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RESPONSE, RESISTANCE AND METASTASIS OF LOCALLY ADVANCED BREAST CANCER (LABC, stage 2B-3C) IN A MULTIETHNIC COHORT

A Phase II International Multicentric Study of Concurrent Paclitaxel and Radiation: Correlation of tumor profiles with pathological response

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SCHEMA

Eligibility: Locally Advanced Breast Cancer: STAGE 2B-3C (AJCC, see Appendix 16.2), HER *2/neu* negative.
Patients with inflammatory breast cancer or distant metastases are excluded. Patients with prior treatment for their breast cancer are excluded.

Baseline Requirements:

Core biopsy for confirmation of diagnosis, assessment of tumor molecular characteristics and tumor banking. Blood samples for special studies and banking.
Staging evaluation, including PET/CT scans of chest, abdomen and pelvis.
Baseline blood tests.
Baseline evaluation by Medical, Radiation and Surgical Oncologist

Treatment Schema:

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Pre-op Chemo*	▼X	▼X	▼X	▼X	▼X	▼X	▼X	▼X	▼X	▼X	X	X			▮		▮	
Radiation**	▼	▼X	▼X	▼X	▼X	▼X	X								▮		▮	
Surgery***												▼			▮X		▮	

* pre-operative chemotherapy consists of Paclitaxel (Taxol or Abraxane) 30 mg/m^2 twice/week
** to the breast, axilla and supraclavicular area, concurrent with chemotherapy
*** within two months from the end of chemo-radiation

1. STUDY OBJECTIVES

1. To test concurrent paclitaxel and radiation in a prospective multiethnic cohort of patients with newly diagnosed stage 2B-3C breast cancer treated at three international academic centers under uniform conditions.
2. To assess the pathological response rate to concurrent paclitaxel-radiation at each collaborating institution and compare it to that achieved in a previous Phase I-II trial.
3. To obtain core biopsies of the original tumor before, and after treatment (from the surgical specimen) for molecular biology and genomic studies (projects 2-5, 7).
4. To acquire descriptive information on patient epidemiological, cultural and behavioral characteristics (for project 6).

2. BACKGROUND AND HYPOTHESIS

2.1 LABC: A Disease of the Underserved

The term ―locally advanced breast cancer‖ (LABC) commonly includes tumors whose maximum diameter is 5 cm (T3) or larger, or which present with involvement of the chest wall or skin. Surprisingly, the simultaneous presence of clinically detectable distant metastases is relatively infrequent (~8%), a peculiar finding since in 73% of these large tumors it is possible to document shedding of tumor cells into the blood [1].

While LABC has become a rare clinical presentation of breast cancer in the general population as a result of improved early detection by mammographic screening, it remains relatively common among minority women of low socioeconomic status [2]. For instance, in a consecutive series of 363 African-American women presenting in a large urban hospital[3], one out of three women newly diagnosed with breast cancer had LABC. It is well documented that although the incidence of breast cancer among African-Americans is lower than among white women, breast cancer mortality in African-Americans is significantly higher [4]. In 1998, the American Cancer Society, the National Cancer Institute and the Centers for Disease Control and Prevention reported an overall downward trend in cancer incidence and mortality between 1990 and 1995 for all cancers combined. Many minority and medically underserved populations, however, did not share equally in these improvements. These patients have continued to encounter multi-factorial barriers to early detection and care, warranting interventions to improve access [5]. At the same time, it is equally important to offer the best chance for survival to those underserved women who have already availed themselves of medical care. Paradoxically, while the medical community is aware of the inadequate accrual of minority patients to clinical trials, only few trials exist for LABC.

2.2 LABC: a Worldwide Problem

The need to understand LABC is especially compelling in populations and countries with limited resources, where breast cancer incidence is relatively low, but mortality is comparably high [6]. In these settings access to appropriate cancer care is characteristically limited or often plainly nonexistent. In contrast to economically developed nations, where on average fewer than 20% of women present with breast cancer at advanced stages [7], LABC and metastatic disease are the most common stages at presentation in 50% or more women in Latin America, Asia and Africa. Although precise data are scarce, reports from areas as diverse as South America (Argentina, Brazil, Peru) [8], India [9] and South Africa [10], compare well with the direct clinical experience reported by our research collaborators in these countries. In 1996, in the city of Trivandrum, in the State of Kerala, south India, stage III LABC accounted for over 40% of new cases of breast cancer, and stage IV for an additional 12.8% [9]. In South Africa, medical records of various large academic hospitals during the 1980s and 1990s offer a picture of striking social differences at presentation, with stages III and IV being the stages at presentation for 77.7% of black patients, as compared to 30.7% among non-blacks [10].

The experience of economically developed countries, like the U.S., show that these incidences can be modified resulting in markedly reduced breast cancer mortality. In fact, while U.S. breast cancer mortality remained constant from the 1930s to the late 1980s, beginning in the early 1990s it has started decreasing by 1-2% annually, a phenomenon that continues [11]. Although the causes remain unclear, it is strongly suspected that it is the result of an effective combination of timely early detection and substantial improvements in therapy. Further gains in terms of reduced mortality in the U.S. and worldwide could be achieved with a deeper understanding of LABC—a multi-dimensional disease that requires a multi-dimensional approach for its study and understanding. These components include behavioral, cultural, epidemiological, genetic, immunological, molecular and possibly ethnic aspects, which need to be investigated concurrently, as they all have the potential to contribute to our understanding. Thus, reaching out to populations comprising a broad range of ethnic, social, economic and health care characteristics—a global perspective for a global disease—is the most effective means for investigating, understanding and eventually impacting on LABC.

2.3 Conventional Approach to LABC

The contemporary management of LABC consists of pre-operative systemic chemotherapy to facilitate the surgical removal of the tumor and to address early systemic treatment of distant micro-metastases [12-13]. This approach has the advantage of enabling *in vivo* assessment of tumor sensitivity or resistance to the pre-operative regimen by measuring the residual disease in the pathological specimen obtained at mastectomy (i.e., pathological response). Moreover, identification of molecular markers from pre-treatment tumor biopsies allows investigators to explore their association with the extent of pathological response [12, 14].

2.4 Pathological Response as a Surrogate Endpoint for Survival

Evidence is emerging that pathological response after primary chemotherapy can be used as a surrogate endpoint for survival. Despite differences in the criteria adopted to measure and report the pathological findings after primary non-invasive treatment, most groups have shown a similar correlation between residual disease found at mastectomy and patient outcome. For instance, Feldman et al. reported lack of residual macroscopic tumor after doxorubicin-based primary chemotherapy as the strongest predictor for overall survival (OS) and disease-free survival (DFS) [15], while Buzdar et al. noted that lack of nodal involvement after therapy was associated with the best prognosis [16]. The Milan National Cancer Institute conducted a series of studies of primary doxorubicin-based chemotherapy. By classifying the pathological findings of 412 consecutive patients, Bonadonna et al. demonstrated conclusively that patients with a pathological complete response had better survival than those who had residual tumor in the specimen ($p=0.034$) [17]. Similarly, Kuerer et al. found a similar association in 372 LABC patients treated at M.D. Anderson Cancer Center [18]. It has thus become clear that therapeutic attempts to improve pathological response to primary therapy are likely to reflect on patient outcomes. In addition, pathological response as a surrogate for outcome provides a much quicker method to evaluate treatments while also offering the opportunity to explore its association with biological correlates [12, 14, 19-21]. In this sense, LABC offers an ideal opportunity to expedite clinical research on how to best –tailor treatment‖ based on specific tumor characteristics. Moreover, the data derived are likely to be relevant to the management of all invasive breast cancers.

2.5 Pre-operative Concurrent Chemotherapy and Radiation: Preliminary Experience

Over the past decade we have investigated the combination of up-front concurrent chemo-radiation as a pre-operative treatment, the extent of pathological response of the primary tumor then being used as a principal endpoint to measure tumor resistance to the tested chemo-radiation regimen. Radiation had traditionally played a role in the management of LABC since local control is also an important endpoint in this disease. However, it is usually added after surgery [13, 15-18]. We have selected concurrent chemo-radiation based on the promising results witnessed in other tumors (cervix, nasopharynx, stomach, lung, etc.) where both local control and distant metastases are significant causes of treatment failure. Moreover, original primary chemotherapy trials reported pathological response rates of less than 10% [13, 17], providing the impetus to investigate the combination of chemotherapy with radiation to increase the proportion of patients who might derive a potential survival benefit associated with a pathological response. We have conducted two sequential studies in LABC: the first one combined continuous infusion 5-FU and radiotherapy while the second combined semiweekly paclitaxel with radiotherapy [14, 19-20, 22].

