communication). It provides a tissue-like environment for testing of tumour growth, invasion and response to chemotherapeutic agents *ex vivo* that more closely models the clinical situation of examining tumour growth in the context of breast cancer treatment protocols. Typically, tumours are surgically resected, followed by adjuvant therapy (which can include chemotherapy), radiation and other biological and hormonal therapies. In this scenario, there would likely be no primary tumour to contend with, only migrating cells seeking to establish themselves as new tumours. Thus, assessing sensitivity to drugs in a migration/invasion assay may be the best predictor of an individual tumour's chemotherapy response at this stage of treatment.

Most *in vitro* culture experiments, such as invasion assays, utilize 2-dimensional monolayer cultures exposed to a variety of agents [27], 3-dimensional spheroids [28, 29], or cultured cells plated in a 3-dimensional overlay culture on matrices in order to explore tumour response to various conditions [30, 31]; co-cultured cells in matrix and/or in transwells may be used to evaluate tissue interactions [32]. Three-dimensional cultures have been reported

in the literature as a tool for exploring tumour invasiveness within the stromal microenvironment *in vitro* [33]. Others have also used this model to determine breast cancer response to chemotherapeutic agents, such as rapamycin [34].

There have been three reported publications using the 3-dimensional human breast cancer invasion assay similar to the OncoScreen® chemosensitivity assay as method of screening for individual sensitivity to chemotherapies [35-37]. As a result, this method was felt to represent an *ex vivo* model, which could use tumour samples obtained by needle biopsy to predict individual chemotherapeutic sensitivities prior to treatment delivery.

Given the poor prognosis of LABC, we proposed the use of an adjuvant regimen in the neoadjuvant setting, using the taxane (docetaxel) concurrently with radiation for radiosensitization. As an addition to a clinical trial (Chapter 2), three separate sub-studies were undertaken to assess the response of LABC tumours to treatment: RNA Integrity Assay (RIN), serial single photon emission computed tomography (SPECT-CT) imaging (sestaMIBI), and serial *ex vivo* studies of biopsied tumours (3D gel invasion assay) assessing the tumour invasiveness in response to chemotherapy.

IV.2. METHODS

IV.2.1 Patients and Therapeutic Regimen

Thirty-two patients with stage III non-metastatic LABC were enrolled at a single institution between 2009 and 2011 (see Chapter 2). They were treated with neo-adjuvant 5-fluorouracil, epirubicin and cyclophosphamide q3 weekly for

4 cycles followed by weekly docetaxel (35mg/m^2) concurrently with regional radiation (45Gy with 9Gy boost in 25 & 5 fractions) for the first 6 of 9 weeks. Patients underwent serial tumour biopsy for biological substudies (14-gauge tumour core biopsy) pre-, mid- and post-treatment and the biopsy specimen was stored in refrigerated phosphate buffered saline for transportation to the laboratory. One mm^3 section was then taken from the biopsies, immersed in RNAlater™, and stored frozen at -80°C. At the completion of the third post-treatment biopsy procedure, the patient then underwent a modified radical mastectomy.

IV.2.2 RNA Integrity Assay

IV.2.2.1 RNA Isolation from Tumour Core Biopsies

RNA was isolated from image-guided tumour core biopsies of the patients pre-, mid-, and post-treatment using Qiagen RNAeasy® Mini kits, as per manufacturer's instructions. Briefly, The biopsies were homogenized and the lysate was then passaged at least 5 times through a 20-gauge needle (0.9mm diameter) fitted to an RNase-free syringe. The sample was centrifuged at high speed in a refrigerated microfuge at 4°C for 3 minutes, with transfer of the supernatant to a new tube. One volume (500µl) of 70% ethanol was then added to the supernatant and the sample mixed well by repeated pipetting. A maximum of 700µl of the sample, including any precipitate, were added to an RNeasy® mini column and placed in a 2ml collection tube. The column was washed twice in RPE buffer and dried by centrifugation as per the manufacturer's protocol. The

RNA was then eluted from the column in 30µl of RNase-free water and the eluate reapplied and eluted from the column to increase the yield and concentration of the RNA obtained.

IV.2.2.2 Assessment of RNA Quality

The obtained RNA samples were applied to RNA 6000 Nano Lapchips™ (Agilent Biotechnologies, Inc.) and subjected to capillary electrophoresis using an Agilent® 2100 Bioanalyzer. The protocol followed was identical to that described in the company's technical brochure for the Agilent® 2100 Bioanalyzer. The amount and quality (RIN value) of RNA from each core biopsy was then determined by the Bioanalyzer.

IV.2.3 Serial SPECT-CT Imaging

IV.2.3.1 Sesta-MIBI Scans

^{99m}Tc-labelled sesta-MIBI scintimammography was performed on the LABC patients enrolled in the study (see IV.2.1) at the following time points: at the time of clinical diagnosis of LABC, in the middle of neoadjuvant chemoradiation therapy with FEC-D (after FEC chemotherapy was completed and prior to initiation of weekly docetaxel/radiation), and at 5 weeks post-treatment (immediately prior to surgery).