2.6 Concurrent 5-Fluorouracil (5-FU) and Radiation: A Phase I-II Study

Inspired by the translational research of Leichman and co-workers in gastrointestinal cancers [21] and by the known radio-sensitization properties of 5-FU [23], the first trial

combined radiotherapy with concurrent continuous infusion (c.i.) 5-FU to optimize drug exposure during the course of radiation. The study included tumor biopsies to study molecular determinants of response (NIH Grant #1ROI CA 60859-01A1, 1994-97. –Response Determinants in Advanced Breast Cancer.", P.I, Drs. Peter Dannenberg and Silvia Formenti). Figure 3 displays the original study design of this trial [14]. The eligibility criteria for this study pre-selected patients with particularly advanced LABC insofar as all patients were deemed –inoperable,‖ i.e., in order to resect the primary tumor the wound could be closed only with the interposition of a skin graft or a myo-cutaneous flap. For each patient, pre-treatment breast cancer biopsies were analyzed by immunohistochemistry for ER/PR hormonal receptors, HER-2/neu, and p53 over-expression. p53 protein over-expression at immunohistochemistry was used as a surrogate for p53 gene mutation [24]. Moreover, initial tumor core biopsies were analyzed by reverse transcriptase quantitative PCR (RT-PCR) to measure expression of genes expected to be associated with response to 5-FU and radiation, e.g., the main target enzyme of 5-FU, thymidylate synthase. Pre-operative c.i. 5-FU, 200 mg/m^2/day was delivered for 8 weeks with radiotherapy, weeks 3-8, to 50 Gray in 2-Gray fractions. The first lesson derived from this study was a definition of pathological response to chemo-radiation. At mastectomy, pathological findings were classified on the basis of persistence of invasive cancer. Pathological complete response (pCR) defined the finding of no residual invasive cells in the breast and axillary contents; pathological partial response described the presence of <10 microscopic foci of invasive cells in either the breast or nodal specimens. All other cases were classified as no pathological response (pNR). This trial achieved a high response rate in large, inoperable LABC with an objective clinical response rate of 71% and a **pathological response rate (pCR+pPR) of 34%**. The pathological response rate of 5FU-RT compares favorably with that of any reported contemporary chemotherapy neo-adjuvant trial in this patient population, as displayed in Table 1 below [18, 25-30].

Table 1: Pathological Response and Outcome In Phase I-II Studies of LABC

AUTHOR	# PTS	TREATMENT	% PATH RESPONSE	5Y DFS	5Y OS
Powles [25] 1995	105	Chemo-Endocrine Therapy/Surgery/RT*	10%	NA	NA
Schwartz [26] 1994	158	Doxorubicin-Based CT**/Surgery/RT	10%	61%	69%
Merajver [31] 1997	89	Prolonged Chemo-Hormonal Therapy/ Surgery Or RT	NA	44%	54%
Formenti [14, 27] 1997	35	**Concurrent FU/RT/ Surgery/ Post-Op AC**	**35%**	**58%**	**74%** pCR 90% Others 65%
Karlsson [28] 1998	128	FEC/Surgery/FEC/RT	NA	36% (actuarial)	49% (actuarial)
Morrell [29] 1998	49	Doxorubicin-Based CT, Surgery/Rt	16%	51%	63%
Ayash [30] 1998	37	High Dose CT With Stem Cell Support/ Surgery /Radiation	14%	64% At 30 Months	NA
Kuerer [18] 1999	372	Doxorubicin-Based CT, Surgery/Rt	12%	p CR 87% Others58%	pCR 89% Others 64%
Zambetti [32] 1999	88	Doxorubicin-Based CT Surgery / CMFX6/RT	2%	52%	62%
Formenti [20] 2001	44	Concurrent paclitaxel-RT / Surgery / Doxorubicin-Based CT	**35%**	NA	NA

* RT= radiation therapy **CT= chemotherapy.

Eighty-four percent of the patients (32/38) in the trial were minority women. Importantly, the 5-FU/RT regimen was very well tolerated with no grade III or IV acute toxicities or increased toxicities during post-operative doxorubicin and cyclophosphamide (AC). With a median follow-up of 5 years, overall survival of the entire group of 38 patients is 74% and disease-free survival is 58% (Table 1), which compares favorably with neo-adjuvant chemotherapy.

We then asked whether a pathological response to chemo-RT could also be a surrogate endpoint for survival in LABC. The patients who achieved a pathological response (pCR+pPR) to 5-FU/RT have both a better DFS and OS than non-responders (p=0.023, and p=0.08, respectively), with only one failure among the women who achieved pathological response [20, 27]. In addition, no isolated chest wall or nodal recurrences have occurred to date.

In addition to achieving promising clinical results, this study proved the feasibility of using biological correlates from pre-treatment tumor biopsies to explore associations with pathological response: patients whose tumors had no evidence of p53 over-expression on immunohistochemistry were significantly more likely to achieve a pathological response to the regimen, compared to patients whose tumors over-expressed p53 (p=0.010, Fisher exact text). On multivariate analysis, lack of p53 over-expression

was the only independent predictive factor for pathological response, generating indirect evidence that wild type p53 tumors were more likely to respond to the 5FU/RT regimen. Since the majority of patients who over-expressed p53 failed to achieve a pathological response, it made sense to investigate the use of agents like taxanes, which are differently affected by p53 status [14].

2.7 Background for Combining Taxanes and Radiation

In vitro and pre-clinical models support the combination of taxanes with radiation. For instance, Wahl et al. have shown that loss of normal p53 function actually confers sensitivity to paclitaxel in some cell types because of an increased G2/M arrest leading to p53 independent cell death [33]. This observation may underlie the preferential activity of paclitaxel against cancer cells compared to normal cells as well as its activity against tumors that are p53 mutated [34]. Moreover, accumulation of cells at the G2/M checkpoint explains some of the in vivo radio-sensitizing effects of the drug [35]. Interestingly, our group was able to document the kinetics of paclitaxel-induced apoptosis and mitotic arrest in human tumor tissue [36]. These findings support the use of a semiweekly regimen as the optimal radio-sensitizing schedule in the clinic [19-20].

Another reason for studying taxanes in a chemo-RT approach to LABC is the fact that several potential markers of sensitivity to taxanes have been identified. Since the microtubule is also a target of paclitaxel, biochemical mediators that could affect microtubule stability are expected to affect paclitaxel activity. For instance, the components of mammalian microtubules are and -tubulin: the latter is the main target of paclitaxel action [37] and elevated class III and class IV -tubulin iso-forms were found in paclitaxel-resistant ovarian tumor cells [38]. Metablastin (also termed p19 and stathmin) is also a regulator of MT dynamics [39] and thus could influence paclitaxel activity. Similarly, MAP4 protein over-expression, which is known to stabilize microtubules and thus would be expected to antagonize paclitaxel, was found to predict for paclitaxel-resistance in vitro [40]. Interestingly, MAP4 is one of the few genes known to be down-regulated by wild type p53, possibly explaining the observed inverse relationship of p53 status and paclitaxel activity. Other potential determinants for response to paclitaxel are genes associated with the apoptotic or cell-cycle regulation pathways. Paclitaxel has been demonstrated to cause the phosphorylation of bcl-2, which is believed to abrogate the anti-apoptotic function of bcl-2 by dissociating it from the pro-apoptotic molecule bax [41]. Finally, HER-2/neu over-expression was shown both to alter -tubulin iso-form expression and to confer resistance to paclitaxel [42]. Finally, Pietras et al. reported resistance to radiation when HER-2/neu over-expression was induced in vitro and in vivo, in a breast xenografts tumor model. They demonstrated that a monoclonal antibody directed against the HER-2/neu receptor affects repair of radiation-induced DNA damage and enhances radio-sensitivity of human breast cancer cells over-expressing HER-2/neu [43].

2.8 Concurrent Paclitaxel and Radiation: A Phase I-II Study

Based on the background described above, the second concurrent chemo-RT study combined a taxane with RT. For each patient studied, pre-treatment breast cancer biopsies were prospectively analyzed by immunohistochemistry for p53 over-expression.