IV.2.3.2 Sesta-MIBI Injection, Scanning Protocol and Analysis

Patients underwent MIBI imaging (GE Infinia GP3 Hawkeye 4 SPECT/CT scanner) using the prone lateral imaging technique, which allows visualization of the breast tumour without contamination by the overlaying structures [38, 39]. ^{99m}Tc-labelled sesta-MIBI (Lantheous, Montreal QC) was injected via gauge 20 catheter placed in the patient's arm contralateral to the breast tumour lesion. Patients underwent IV injection of 750MBq (20mCi) ^{99m}Tc-labelled sesta-MIBI, followed by 30ml saline flush. Prone lateral imaging was performed 10min after injection, and then at 3 hours post-injection. Images of the anterior, left lateral and right lateral positions were acquired for each patient, using a high-resolution, low-energy, parallel hole collimator, 512x512 matrix, no zoom, 15% energy window centered at 140keV.

To measure MIBI washout [22, 40], a second MIBI scan was performed 3 hours post-injection. Care was taken to reproduce breast positioning compared to early image; timing of both early and late scans relative to tracer injection was carefully noted.

MIBI images were analyzed on XELERIS station (GE) using a method routinely used at our centre. Briefly, circular region of interest (ROI) was placed over the tumour on axial slice, which represented a maximum count from the tumour. Background (Bkg) counts were obtained from the same ROI/area from the opposite breast. The same process was repeated on early and delayed images. Count number was corrected for decay to obtain accurate calculations. Wash-out calculation was performed using the following formula:

$$washout = 100\%\, x\ \left(early\ \frac{tumour}{bkg}\right) -\ delayed\ \left(\frac{tumour}{bkg}\right) / early\ \frac{tumour}{bkg}$$

Lesion-to-normal breast (L:N) ratios were used to analyze MIBI uptake. Changes in MIBI uptake with therapy were expressed as the percentage of baseline L:N ratio and were compared with different categories of response to therapy. If the efflux was more than 30% of the baseline, the tumour was classified as chemotherapy resistant (R); conversely, equal to, or less than 30% washout led to tumour classification as chemotherapy sensitive (S) [40]. Statistical analysis was performed using SAS statistical software, with significance level set to $p<0.05$.

IV.2.4 *Ex vivo* Tumour Studies

IV.2.4.1 Protocol Rationale and Patient Recruitment

The OncoScreen® chemosensitivity assay was selected to test whether a 3D human tumour culture tool could be used to individually predict patient responsiveness to neoadjuvant chemotherapy. Patients were recruited as described in Section IV.2.1 and Chapter 2. Tissue samples were obtained from 32 adult females between the ages of 35 and 88, diagnosed with invasive mammary carcinoma and undergoing a neoadjuvant clinical trial of FEC chemotherapy followed by weekly docetaxel concurrent with locoregional radiation prior to modified radical mastectomy (see Chapter 2). Samples were at

baseline (i.e. prior to all chemotherapy), mid-way through FEC regimen (but prior to radiation), and following docetaxel and radiation, just prior to surgery.

IV.2.4.2 Tumour Tissue Sample Handling

Tissue samples were received directly from the diagnostic imaging department where image guided biopsy samples were taken, and placed in a sterile phosphate-buffered saline solution at 4^{o}C, within minutes of acquisition. The sample was transported to the laboratory where it was placed into a dissecting dish with a small amount of sterile buffer solution to cover the tissue. Using a scalpel and forceps, the tissue was cut into 1-3mm pieces, dissecting away any normal or non-viable material. The pieces were then washed with sterile saline to remove any remaining blood and debris.

IV.2.4.3 Tumour Invasion Assay

The tumour invasion of the biopsied tumour pieces was assayed using a collagen gel system. Briefly, a single piece of tissue was placed into the center of each well of a 48-well plate containing 0.25ml matrix mixture, ensuring the placement of each tissue fragment as close to the center of the well as possible. The gel was then permitted to set at room temperature, or at 37^{o}C non-CO_2 incubator. Each well was overlaid with 0.25ml tissue culture media containing 20% serum to achieve a final volume of 0.5ml per well. Collagen type I gel (Vitrogen 100, Cohesion Technologies, Palo Alto, CA) was added to the matrix buffer at a concentration of 1mg/ml and mixed, adjusting the pH to 7.4.

To assess tumour invasion in response to treatment of breast cancer cells with standard breast cancer chemotherapies, 48-well plates were again seeded with fresh tissue from patients in six replicates. Tissues were either left untreated (control) or treated with the following individual chemotherapeutic agents: FEC (5-fluorouracil (5-FU), epirubicin (epi), cyclophosphamide (cyclo)) or docetaxel (doc). The recommended intravenous therapeutic dosage for patients was used and reconstituted into 0.5ml total, and added to each well (5-FU 10µg/ml; epi 4µg/ml; cyclo 20µg/ml and doc 3µg/ml). Plates were maintained at 37^{o}C with 5% CO_2 for 5 days, monitoring cell movement and invasion on days 1, 3 and 5. A screen was only deemed valid if there was cell movement or outgrowth from the main tumour sample in at least two of the six replicate wells. This was done on two separate 48-well plates per patient: one plate was irradiated (0.8Gy using ^{60}Cobalt γ-radiation) while the second plate was not, in order to mimic *ex vivo* the treatment being received with concurrent chemoradiation *in vivo* (R-control, R-FEC, R-Doc).

IV.3 RESULTS

IV.3.1 Clinical Responses and Toxicities of FEC-D Neoadjuvant Chemoradiotherapy

While 30 of the 32 patients (94%) completed the treatment protocol described above, patients did experience significant toxicities. Twenty-seven patients (84%) had grade 3 or greater toxicities, including grade 3 resolving pneumonitis (6 patients), grade 3 dermatitis (6 patients) and one treatment-

related death. Eight of these patients (23%) exhibited a pathologic complete response (pCR) to treatment (Table 2.3), which is approximately twice the Ontario pCR rate for locally advanced breast cancer (10%, unpublished data) and significantly higher than the 14% pCR rate seen in 81 matched controls (see Chapter 2). Moreover, at three years median follow-up, the relapse-free survival rate was 100% in the pCR cohort and 65% among partial responders (PRs). This suggests that the regimen, while exhibiting strong toxicity, appears to enhance the pCR and shows a trend toward a 15% improvement in disease-free and overall survival in locally advanced breast cancer patients.

Tumours that exhibited pCRs were distributed almost equally amongst the basal (2 of 5 tumours = 40%), Her2 (3 of 3 tumours = 100%), and luminal B (3 of 6 tumours = 50%) subtypes. No pCRs were found among the 11 patients with luminal A tumours (0%). While the numbers are small, the data suggests that FEC-D regimen with concurrent radiation appeared able to induce pCRs across a variety of breast cancer subtypes, except for the luminal A subtype. This supports existing data in the literature that pCR is a good surrogate for survival with the exception of luminal A subtype [41].

IV.3.2 Changes in Tumour RNA Content in Response to FEC-D Neoadjuvant Chemoradiotherapy

We then assessed whether, similar to the NCIC-CTG-MA.22 clinical trial, changes in tumour RNA quality or quantity could be observed during or in response to treatment and whether low RNA quality was associated with a strong

clinical response the completion of treatment (i.e. pCR). Figure IV.1 illustrates the RNA concentration values for all patient biopsies isolated prior to treatment, in the middle of treatment, and post-treatment. The plot shows that there was some significant variability in the quantity of RNA isolated from the biopsies throughout treatment, including pre-treatment biopsies. This suggests possible variations in the preservation of RNA in the collected biopsies and time-dependent degradation at the time of tissue processing. In addition, the data suggests little difference in RNA content between pre-treatment biopsies and biopsies collected after FEC chemotherapy (mean tumour RNA concentration of 50.0±15.1 and 50.0±11.9ng/μl, respectively). In contrast, the mean tumour RNA concentration fell significantly after the completion of the FEC-D regimen with concurrent radiation (10.6±2.1ng/μl, $p<0.05$). These findings suggest that the FEC chemotherapy alone is insufficient to induce reductions in tumour RNA content, but upon treatment with concurrent radiation therapy and docetaxel, tumour RNA content falls dramatically. Despite this treatment effect, no significant differences in tumour RNA content were observed amongst patients that exhibited a pathologic complete response post-treatment (pCR), patients that exhibited a partial response to treatment (PR), and patients with stable or progressive disease (SD or PD) post-treatment (Figure IV.2).

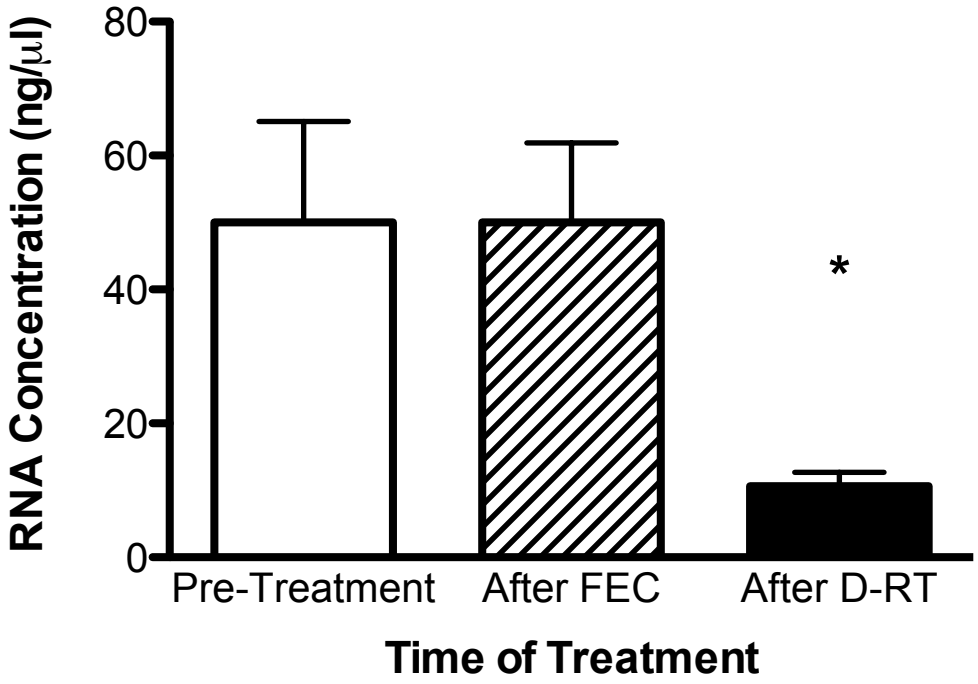

Figure IV.1. Changes in tumour RNA concentration during treatment of locally advanced breast cancer. There was a significant decrease in tumour RNA content in response to treatment with docetaxel followed by radiation therapy (* signifies $p<0.05$).

FEC, fluorouracil-epirubicin-cyclophosphamide; *D-RT*, docetaxel concurrent with radiation therapy.