Estrogen receptor (ER), HER-2/*neu*, metablastin, -tubulin III and IV, MAP 4, *blc-2*, *bax* gene expression were measured using RT-PCR. The regimen consisted of pre-operative concurrent paclitaxel (30 mg/m^2 semiweekly) for a total of 8 weeks, while radiation was delivered to the breast and regional nodes during weeks 2-7 to a dose of 45 Gy at 1.8 Gy per fraction. Eighty-nine per cent of the patients were minority women. Pathological response (pCR + pPR) occurred in 33%. Concurrent chemo-radiation was well tolerated (with acceptable toxicities) and did not worsen the toxicity profile of post-operative AC or AT. When associations between pathological response and molecular markers were explored, tumors with low HER-2/*neu* gene expression by RT-PCR and negative estrogen receptors were more likely to respond to the tested regimen (p=0.009 and p=0.006, respectively). **The findings suggest that tumors without HER-2/*neu* over-expression are more likely to respond to paclitaxel/RT.** Conversely, p53 protein expression measured by IHC did not appear to be associated with pathological response (p=0.67) [14].

Our two consecutive chemo-RT phase I-II studies proved the feasibility of concurrent chemotherapy and radiation as primary treatment for LABC. With the limitations associated with a Phase I-II study, 5-year DFS and OS data from the FU/RT study are better than those reported by other investigators that have employed sequential treatment with post-operative radiation in this disease. In both trials one out of three patients achieved a pathological response (pCR or pPR, as per our definition), significantly higher than the 5-15% pathological response rates reported in contemporary studies of preoperative systemic chemotherapy alone.

2.9 Hypotheses

LABC remains a challenging presentation of breast cancer that is particularly common among underserved, minority women in the United States and is the most common presentation of breast cancer in developing countries. We have hypothesized that:

1. An international, multi-disciplinary approach is essential for the study and development of more successful intervention in LABC, a global, multi-dimensional disease.
2. Many components are likely to contribute to LABC, including behavioral, cultural, epidemiological, genetic, molecular, immunological and possibly ethnic factors.
3. To understand LABC, which varies in incidence and progression in a worldwide manner, these components need to be studied concurrently, under standardized conditions applied to the same multi-ethnic cohort of patients, treated consistently by the same treatment regimen.
4. Pathological response to the same treatment regimen will provide an understanding of the molecular and genetic profiles of the tumors and surrounding tissues that contribute to tumor response to treatment or progression. There is also a need to investigate the epidemiological, cultural and behavioral characteristics of LABC to develop more effective treatment strategies.

3. PRELIMINARY DATA

3.1 Association of Molecular Markers with Pathological Response: Preliminary Data

The two preliminary studies which combined taxanes and radiation (sections 2.7-2.8) also demonstrated that different molecular markers are associated with pathological response, supporting the hypothesis that different drugs combined with concurrent radiation are effective on different tumor types. In the 5FU/RT study, lack of p53 over-expression (i.e. wild type p53 tumors) was significantly associated with the achievement of a pathological response, while in the paclitaxel/RT study p53 status had no relevance, but tumors without HER-2*/neu* over-expression were more likely to respond to paclitaxel/RT. Based on these findings, selecting a particular chemo-RT regimen based on the molecular characteristics of the tumor should improve pathological response rates, thus potentially improving survival.

While intriguing, the data reported require validation in a much larger sample of patients, ideally in a multi-institutional fashion that represents the multiethnic characteristics of LABC. Moreover, the availability of a modern, comprehensive basic research matrix of collaborating researchers enables the multi-dimensional research strategy necessary to the understanding of the complexity associated with LABC.

3.2 Rationale for a Single Study of Preoperative Chemo-radiation

The publication in JCO of our Phase I-II trial of concurrent paclitaxel and radiation [44] has generated interest from a number of groups of investigators, including those from developing countries interested in adopting this regimen. Similarly, the two foreign academic institutions participating in the COE were among those that had originally contacted us to develop a common preoperative trial for LABC.

The current choice for a common pre-operative regime is the result of a careful assessment of the available resources for clinical care at each of the institutions outside the U.S. State of the art radiology, pathology and radiation oncology equipment are available. In addition, a qualified medical oncology clinic is in place at each site. To cope with the reality of the existing health system of the two institutions outside the U.S., the current protocol was developed from the preliminary work published in the Phase I-II study of pre-operative paclitaxel and radiation [44]. This protocol proved to be the most realistic and feasible common approach to LABC. The advantages of adopting this preoperative regimen of concurrent chemo-radiation consist of: 1) availability of safety and toxicity data, demonstrating only grade 3 toxicity consisting of skin desquamation; 2) the pathological response rate achieved (34%), which was at least comparable to that of much more prolonged, costly, multi-agent pre-operative chemotherapy regimens; 3) independent evidence that the use of neo-adjuvant single agent paclitaxel can achieve results comparable to those of a more conventional preoperative regimen for LABC, consisting of 5-fluorouracil, doxorubicin and cyclophosphamide [45]; and 4) because of its generic formulation, paclitaxel has resulted as the most convenient available drug for the study. As a result, the current study will be the common clinical platform to treat patients at each center. As described below, the same standardization procedures implemented at each center to assure homogeneity of methods within the network of participating cancer centers. All patients will be concurrently studied by the research matrix described above.

3.3 Establishment of an International Network of Three Participating Clinical Institutions

A network of clinical academic centers has been established to provide the unified methodology, treatment protocols, specimens and data acquisition that are necessary to effectively study LABC. In addition, a multidisciplinary research approach was developed to describe and understand behavioral, cultural, genetic, immunological and molecular components. **Our central hypothesis is that by studying LABC from a global perspective and in a multi-dimensional manner, those features that are common across cultures and those that differ across cultures can be identified.**

LABC is possibly the most common stage at presentation of breast cancer worldwide. In many economically disadvantaged countries where LABC is extremely common, an unknown, but likely non-trivial number of breast cancer patients never reach a formal ―clinical diagnosis‖ since they die before ever receiving medical treatment of any kind. Thus, the burden of breast cancer incidence and mortality is likely to be substantially underestimated. In addition, access to screening, early detection and timely medical care are not options available to the vast majority of women worldwide, including a sizable subset of minority, often underserved women in the U.S. These women are much more likely than those living in affluent communities to present to medical attention with large, palpable breast tumors, often LABC. We are convinced that improvements in our understanding of how to manage, treat and minimize the complications of LABC represents an untapped opportunity for investigations aimed at reducing the burden of mortality for breast cancer. To this aim, a clinical research network of academic institutions in 3 distinct international sites capable of delivering the same standardized pre-operative therapy and surgery have been established (Table 2). These centers are characterized by broadly different cultural, social and economic contexts, diverging incidence of breast cancer and different burden of LABC (areas where breast cancer incidence is high have the smallest frequency of advanced stages at presentations and vice versa) (Table 2). This unique global network enables the establishment of a common clinical trial in well-characterized distinct populations of LABC patients, while conducting prospective tissue acquisition, molecular and genomic profiling. Key to the success of this initiative has been the enthusiastic endorsement provided by each site, with the proposed commitment to contribute 25 patients over a four year accrual period. Each patient's characteristics are entered into the COE Oracle Clinical database linked through a website available to the P.I., regularly updated during the five years of follow-up, to enable the exploration of the relationships between biological profiles and outcomes.

Table 2

Geographic Location	Main Ethnic Groups Served	Breast Cancer Incidence per 100,000 (ASR-World)	Participating Academic Institution	Local Principal Investigator
South Africa	Black Asian White	11-70[a]	Tygerberg Hospital (Stellenbosch University)	Dr. Justus Apffelstaedt
New York State (U.S.A).	Hispanic Asian Black White	66-90[d]	NYU Hospitals (New York University)	Dr. Silvia Formenti

[a]Only age-standardized rates available, 1993-95 (CANSA). Range: 11.3, blacks; 14.7, Malay (‒coloured‖); 42.1, Asians; 70.2, whites. [b]Personal No available cancer registry data for Mexico. Participation not confirmed. [d]New York State Cancer Registry, 1993-97[46]. Range: 66.3, blacks; 90.6, whites.