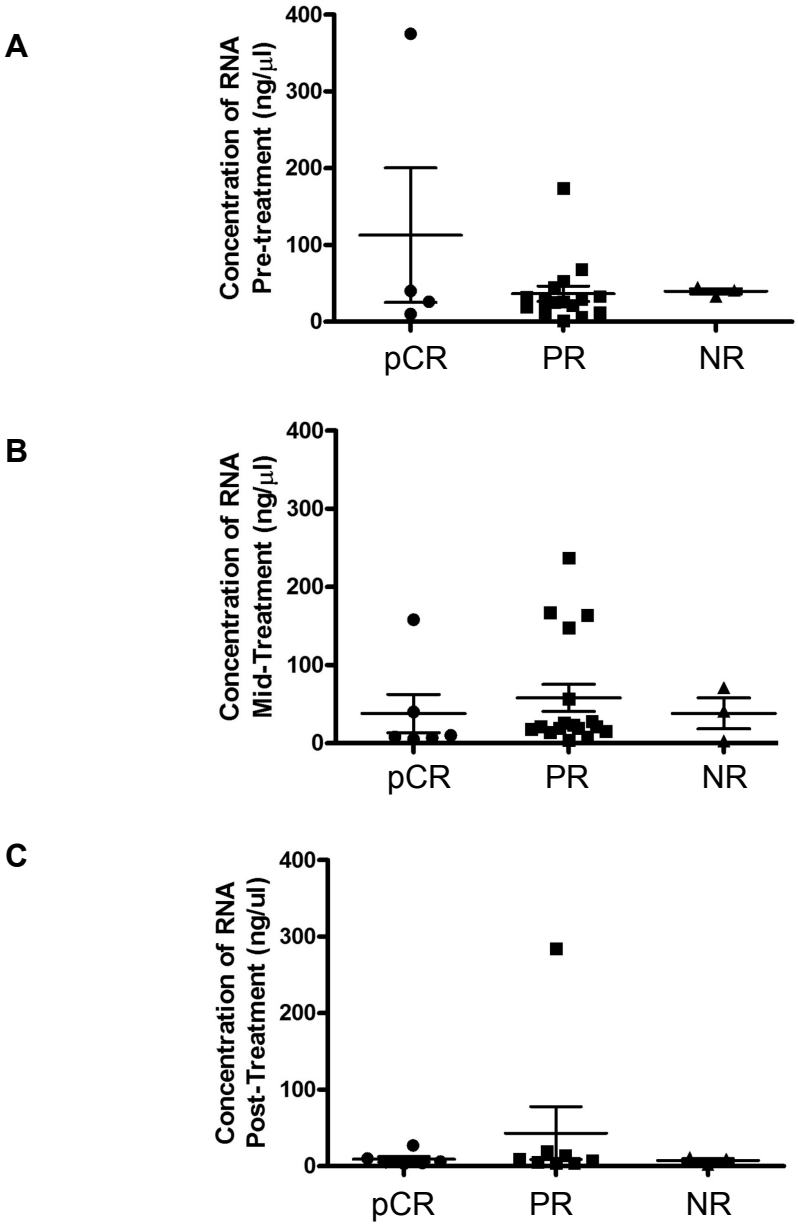

Figure IV.2. Changes in tumour RNA content (A) pre-treatment, (B) during treatment and (C) post-treatment of locally advanced breast cancer. No changes in tumour RNA content were observed amongst patients exhibiting pCR, PR or NR (both SD and PD).

pCR, pathological complete response; *PR*, partial response; *NR*, no response; *PD*, progressive disease; *SD*, stable disease.

IV.3.3 Changes in Tumour RIN Values in Response to FEC-D Neoadjuvant Chemoradiotherapy

To assess changes in tumour RNA content during FEC-D chemotherapy with concurrent radiation treatment, all samples that were noted as “insufficient signal” for mathematical analysis were omitted. As shown in Figure IV.3A, in the three samples post-FEC chemotherapy but before docetaxel/radiation treatment (which achieved a pCR at the end of treatment), 2 out of 3 samples demonstrated RIN values indicative of high RNA integrity or minimal RNA degradation (RIN >7). Only one patient sample had a very low RIN value suggestive of significant loss of RNA integrity (RIN value = n/a or 0). In the samples from non-responding patients (patients who did not achieve pCR post-treatment), no effect of FEC treatment could be discriminated using RIN. These results suggest that FEC treatment may have an effect on RNA disruption, but this effect did not appear to be associated with a pCR post-treatment, since only 1 out of 3 adequate samples demonstrated this effect.

When tumour RNA integrity was assessed, after both the FEC chemotherapy and docetaxel/radiation treatment, only two samples from patients that achieved a pCR post-treatment had sufficient RNA for mathematical analysis (Figure IV.3B), presumably because there was no viable tumour left in the biopsy sample from which to extract RNA, and/or that what was thought to be tumour by image guidance actually represented only stromal fibrosis rather than residual tumour. For these two samples, both had RIN values of n/a or zero, indicative of very strong loss of RNA integrity. These two responders to treatment were

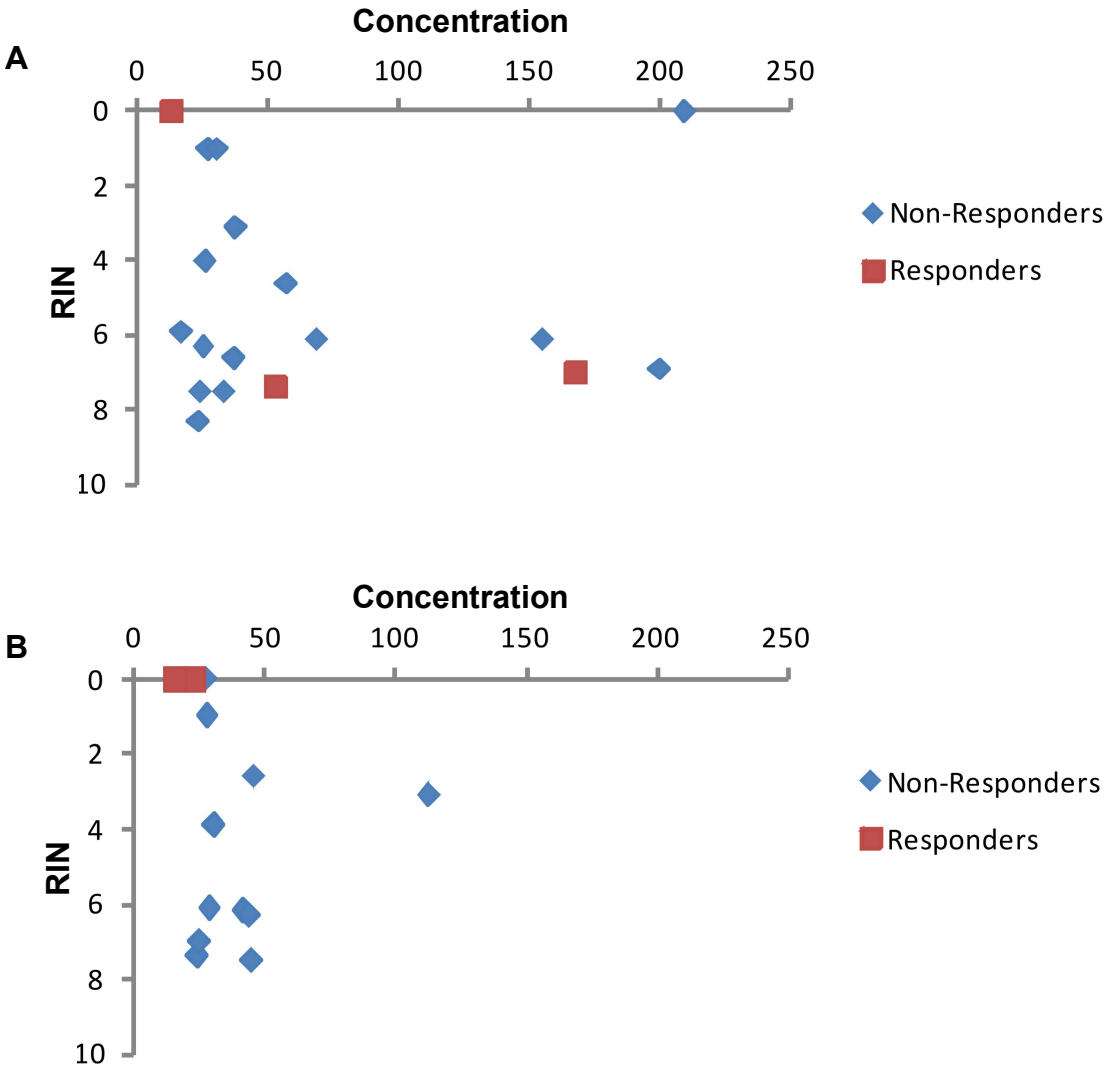

Figure IV.3. Changes in tumour RNA integrity in response to treatment of locally advanced breast cancer: (A) mid-treatment and (B) post-treatment. Following FEC (but prior to docetaxel) and radiation, the responders (patients achieving pCR) had a varied RIN level similar to non-responders (patients NOT achieving pCR), while after concurrent chemoradiation, all responders had low RIN levels, consistent with a treatment-related effect on RNA integrity. *RIN*, RNA integrity number

strongly distinct from non-responders based on RNA concentration and RIN values. The low RIN values are indicative of loss of normal RNA. In the non-responders, a wide range of RIN values are noted which is indicative of a spectrum of change in tumour RNA from highly fragmented to highly intact. These results suggest that loss of RNA integrity occurred with radiation and docetaxel resulting in decreased RIN values and a loss in RNA concentration. This loss of RNA integrity correlated with a strong response to treatment (pCR).

IV.3.4 Sesta-MIBI Serial SPECT-CT Imaging of LABC Tumours in Response to FEC-D Neoadjuvant Chemoradiation Treatment

Of 32 patients included in the study, 2 patients failed to complete the full MIBI protocol; these were then excluded from the study. Of the remaining 30 patients, tumours of 25 patients (83%) were found to be chemotherapy sensitive at baseline, 28 during mid-treatment (93%), and 22 out of 22 post-treatment (100%) (Figure IV.4). Chemotherapy sensitivity is presumed when a washout index of less than 30% is seen. With a low washout index, the cell's inability to efflux the cytotoxic chemotherapy is expected to result in damage to the cell in accordance with the molecular mechanism of cell death specific to whichever chemotherapy is delivered. Of those patients who achieved a pCR response to neoadjuvant chemoradiotherapy, pCR sensitivity was 8 out of 8 (100%). The sensitivity of the MIBI SPECT-CT imaging in the PR/SD cohort of patients was 17 out of 22 (77%) (difference of 23%) (Figure IV.5), which was not statistically significantly significant (p=0.287).