An important issue related to the feasibility of the current trial is the communication and diffusion of standard procedures that will enable the clinical and basic scientists to merge the data to perform the proposed clinical and laboratory analyses. The leadership of the COE has identified this issue as central and has developed the necessary tools to assure international standardization of information, data acquisition, tissue acquisition and handling, tissue shipment procedures and treatment data review (Appendix). The process included each of the P.I.s from the participating clinical cancer centers. Since August 2003, each Principal Investigator from the other sites have visited United States and met with Drs. Formenti and Schneider to discuss the current methods and process at their respective institutions and worked together to achieve standardized common guidelines that can be realistically implemented internationally.

Specifically:

1. Standard diagnostic criteria have been developed.
2. Standard eligibility criteria have been developed.
3. Informed Consent documents have been provided in English (South Africa and U.S.) and Spanish (U.S.) (Appendix, Section 16.5).
4. A standard clinical trial protocol was been agreed upon by all of the centers.
5. Specific Pathology Guidelines were developed in collaboration with each of the clinical centers. In addition, an informative Slide Show was developed for training the clinical teams in tissue acquisition, handling and shipment (Appendix, Sections 16.3 and 16.4). Centralized review of diagnostic tissue has been established and will be conducted at NYU by Drs. Singh and Cangiarella.
6. Centralized review of radiation simulation films has been established and will be conducted at NYU. The South Africa have digital films available and will send the images via the Internet.

7. Site visits to the international sites have taken place to assure that standardized procedures and methods are fully in place before starting the protocol.
8. Data Collection Forms (Appendix, Section 16.1) were developed and will be available in Spanish (for the U.S) and Afrikaans (for South Africa). The instruments were closely reviewed by the P.I.s from each site to ensure that the instruments are clear, culturally appropriate and adherent to standardized guidelines of data collection.

3.4 Establishment of a Research Matrix Composed of Investigators with Complementary, Synergistic Research Expertise

This clinical protocol is the basis for a standardized, unified approach to a clinical presentation of breast cancer, LABC, common among the three participating international institutions. From each of the three centers the clinical P.I.s will work together as a network of clinical investigators committed to treat consistently, as described in the protocol, three parallel, synchronous cohorts of LABC patients.

The NYU Clinical Investigators' team will lead the effort. The team has extensive experience in LABC and funded clinical, translational and epidemiological programs that are ongoing at our institution. NYU School of Medicine serves Tisch Hospital, Bellevue Hospital, NYU Downtown Hospitals. The latter have historically been the flagship of Manhattan's public hospitals. Bellevue Hospital has a multi-centennial tradition of providing medical care to immigrant populations and it is **a true microcosm of multi-ethnicity in health care**. Building on their successful tradition for conducting clinical trials among underserved, minority populations, the team has converged a second network, composed of basic and behavioral research scientists interested in collaborating to deepen the understanding of LABC. Premier investigators from NYU, Cold Spring Harbor Laboratory (CSH), Princeton University and the University of Arkansas in collaboration with a team of very active patient-survivor advocates have generated a **Matrix Program based at NYU that has brought together the expertise of multiple investigators and institutions to work collectively on a single problem, the description of the presentation of locally advanced breast cancer, the response of locally advanced breast cancer to treatment, and disease progression.**

The following 6 research studies have been developed. They will be enabled by this Clinical Research Protocol (**Project 1**) which is the platform to enable all scientific research projects.

Project 2, Functional genomics of pre-treatment LABC tumors. This research is directed to identifying gene expression signatures associated with treatment response, resistance, and progression to local recurrence and/or metastatic disease. These studies will be led by Dr. Schneider-NYU School of Medicine. This research also involves extensive collaboration with the Dr. Goldberg (NYU) for study design and statistical analysis. While there are many research efforts underway to identify gene expression signatures associated with response and failure of different tumors, including breast cancers, this approach is novel in its examination of heterogeneous global cohorts and the focus of the data analysis on comparison of results within and among cohorts. This approach, in conjunction with molecular biology studies of LABC and genomic mutational studies

(described below), are expected to expedite identification of gene expression responses that will ultimately lead to tailored treatment strategies and new targets for intervention.

Project 3, Molecular and tumor cell biology. This research effort will identify molecular and biochemical signatures in LABC tumor cell responses that are linked to tumor sensitivity or resistance (progression) to treatment, including hypoxia, angiogenesis, apoptosis and protein synthesis. These studies will be directed by Dr. Schneider (NYU). These studies are novel in their ability to provide a biological context for interpreting and understanding the signatures generated by the functional genomics program at the molecular level of gene expression, without which gene expression profiling remains largely descriptive.

Project 4, Genomic mutational analysis. This research project will be directed by Drs. Wigler and Lucito (co-leaders, CSH). Using a state-of-the-art gene chip analytical approach developed by these two investigators, this research focuses on identification of tumor suppressor and tumor promoting mutations associated with LABC incidence and metastatic progression of disease. This is a powerful and unique research project which has never been applied to LABC and promises to develop a new genetic understanding of this disease which can be used to identify new targets for future therapeutic intervention and characterization of this heterogeneous patient population for tailored treatment approaches.

Project 5, Association of endogenous reproductive hormones in LABC progression. This project, directed by Dr. Toniolo (NYU), will continue efforts to elucidate the role of endogenous reproductive hormones in breast cancer by investigating the role of estrogenic and genotoxic estrogen metabolites in breast cancer prognosis.

Project 6, Common and distinct cultural and social barriers to treatment. This project will be directed by Dr. Bright (NYU) and Dr. Erwin (University of Arkansas) and will aim to develop multicultural and multilingual psychological scales related to traditional barriers to screening. This work is unique and important as it seeks to produce narratives that describe the experiences of each group in the multi-cultural, multi-national sample. Also, the study will develop qualitative approaches and data that have the potential to greatly improve models for cross-cultural behavior, and to aid in the development of culturally appropriate educational and behavioral interventions.

Project 7, Role of single nucleotide polymorphisms (SNPs) in the p53 signaling pathway in LABC. This project is directed by Dr. A. Levine (Princeton University and the Rutgers University Cancer Research Program). SNPs have been identified in the p53 signal transduction pathway, one of which (SNP 309) is localized in the promoter of the MDM-2 gene and reduces the p53 response to DNA damage. At the clinical level, individuals with this SNP (12% of people tested) are diagnosed with cancers (including breast cancers in Li-Fraumeni patients) at much earlier ages than those with the wild type sequence. Using LABC tumor core biopsy DNA extracted by Dr. Schneider's group in project 2, the SNP 309 profile will be determined in precisely the same cohorts under analysis for the other projects. These data will examine whether age of onset and

progression of LABC are associated with this important SNP in a manner related to ethnicity and global pattern.

4. STUDY DESIGN/SUBJECT ELIGIBILITY

4.1 Study Design: Overview

The primary objective of this study is to confirm the pathological response rate of 34% (pCR+pPR), reported for patients in the U.S. Phase I-II trial [44]. This is a phase II trial because it is designed to extend and confirm the smaller U.S. Phase I/II trial reported previously. With 100 cohorts at NYU/Bellevue and 25 patients accrued at each of the two other funded sites (S. Africa), patients in each cohort, pathological response rates will be estimated in each cohort individually and for the combined cohort of 150 patients. Progression free survival and overall survival will also be estimated for each cohort individually and across cohorts. Patients will be randomly selected within cohorts to participate in the studies of genomics, genetics, biomarkers, and hormonal markers. The approach of the Center will be to use a common stratified random sample for each of the research projects that will not be conducted on all of the 150 enrolled patients. This approach, which ensures that a random sample of 75 patients are included in all of the research studies, will facilitate the identification of characteristics that can be used to predict pathological response, progression, and survival from the multi-ethnic cohorts under study and to identify hypotheses for the generation of further studies. Further details of the design and analysis strategy are included in Statistical Considerations, Section 13.

4.2 Projected Accrual

150 patients over 4.5 years at the three centers (100/NYU-Bellevue; 25/site x 2).

- 4.2.1 Recruitment at the NYU/Bellevue site will involve 20 patients in year 1 during the phase-in period; 30 patients in years 2 and 3; and 20 patients in year 4, while accrual at the other sites will be 5 patients in year 1; 7 to 8 patients in years 2 and 3; and the remainder in year 4.
- 4.2.2 Recruitment will continue into the mid-fourth study year to permit each enrolled patient to complete at least 12 months of follow-up from time of diagnosis to the evaluation of pathologic response, the primary endpoint of this study.