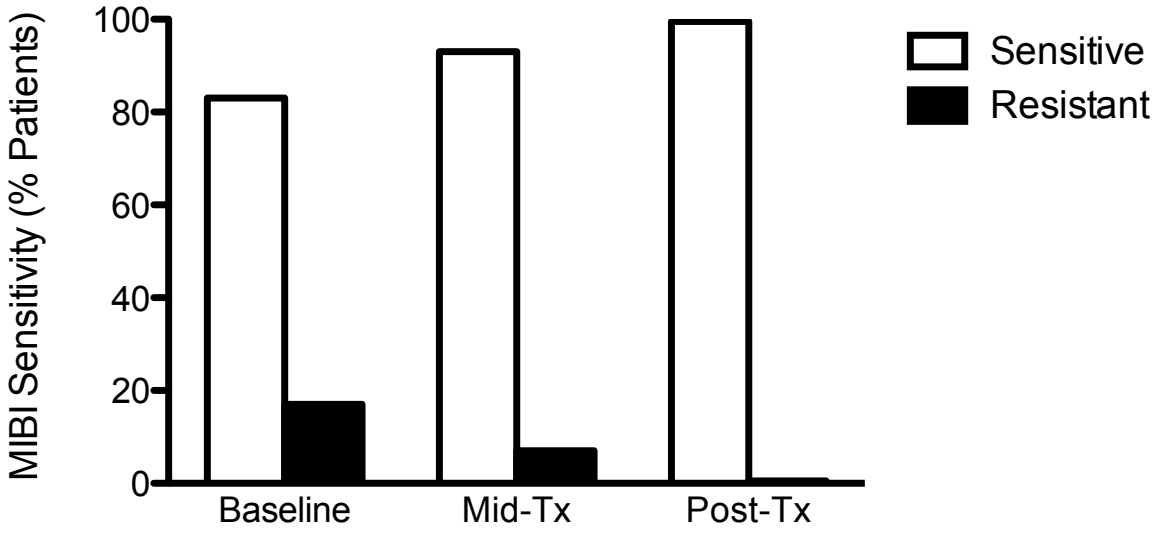

Figure IV.4. The tumour sensitivity to concurrent neoadjuvant chemo-radiation, as demonstrated by SPECT-CT imaging of sesta-MIBI washout. At baseline, 83% of patients demonstrated sensitivity to chemotherapy; at mid-treatment, 93% of patients were sensitive, and post-treatment, 100% sensitivity was achieved.

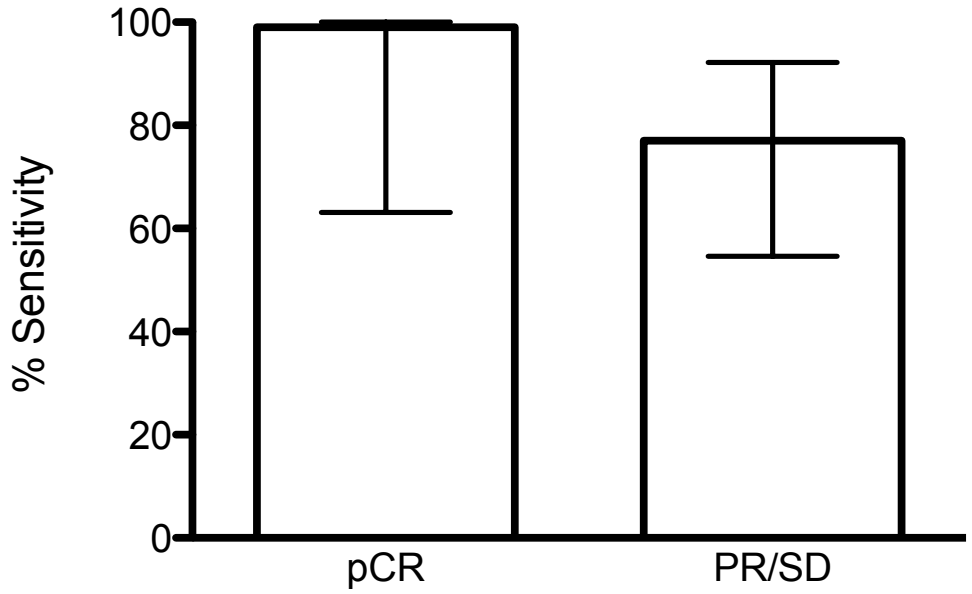

Figure IV.5. The tumour sensitivity (mean ± 95% exact confidence interval) to concurrent neoadjuvant chemo-radiation, as a function of pCR. Among the 8 patients who achieved a complete pathological response, low washout index (and, therefore, chemosensitivity) was seen in all 8/8 (100%) patients. In the remaining 22 patients who demonstrated a partial response or stable disease to chemotherapy, 17/22 (77.3%) patients demonstrated chemosensitivity by washout index (p=0.287, Fisher exact test).

IV.3.5 *Ex vivo* Tumour Studies

The number of tumour samples demonstrating growth (invasive cells seen beyond the tumour sample invading into the surrounding Matrigel®) was significantly impacted by the *in vivo* treatment. At baseline, prior to chemotherapy, 100% of the 32 patients had tumours that exhibited growth using this invasion assay. After FEC chemotherapy, 17/32 (53%) of tumour samples exhibited growth. Following combined docetaxel and radiation, 7/23 (30%) of tumour samples demonstrated growth using this *ex vivo* model. Therefore, the *ex vivo* model appears to be most effective as an invasion assay when the baseline untreated patient tumour samples are used. The pre-treatment, mid-treatment and post-treatment mean tumour growth (as a percentage of control to account for intra-tumoural heterogeneity) was calculated for tumour samples cultured with FEC chemotherapy, DOC (docetaxel), R-FEC (FEC chemotherapy while also radiated at 0.8Gy to mimic radiosensitizing chemotherapy) and R-DOC (docetaxel chemotherapy while also radiated at 0.8Gy) (Figure IV.6). There was wide variety of responses both within patient wells and between patients treated with the same chemotherapy regimens, resulting in large standard error bars. As a result, these results do not demonstrate a clear reduction in growth with any particular chemotherapeutic agent, even when tumour growth is personalized (calculated as a proportion of the same tumour sample cultured alone, as a control). The only visual difference is seen in the radiated docetaxel samples, where the mid- and post-treated samples appear to show less tumour growth