4.3 Inclusion Criteria

- 4.3.1 Biopsy proven locally advanced breast cancer: IIB, IIIA and IIIB.
- 4.3.2 Metastatic breast cancer: limited to the subset of patients with intact breast, locally advanced tumor and involved ipsilateral supraclavicular nodes.
- 4.3.3 Measurable disease required.
- 4.3.4 Adequate laboratory values:

  Hgb > 10

  ANC >1500

Platelets >150,000
Creatinine < 1.5
Liver function < 3 X normal.

4.3.5 Patient ≥ 18 years of age.
4.3.6 Medically and psychologically able to comply with all study requirements.
4.3.7 ECOG performance score 0-1.
4.3.8 CT chest, abdomen, and pelvis performed.
4.3.9 Mammogram or USG performed.
4.3.10 Signed informed consent.

4.4 Exclusion Criteria

4.4.1 Breast cancer patients with Stage 0, Stage I, Stage IIA.
4.4.2 Previous XRT or chemotherapy.
4.4.3 Presence of distant metastases documented clinically or radiographically with the exception of ipsilateral supraclavicular nodes.
4.4.4 Pregnancy.
4.4.5 Inflammatory breast cancer.
4.4.6 Patients under treatment (or who will have recently been treated) with anti-neoplastic, immunosuppressive or hormonal medications
4.4.7 Patients who are found to have a cancer positive for the marker HER-2/*neu* (*Note*: this exclusion criterion applies only to patients at the NYU Tisch and Bellevue sites where there is currently a protocol open and available for patients who are HER-2/*neu* positive. This exclusion criterion does not apply to patients at the South Africa site).

5. DATA AND TISSUE STANDARDIZATION PROCEDURES

5.1 Epidemiology and Data Collection (EDC) Core

The EDC core, based at NYU, will coordinate the procurement of samples and data collection forms at each of the centers and will provide on-site quality assurance. In addition, the EDC will distribute the samples to the various labs according to the pre-specified sampling plan. As part of its distribution plan, the EDC core will provide each site with bar-coded adhesive tags bearing a site-specific number series for all tissue specimens, slides, bloods, and data forms, etc. Two training slide shows were developed to enable uniform procedures among the centers (Appendix, Sections 16.3 and 16.4).

5.2 Tissue Acquisition and Handling

Protocol for tissue sampling (Slide Shows in Appendix, Sections 16.3 and 16.4)

5.2.1 Patient with locally advanced breast cancer will be consented for tissue acquisition to collect core biopsies before beginning therapy and samples from the surgical specimen.
5.2.2 Sampling kits, provided by NYU will be available for each patient. A sampling kit will contain core biopsy needles, glass slides and vials containing RNA*later*. Bar coded labels for de-identification purposes will also be included.

5.2.3 Patient will undergo a core biopsy of the breast using 18 gauge spring loaded core biopsy gun (Tenmo). Four core biopsies will be taken from the mass.

5.2.4 A pathologist will be present at the time of core biopsy to perform concomitant touch preparations to determine the adequate presence of malignant cells: viable cores will be considered those with at least 50% neoplastic cells.

5.2.5 Touch preparations will be performed by imprints of the core biopsy onto glass slides. Each core biopsy specimen will be imprinted on a separate corresponding glass slide. Glass slides will be stained by a modified Giemsa stain (Diff-quik) stain and assessed for the presence of malignant cells.

5.2.6 Once the cores are determined to be adequate, two cores will be fixed in formalin for paraffin embedding to generate tumor blocks. One representative tumor block will be retained by the participating institution for records and routine histologic analysis with hematoxylin and eosin stains. The remaining tumor blocks will be sent to NYU for research studies. The remaining two cores will be placed in vials of RNA later.

5.2.7 All samples will be de-identified and bar coded labels will be affixed to all the vials and all the specimens before shipment to NYU at described below under Shipment (also see Appendix Shipment Slide show).

5.2.8 In collaboration with the EDC each regional study manager will catalogue, code and ship core biopsies, bloods samples and surgical samples (made anonymous) to NYU on a bimonthly basis.

5.2.9 In addition, Amrita Institute of Medical Sciences (AIMS) in Cochin India has entered into a data-sharing agreement with NYUSOM to make available the following de-identified LABC data for analysis at NYUSOM. *Please note that AIMS is not taking part in the DOD sponsored treatment trial—rather, it is participating in the sharing of de-identified archived LABC specimens and data that will enrich the diversity and scientific robustness of the overall international study*.

a. fresh tumor samples collected at biopsy from LABC trial patients and again at surgery at AIMS, and preserved in RNA*later* for subsequent extraction and purification of genetic material at NYUSOM;
b. a small portion of each fresh tumor sample preserved in paraffin for local banking in the Pathology Department at AIMS Cancer Institute;
c. a slice of paraffin block stored on slides and shipped to NYUSOM;
d. de-identified clinical forms that contain key baseline, demographic, clinical and pathological response information for these patients.

5.3 Protocol upon Receipt of Tissue at NYU

5.3.1 Upon receipt, one core will be paraffin embedded for routine histologic analysis with hematoxylin and eosin stains. Pathologic diagnoses will include nuclear grade, architectural grade, mitotic count, presence and extent of intraductal component, lymphatic and vascular invasion.

5.3.2 Immunohistochemical staining for estrogen and progesterone receptor, Her-2- neu, p27 and p53 will be performed.

5.3.3. One of the 2 cores in RNA*later* will be banked and frozen in a –80 degree locked freezer owned by the COE and backed-up with a liquid carbon dioxide sentinel system. Of the two core biopsies obtained for research purposes, one will be banked as whole tissue and the other micro-dissected and used for Trizol extraction and separation of DNA, protein and RNA. Extraction is described under project 2 in the COE grant proposal and will be carried out by Dr. Schneider's group, distributed to the other research groups and banked.

5.3.4 The second core biopsy preserved in RNA*later* will be utilized for gene expression profiling.

5.3.5 Paraffin embedded LABC blocks collected by the EDC core will ultimately be used to produce tissue microarrays at NYU. Initial hematoxylin and eosin stained slides will be examined and marked to determine the best area for punch biopsy of the core. A 4 mm punch biopsy will be performed on each paraffin block and multiples cores from different patients will be re-embedded in paraffin to produce a tissue microarray.

5.3.6 Dr. Hiran, Assistant Professor of Pathology at AIMS, will travel to NYUSOM with a collection of archived paraffin LABC specimens, and under the supervision of Drs. Robert Schneider (Microbiology) and Baljit Singh (Pathology) develop expertise in and a common practice approach to the analysis of the specimens consisting of: (1.) micro-dissection of tumor specimens, (2.) use of non-conventional immunohistochemical techniques and antibodies vital to the LABC study; (3.) specific biochemical purification techniques for preparation and purification of RNA and DNA from specimens; and (4.) production of tissue microarrays.

5.4 Protocol for Assessment of Pathologic Response

5.4.1 The primary study outcome will be pathological response to therapy. Pathologic samples (mastectomies or segmental mastectomy) will be serially sectioned and evaluated macroscopically. Approximately 30 sections of the post-treatment tumor bed should be taken for histologic analysis.

5.4.2 Important pathologic information from local pathologists will include:

1. measurement of the greatest diameter of invasive carcinoma
2. size of the residual tumor bed in three dimensions
3. assessed proportion (%) of tumor bed that contains invasive carcinoma
4. location of the tumor bed in the breast specimen (quadrant, o'clock position)
5. number of axillary lymph nodes and presence of tumor within the nodes

5.4.3 Banking of tumor and normal tissue from the mastectomy or segmental mastectomy specimens will be performed. In each patient both paraffin embedded and RNA*later* preserved samples will be obtained, similar to that described in 5.3.3 and 5.3.4.

5.5 Pathologic complete response: no residual invasive carcinoma in breast or axillary lymph nodes.

5.5.2 Pathologic partial response: presence of less than 10 microscopic (X40

magnification) foci of invasive tumor cells in either the breast or axillary lymph nodes.

5.5.3 All other cases will be classified as no pathological response.

5.6 Peripheral Blood Sampling and Handling

5.6.1 For each patient 30 cc of peripheral blood (from which we will extract 5 cc of serum and plasma) will be obtained, in three occasions;
1. when the core biopsies are performed at baseline before the start of treatment; 2. at the end of second week of radiation therapy and 3. at the time of surgery, after chemo-radiation.

5.6.2 Peripheral Mononuclear Blood Cells (PBMC) and polymorphonuclear cells will be isolated after gradient separation, cryo-preserved in DMSO and stored at -80°C at each of the centers.

5.7 Standardization of Pathology Procedures

5.7.1 All head pathologists at each site will visit NYU early in the developmental phase to review cases and discuss/agree on procedures; these pathologists will form the standardization committee.

5.7.2 100% of diagnoses and path reports in year 1 will be reviewed by all head clinicians and pathologists at each site (the committee): disagreements are discussed in periodic teleconferences and resolved by consensus.

5.7.3 In subsequent years, a random sample of all diagnoses and pathologies will be reviewed by all committee members and disagreements discussed in monthly teleconferences for consensus resolution.

5.7.4. All controversial cases will be reviewed at NYU and unresolved divergences, if any, are discussed at the full committee level, in teleconferences.

5.8 Shipment and Storage of Tissue Samples

Details on international shipment procedures are included in Appendix 16.4.

At NYU, all samples will be stored in a –80 degree locked freezer owned by the COE and backed-up with a liquid carbon dioxide sentinel system. Of the two core biopsies obtained for research purposes, one will be banked as whole tissue and the other micro-dissected and used for Trizol extraction and separation of DNA, protein and RNA. Extraction methods are described under project 2 and will be carried out by Dr. Schneider's group, distributed to the other groups and banked. Samples collected for this study will be stored at NYU for 10 years or until the completion of the study whichever is later. Only investigators on the trial will have access to the tissue.

6. TREATMENT

6.1 Radiation Therapy

6.1.0 Quality assurance measures.

Central review of all simulation films will occur at NYU. Simulation films images will be sent from S. Africa via the Internet and approved electronically.

6.1.1 Breast Irradiation

6.1.1.1 Treatment planning on a simulator is required in all patients and for each field treated with photon radiation. The whole breast and underlying chest wall will be treated through opposed medial and lateral tangential fields. Non-divergence of the deep margins will be assured by half-beam block or multi-leaf collimator (MLC) blocking.

6.1.1.2 Patient Position: The patient will be supine, +/- table (breast) wedge, with the ipsilateral arm abducted approximately ≥ 90∞ and supported appropriately in the horizontal position (with or without elbow flexion).

6.1.1.3 Field Margins: As clinically determined, these will be 1.5-2 cm around palpable breast tissue in the medial, lateral and inferior dimensions. The superior field margin will be at the matchline with the inferior margin of the regional lymphatic field and will generally be at the level of the inferior aspect of the sternoclavicular joint.

6.1.1.4 Blocks: Blocking material with ≥ 5 HVL will be utilized in the half beam block technique to comply with field margin and normal tissue dose requirements. In addition, blocking at the superior border of the tangential fields will be necessary to obtain a precise matchline with the nodal field. MLC is accepted.

6.1.1.5 Normal Tissues: No more than 3 cm (demagnified) of lung will be included within the tangential fields as measured along the transverse plane of the central axis. For left-sided primary tumors, care will be taken to exclude as much of the heart as possible from the tangential fields. If it is impossible to avoid including part of the heart in the tangent field, the heart will be blocked after the first 36 Gy.

6.1.1.6 Treatment Equipment: Megavoltage units with peak photon energies of ≤ 6 MV will be used. An occasional (large breasted) patient may be treated with a > 6 MV linear accelerator if necessary to comply with dose homogeneity requirements. A source-axis distance or source-skin distance of ≥ 80 cm is used.

6.1.1.7 Beam Verification (Port) Films: Beam verification films on the radiation treatment machine will be obtained of each treatment field until satisfactory. Thereafter, beam verification films of each treatment field will be obtained each 5 treatments and at the time of any field modification(s).

6.1.1.8 Dosimetry: Transverse contours at the central axis, 2 cm inferior to the superior field border and 2 cm superior to the inferior field border will be obtained. Employing computerized dosimetry, without tissue inhomogeneity correction, for the above-mentioned contours, the maximum dose inhomogeneity allowed will be + 10% (measured by an isodose area > 2 cm^2 rather than ‒hot spots‖) and –5%. It is expected that compensating blocking and wedge filters or tissue compensating devices will be used to maximize dose homogeneity.

6.1.2 Regional Lymphatic

6.1.2.1 Supraclavicular ± full axillary lymph node irradiation will be given concurrent with whole breast or chest wall irradiation. No attempt will be made to include the internal mammary lymph nodes within a separate treatment field, within the supraclavicular ± full axillary nodal fields (i.e., the "hockey stick") or within the breast tangential treatment fields. Treatment planning on a simulator is required in all patients and for each field treated with photon radiation.

6.1.2.2 Patient Position: The patient will be supine +/- table (breast) wedge, with the ipsilateral arm abducted approximately ≥ 90° and supported appropriately in the horizontal position (with or without elbow flexion). Patients with large, pendulous breasts will be treated prone to limit the extent of infra-mammary wet desquamation.

6.1.2.3 Technique: Treatment will be administered through a photon beam field angled into the ipsilateral side at approximately 10°-15° so as to avoid irradiation of the entire trachea, esophagus, and spinal cord (e.g., for treatment of the right side, the field direction would be left anterior oblique).

6.1.2.4 Field Margins: supraclav ± full axillary field: The field margins will be defined as follows

Superior: level of the thyro-cryoid membrane. If necessary, a trapezial ridge "splash" block to avoid fall off over the skin surface may be used.

Inferior: to coincide with the superior border of the breast/chest wall tangential field. Commonly, this will be at the level of the inferior aspect of the sternoclavicular joint. A half-beam block technique, so as to avoid field divergence, is required.

Medial: 1 cm to the contralateral side of the anterior mid-line. A superior-medial block to shield portions of the spinal cord will, if necessary, be used.

Lateral: to the level of the humeral head or, when so treated, to include the full axillary contents. Blocking will, if necessary, be used to shield the humeral head.

6.1.2.5 Field Margins axillary field: posterior axillary boost In order to administer the target dose at the axillary midplane, a PA field will be used to supplement dose "fall-off" from the anterior-oblique field, and will have field margins defined as follows:

Superior: coracoid process; blocking bisects clavicle in craniocaudad dimension.

Inferior: to coincide with the inferior margin of the supraclav/axillary field.

Medial: junction of 1st rib and clavicle, medially.

Lateral: to coincide with the lateral margin of the supraclav/axillary field; if necessary, blocking will be used to shield the humeral head.

6.1.2.6 Treatment equipment.

Megavoltage units with peak photon energies < 6 MV will be used. A source-skin distance of > 80 cm will be used.

6.1.2.7 Beam Verification (Port) Films Beam verification films on the radiation treatment machine will be obtained of each treatment field until satisfactory. Thereafter, beam verification films of each treatment field will be obtained following each five treatments.

6.1.2.8 Total dose to the breast, axilla and supraclavicular area will be 45 Gy @1.8 Gy/fraction, + 14 Gy to the area of original palpable tumor @ 2 Gy/fraction (total 32 fractions).

6.1.3 Expected toxicities

6.1.3.1 Acute reactions: Common acute side effects of radiation treatment include skin desquamation, with possible blistering and tumor ulceration. The skin reaction is more brisk due to the sensitizing effect of systemic therapy. Tiredness is commonly reported and it subsides at the completion of radiotherapy.

6.1.3.2 Intermediate Reactions: After treatment it is common to find darkening of the skin within the field. Wound closure delay also tends to occur more often, when compared to non-irradiated patients.

6.1.3.3 Late Reactions: Rare late reactions are rib fractures and radiation pneumonitis (less than 3% of treated patients). If radiation pneumonitis is not treated with low dose steroids for 2-3 months complications may occur and (if mismanaged) can evolve in death.

6.1.4 Treatment Modifications

Radiation therapy will be interrupted for any Grade III toxicity, except for Grade III skin toxicity.

6.2 Chemotherapy

6.2.1 Paclitaxel (including Abraxane), 30 mg/m^2 twice per week. Paclitaxel will be given IV over 1 hour, Abraxane® will be administered over 30 min, and administered on a Monday/Thursday or Tuesday/Friday schedule.

6.2.2 Pre-medication for paclitaxel should be based on institutional standards; it is suggested that decadron, 20 mg IV, be given with the first paclitaxel dose. If the patient tolerates the treatment, the decadron may be tapered and/or discontinued for subsequent doses. While reactions can occur to any medication, severe allergic reactions to ABRAXANE are uncommon and premedication is not required.