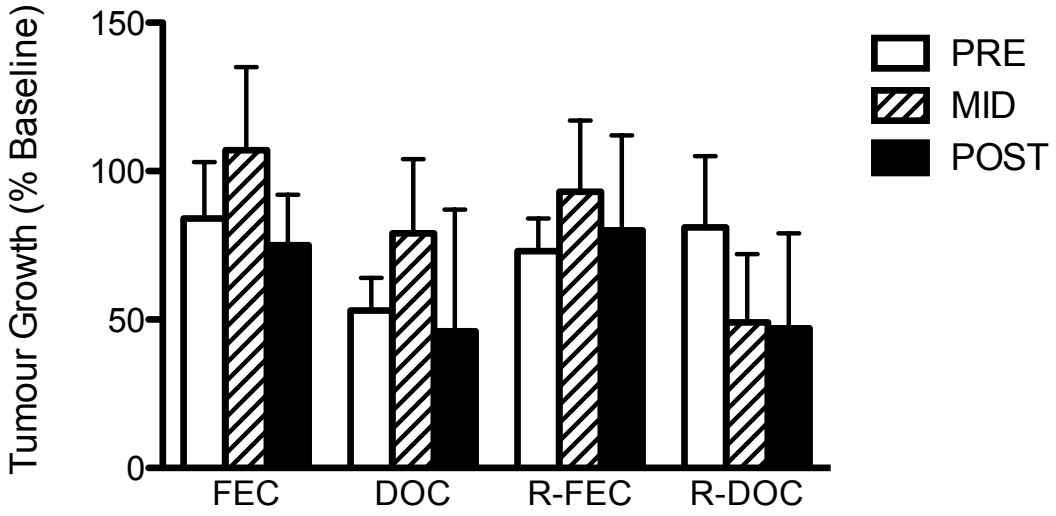

Figure IV.6. The tumour sensitivity to concurrent neoadjuvant chemo-radiation, as demonstrated by the *in vivo* 3D gel invasion assay. Tumour growth sizes were obtained from core biopsies pre-treatment, mid-treatment and post-treatment. While not statistically significant, docetaxel appeared to exhibit a radiosensitizing trend. *PRE*, pre-treatment; *MID*, mid-treatment; *POST*, post-treatment.

than the pre-treated samples, perhaps demonstrating a radiosensitizing trend with the use of docetaxel not seen with chemotherapeutic agents alone.

Using the baseline pre-treatment samples of tumours exposed to FEC, DOC, R-FEC and R-DOC, the patients who achieved a pCR in response to the neoadjuvant regimen were compared to the non-pCR patients treated the same way (Figure IV.7). Of the patients that had achieved pCR, docetaxel alone resulted in 43% of baseline tumour growth, while addition of radiation further reduced the tumour growth to 34% of baseline. The radiated tumour samples appear to differ in the pCR cohort from the non-pCR cohort. The tumour samples radiated while exposed to FEC appear to have a higher growth in the pCR cohort, which is difficult to explain. In the pCR patient cohort, the tumour growth was much lower when exposed to concurrent docetaxel and radiation (R-DOC) than in the non-pCR cohort ($p=0.046$).

The tumour growth seen in baseline untreated tumour samples exposed to FEC, DOC, R-FEC and R-DOC were statistically analyzed using Wilcoxon Rank Sums two-sided test to determine whether baseline growth predicted for death or recurrence of disease. The baseline DOC growth *ex vivo* appeared to best predict for recurrence or death ($p=0.039$), while the others did not (FEC $p=0.71$; R-FEC $p=0.14$; R-DOC $p=0.29$).