6.2.3 Duration of pre-operative systemic therapy.

Systemic therapy will be given for 12 weeks total. Radiation therapy should commence within the first week of chemotherapy so that patient is receiving 6 weeks of *concurrent* chemotherapy and radiation therapy. Chemotherapy should be continued for 5 weeks after radiation. During pre-operative therapy, tumor assessments will be performed every two weeks; patients with progressive disease will be taken off study treatment.

Other reasons for removal of patients from treatment are:

(a) patient choice or failure to keep appointments, follow directions or take medications as instructed

(b) a serious adverse reaction to drug therapy

(c) the need for treatment that is not allowed in the study
(d) termination or cancellation of the study by study sponsor.

6.2.4 Dose Modifications Guidelines for Paclitaxel
Paclitaxel doses should be adjusted according to the following table for all toxicities, *except for radiation dermatitis*, anemia, lymphopenia and any not significant laboratory abnormality.

Toxicity Grade (NCI CTC)	**Continue/Hold therapy**	**Dose Modification**
1 and 2	Continue treatment	None
3	Hold until resolution to grade 1 or 0 For absolute neutrophil count (ANC) < 1,000 or Platelets < 75,000, hold chemotherapy and monitor counts weekly. Resume therapy after counts have recovered to ANC > 1,000 or platelets > 75,000. If a patient is delayed more than one week, reduce chemotherapy dose by 25%. Other Hematological toxicities (i.e., lymphopenia) will not require dose modification.	1st event: Decrease dose by 25%. 2nd event: Decrease dose by 50%
4	For CTC grade 4 hematologic toxicity, reduce chemotherapy dose by 25%.	

6.2.5 Patients who experience recurrent toxicity that would lead to a dose reduction below 50% of the starting dose should be withdrawn from study treatment.

6.2.6 Dose Modifications Guidelines for Abraxane.
Patients who experience severe neutropenia (neutrophil <500 cells/mm3 for a week or longer) or severe sensory neuropathy during Abraxane therapy should have dosage reduced to 220 mg/m^2 for subsequent courses of Abraxane. For recurrence of severe neutropenia or severe sensory neuropathy, additional dose reduction should be made to 180 mg/m^2. For grade 3 sensory neuropathy hold treatment until resolution to grade 1 or 2, followed by a dose reduction for all subsequent courses of Abraxane. Patients should not receive Abraxane if AST > 10 x ULN or bilirubin > 5.0 x ULN.

6.3 Surgery

6.3.1 Patients will undergo lumpectomy and axillary node dissection or modified radical mastectomy within two months from the end of chemo-radiation, contingent upon recovery of skin toxicity.

6.3.2 For patients achieving clinical CR or PR, the decision regarding the type of surgery will be based on the preferences of the patient and the

surgeon's recommendation based on the clinical assessment of the residual tumor[47].

6.3.3 In the absence of clinical response, if operable, patients will undergo modified radical mastectomy.

7. STUDY PARAMETERS

The research procedures will be conducted as indicated in the following table:

Parameter	Baseline	During Preoperative Treatment	During Long-term Follow-up
Tumor tissue Core Biopsy	Required	None	At the time of surgery
Blood for Serum and Plasma Banking	Required at time of core biopsy.	Required at the end of second week of radiation therapy and after completion of pre-op therapy	None
History and Physical	Complete H & P required at baseline.	Complete physical q 2 weeks	Complete physical every 3 months during the first 2 years post therapy; every 6 months during years 3, 4 and 5 post therapy; thereafter once a year (up to 10 years)
ECOG PS	Required	q 2 weeks	At each F/U visit
Tumor Measurement	Bi-dimensional measurements.	q 2 weeks	N/A
Lymphedema Measurement	Required	q 2 weeks	Required at each F/U
Mammogram	Required pre-therapy.	None	For the involved breast, q 6 mos (lumpectomy pts.); standard annual mammogram of the uninvolved breast.
PET/CT (or CT Chest, Abdomen, Pelvis with Bone scan)	Required.	None	As medically indicated.
Serum Pregnancy Test	Required for patients of child-bearing potential.	None	None
CBC,diff	Required.	weekly	As medically indicated.
Electrolytes, BUN, Cr, LFT'S	Required.	q 2 weeks	As medically indicated.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

Clinical Measurement

Clinical response will be measured by the following criteria:

Complete Response (CR): complete disappearance of all known tumor masses and the appearance of no new lesions.

Partial Response (PR): greater than 50% reduction in the product of perpendicular measures of all measurable tumor masses and the appearance of no new lesions.

Stable Disease (SD): less than a 50% decrease or less than 25% increase in the product of perpendicular measures of tumor masses and the appearance of no new lesions.

Progressive Disease (PD): greater than a 25% increase in the product of perpendicular measures of tumor masses or the appearance of new lesions.

Pathologic Measurement

Mastectomy specimens will be processed and sectioned to identify residual tumor. Accurate measurements will be performed for quantification of pathologic response. Residual tumor measurements will be carried on at pathology and will be classified as:

Complete Pathological Response (pCR): absence of residual invasive tumor cells both in the removed breast and axillary contents specimens (persistent DCIS is accepted).

Partial pathological response (pPR): <10 persistent microscopic foci of invasive cancer cells in either the breast or nodal specimens;

No pathological response (pNR): microscopic confirmation of persistence of invasive tumor (in more than 10 microscopic foci).

9. PRODUCT INFORMATION

9.1 PACLITAXEL[22, 48-52]

OTHER NAMES Taxol, NSC 673089

CLASSIFICATION: Antimicrotubule agent.

MODE OF ACTION: Promotes microtubule assembly and stabilizes tubulin polymers by preventing their depolarization, resulting in the formation of extremely stable and nonfunctional microtubules, and consequently inhibition of many cell functions.

STORAGE AND STABILITY: The intact ampules are stored at controlled room temperature, 2-25C. Freezing does not adversely affect the product. Solutions diluted to a concentration of 0.3 to 1.2 mg/ml in normal saline or 5% dextrose are stable for up to 27 hours when stored at room temperature and normal room light. Analyses of solutions filtered through IVEX-2 and IVEX-HP (Abbott) 0.2 micron filters showed no appreciable loss of potency.

PREPARATION: The concentrated solution must be diluted prior to use in normal saline, 5% dextrose, 5%dextrose and normal saline, or 5% dextrose in Ringer's solution to a concentration of 0.3 to 1.2 mg/ml. Solutions exhibit a slight haze, common to all products containing nonionic surfactants. Glass, polypropylene, or polyolefin containers and non-PVC-containing (nitroglycerin) infusion sets should be used. A small number of fibers (within acceptable limits established by the USP) have been observed after dilution. Solutions exhibiting excessive particulate formation should not be used.

ADMINISTRATION: Usually administered as an intravenous infusion with a hydrophilic in-line 0.22 micron filter.

INCOMPATIBILITIES: Avoid the use of PVC bags and infusion sets, due to leaching of DEHP (plasticizer). Prior administration of cisplatin may increase myelosuppression because of reduced clearance of taxol. Ketoconazole may inhibit taxol metabolism, based on *in vitro* data.

AVAILABILITY: A concentrated solution of 6mg/ml in polyoxyethylated castor oil (Cremophor EL) 50% and dehydrated alcohol 50% is commercially available in 5 ml ampules.

SIDE EFFECTS:
1. Hematologic: Myelosuppression (neutropenia, leukopenia, thrombocytopenia, anemia).
2. Hypersensitivity: Thought to be caused by the Cremophor vehicle. Minor symptoms include hypotension, flushing, chest pain, abdominal or extremity pain, skin reactions, pruritus, dyspnea, and tachycardia. More severe reactions include hypotension requiring treatment, dyspnea with bronchospasm, generalized urticaria, and angioedema. The majority (53%) of the reported reactions occurred within 2-3 minutes of initiation of treatment and 78% occurred within the first 10 minutes. Reactions usually occurred with the first and second doses.
3. Cardiovascular: Atrial arrhythmia (sinus bradycardia [usually transient and asymptomatic], sinus tachycardia, and premature beats); significant events include syncope, hypotension, other rhythm abnormalities (including ventricular tachycardia, bigeminy, and complete heart block requiring pacemaker placement), and myocardial infarction. Hypertension, possibly related to concomitant administration of dexamethasone, may also occur.
4. Neurologic: Sensory changes (taste changes); peripheral neuropathy; arthralgia and myalgia (dose-related, more common when colony-stimulating factors are also administered); seizures; mood alterations; neuroencephalopathy; hepatic encephalopathy; motor neuropathy; and autonomic neuropathy (paralytic ileus and symptomatic hypotension).
5. Dermatologic: Alopecia, universal, complete, and often sudden, between days 14-21; injection site reactions (erythema, induration, tenderness, skin discoloration); infiltration (phlebitis, cellulitis, ulceration, and necrosis, rare); radiation recall; and rash.
6. Gastrointestinal: Nausea, vomiting, diarrhea, mucositis, pharyngitis, typhlitis (neutropenicenterocolitis), ischemic colitis, and pancreatitis.
7. Hepatic: Increased SGOT (SAST), SGPT (ALT), bilirubin, alkaline phosphatase; hepatic failure, and hepatic necrosis.
8. Other: Fatigue, headaches, light-headedness, myopathy, elevated serum creatinine, elevated serum triglycerides, and visual abnormalities (sensation of flashing lights, blurred vision).