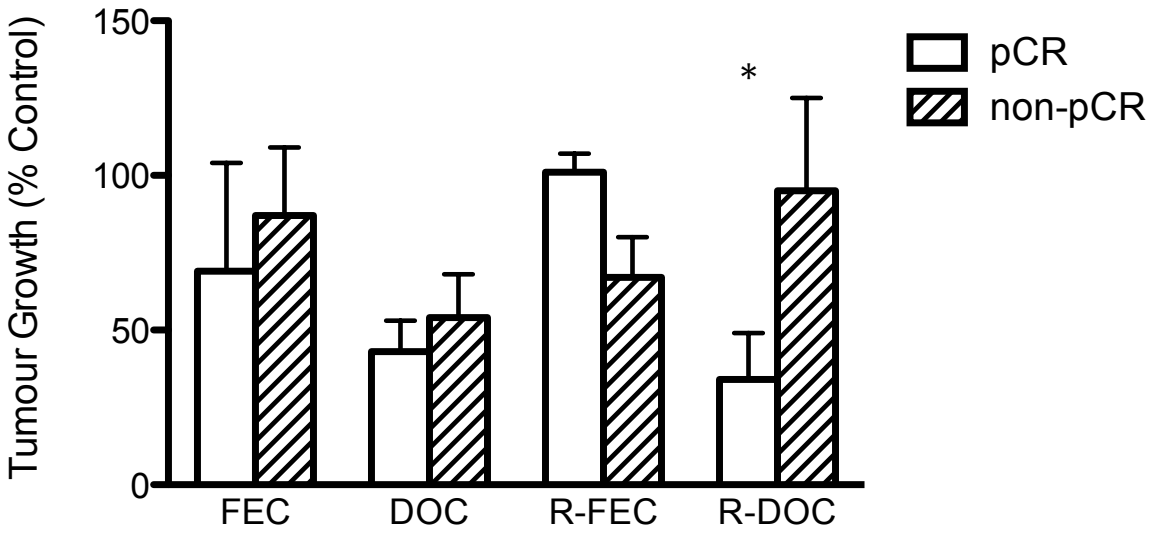

Figure IV.7. The tumour sensitivity to concurrent neoadjuvant chemo-radiation, as a function of pCR, in the *in vivo* 3D gel invasion assay. Tumour growth sizes were obtained from core biopsies. Docetaxel with radiation appeared to exhibit a radiosensitizing trend in patients exhibiting pCR response (p=0.046).

*p<0.05 from non-pCR group

FEC, fluorouracil-epirubicin-cyclophospamide; *DOC*, docetaxel; *R-FEC*, radiation with FEC; *R-DOC*, radiation with DOC.

IV.4 DISCUSSION AND CONCLUSIONS

This study was the first to use a full chemotherapy regimen with radiation in the neo-adjuvant setting for LABC. Although this regimen was not without toxicity, concurrent chemo-radiation significantly improved the surrogate marker for survival in this high-risk group, resulting in a much-improved outcome, even at short-term follow-up.

Of the tumour samples that provided sufficient RNA for analysis, RIN values appear to predict treatment response, particularly to taxane-based chemotherapy regimens. RNA concentration was lowest in tumour samples after concurrent chemoradiation with docetaxel. It may be that the radiosensitizing effects of docetaxel amplifies the genomic damage induced by external beam radiation, mimicking the improvement in clinical outcomes as a result of this combined therapy. Unfortunately, RNA testing revealed that many samples had undergone RNA degradation, likely as a result of prolonged transport time in PBS prior to being placed in RNA preservative. In order to fully evaluate the impact of RNA integrity as a predictive test for both neoadjuvant chemotherapy and radiation, these tests should be repeated prospectively, with samples being placed directly from the biopsy needle into RNA preservative.

The sesta-MIBI SPECT-CT imaging study demonstrated that, among the pCR cohort, this test had 100% sensitivity in predicting patient sensitivity to chemoradiation treatment. However, because the sensitivity for partial responders and non-responders was still high at 77%, this test does not discriminate sufficiently whether patients will respond to systemic treatment to be

a clinically useful test. The serial use of sesta-MIBI SPECT-CT imaging appears to show an increasing sensitivity to treatment over time. This is probably due to the possibility that a maintained efflux of MIBI substrate resulting in a low washout index over time in patients receiving chemotherapy and/or radiation may no longer be indicative of drug efflux capacity in tumour cells, since the post-treatment sensitivity to chemotherapy was 100%, and yet only a quarter of these patients achieved a pCR in response to chemotherapy. Perhaps it becomes difficult to determine where to measure washout of MIBI substrate when there is a treatment change in the imaged tumour, resulting in a falsely low washout index as treatment progresses. Further evaluation of this functional imaging modality would be helpful in elucidating the mechanisms around substrate and drug washout as measured serially in breast tumours receiving neoadjuvant treatment.

The 3D ex vivo OncoScreen® chemosensitivity assay model showed a wide variety of growth rates in response to the regimens given to these patients when assessed serially during treatment. As a result, there does not seem to be any visible trend in tumour growth over time, other than an apparent reduction in tumour growth in samples treated with docetaxel while concurrently radiated, perhaps demonstrating a radiosensitizing phenomenon. Further studies are required to elucidate this. The most interesting finding was that tumour growth appeared significantly inhibited by concurrent radiation and docetaxel in the patients who achieved a pCR compared to those who did not. Again, these are

preliminary findings, but do suggest that more studies should be done to exploit the radiosensitizing effects of taxane chemotherapies in breast cancer patients.

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V.2 Acta Oncologica

Radiation-Induced Lung Injury after Concurrent Neoadjuvant Chemoradiotherapy

for Advanced Breast Cancer; Acta Oncologica 2014, vol. 53(5): 697–701.

V.3 Current Oncology

Locoregional therapy of locally advanced breast cancer: a clinical practice guideline; Curr Oncol 2015, vol. 22(Suppl 1): S54-66.

Concurrent chemo-radiotherapy for locally advanced breast cancer-time for a new paradigm?; Curr Oncol 2015, vol. 22(1): 25-32.

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Subject: RE: permission request

Dear Dr Brackstone,
Permission is granted. Kindly be sure to give credit to Current Oncology.

Thank you,

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66 Martin St.
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Sent: Wednesday, September 23, 2015 1:38 PM
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Hello,

Please find the attached Permission Request Form, on the behalf of Dr Muriel Brackstone, for the articles titled 'Locoregional therapy of locally advanced breast cancer: a clinical guideline', published in 2015 issue of Current Oncology, vol 22 (Suppl 1): S54-66 and 'Concurrent chemo-radiotherapy for locally advanced breast cancer – time for a new paradigm?' published in 2015 issue of Current Oncology, vol. 22 (1): 25-32.

Dr Brackstone would like to use the articles as an appendix in her PhD thesis.

If you have any questions or concerns, do not hesitate to contact me, or Dr Brackstone directly.

Best regards,

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