NURSING IMPLICATIONS
1. Monitor CBC and platelet count prior to drug administration.
2. Symptom management of expected nausea, vomiting, and stomatitis.
3. Monitor for and evaluate abdominal pain occurring after paclitaxel administration (especially in severely neutropenic patients and in those receiving G-CSF) due to the risk of ischemic and neutropenic enterocolitis.
4. Advise patients of possible hair loss.
5. Cardiac monitoring for assessment of arrhythmias in patients with serious conduction abnormalities.
6. Monitor liver function tests.
7. Advise patient of possible arthralgias and myalgias which may occur several days after treatment. Monitor for symptoms of peripheral neuropathy.
8. Monitor for signs and symptoms of hypersensitivity reactions. Insure that the recommended premedications have been given. Premedications (diphenhydramine, steroids, and H2 blocker) appear to reduce the incidence and severity of hypersensitivity reactions but do not provide complete protection. Emergency agents (diphenhydramine and epinephrine) should be available.
9. Evaluate IV site regularly for signs of infiltration. It is not known if taxol is a vesicant; however, the Cremophor vehicle for this drug can cause tissue damage.
10. In-line filtration with a 0.22 micron filter should be used.

9.2 ABRAXANE® OTHER NAMES:
paclitaxel CLASSIFICATION:
Antimicrotubule agent.

MODE OF ACTION: Promotes microtubule assembly and stabilizes tubulin polymers by preventing their depolarization, resulting in the formation of extremely stable and nonfunctional microtubules, and consequently inhibition of many cell functions.
STORAGE AND STABILITY: Retain in the original package to protect from bright light. Unopened vials of Abraxane® are stable until the date indicated on the package when stored between 20°C to 25°C (68°F to 77°F), in the original package. Neither freezing nor refrigeration adversely affects the stability of the product. Reconstituted Abraxane® should be used immediately, but may be refrigerated at 2°C to 8°C (36°F to 46°F) for a maximum of 8 hours if necessary. If not used immediately, each vial of reconstituted suspension should be replaced in the original carton to protect it from bright light. Discard any unused portion. The suspension for infusion prepared as recommended in an infusion bag should be used immediately, but may be stored at ambient temperature (approximately 25° C) and lighting conditions for up to 8 hours.
PREPARATION: Abraxane® is supplied as a sterile lyophilized powder for reconstitution before use. AVOID ERRORS, READ ENTIRE PREPARATION INSTRUCTIONS PRIOR TO RECONSTITUTION.

1. Aseptically, reconstitute each vial by injecting 20 mL of 0.9% Sodium Chloride Injection, USP.
2. Slowly inject the 20 mL of 0.9% Sodium Chloride Injection, USP, over a minimum of 1 minute, using the sterile syringe to direct the solution flow onto the INSIDE WALL OF THE VIAL.
3. DO NOT INJECT the 0.9% Sodium Chloride Injection, USP, directly onto the lyophilized cake as this will result in foaming.
4. Once the injection is complete, allow the vial to sit for a minimum of 5 minutes to ensure proper wetting of the lyophilized cake/powder.
5. Gently swirl and/or invert the vial slowly for at least 2 minutes until complete dissolution of any cake/powder occurs. Avoid generation of foam.
6. If foaming or clumping occurs, stand solution for at least 15 minutes until foam subsides.

Each mL of the reconstituted formulation will contain 5 mg/mL paclitaxel.
Calculate the exact total dosing volume of 5 mg/mL suspension required for the patient: Dosing volume (mL) = Total dose (mg)/5 (mg/mL)
The reconstituted suspension should be milky and homogenous without visible particulates. If particulates or settling are visible, the vial should be gently inverted again to ensure complete resuspension prior to use. Discard the reconstituted suspension if precipitates are observed. Discard any unused portion. Inject the appropriate amount of reconstituted Abraxane® into an empty, sterile IV bag.
ADMINISTRATION: Intravenous infusion in sterile IV bag (plasticized polyvinyl chloride (PVC) containers, PVC or non-PVC type IV bag). The use of specialized DEHP-free solution containers or administration sets is not necessary to prepare or administer Abraxane® infusions. The use of an in-line filter is not recommended. Infusion rate: administer intravenously over 30 minutes.
INCOMPATIBILITIES: Abraxane® should not be used in patients who have baseline neutrophil counts of < 1,500 cells/mm^3. The metabolism of paclitaxel is catalyzed by CYP2C8 and CYP3A4. In the absence of formal clinical drug interaction studies, caution should be exercised when administering Abraxane® concomitantly with medicines known to inhibit (e.g. ketoconazole and other imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or induce (e.g. rifampicin, carbamazepine, phenytoin, efavirenz, nevirapine) either CYP2C8 or CYP3A4.
SIDE EFFECTS:
1. Hematologic: Myelosuppression (neutropenia, leukopenia, thrombocytopenia, anemia).

2. Hypersensitivity: In the randomized controlled metastatic breast cancer study, Grade 1 or 2 Hypersensitivity Reactions occurred on the day of Abraxane® administration and consisted of dyspnea (1%) and flushing, hypotension, chest pain, and arrhythmia (all <1%).
3. Cardiovascular: Hypotension, sinus bradycardia (in <1% of patients) - these vital sign changes most often caused no symptoms and required neither specific therapy nor treatment discontinuation; severe cardiovascular events possibly related to single-agent Abraxane® occurred. These events included chest pain, cardiac arrest, supraventricular tachycardia, edema, thrombosis, pulmonary thromboembolism, pulmonary emboli, and hypertension. Cases of cerebrovascular attacks (strokes) and transient ischemic attacks have been reported rarely. ECG abnormalities on study did not usually result in symptoms, were not dose-limiting, and required no intervention. The most frequently reported ECG modifications were non-specific repolarization abnormalities, sinus bradycardia, and sinus tachycardia.
4. Respiratory: Dyspnea and cough were reported after treatment with Abraxane®. Rare cases of (<1%) of pneumothorax were reported. Rare reports of interstitial pneumonia, lung fibrosis, and pulmonary embolism have been received as part of the continuing surveillance of paclitaxel injection safety and may occur following Abraxane® treatment. Rare reports of radiation pneumonitis have been received in paclitaxel injection patients receiving concurrent radiotherapy. There is no experience with the use of Abraxane® with concurrent radiotherapy.
5. Neurologic: The frequency and severity of neurologic manifestations were influenced by prior and/or concomitant therapy with neurotoxic agents.
In general, the frequency and severity of neurologic manifestations were dose-dependent in patients receiving single-agent Abraxane®. The frequency of sensory neuropathy increased with cumulative dose. No incidences of grade 4 sensory neuropathies were reported. Only one incident of motor neuropathy (grade 2) was observed.
Cranial nerve palsies and vocal cord paresis have been reported during postmarketing surveillance of Abraxane®. Because these events have been reported during clinical practice, true estimates of frequency cannot be made and a causal relationship to the events has not been established.
Reports of autonomic neuropathy resulting in paralytic ileus have been received as part of the continuing surveillance of paclitaxel injection safety.
Ocular/visual disturbances occurred in patients treated with Abraxane® in single arm and randomized trials and 1% were severe. The severe cases (keratitis and blurred vision) were reported in patients in a single arm study who received higher doses than those recommended (300 or 375 mg/m^2). These effects generally have been reversible. However, rare reports in the literature of abnormal visual evoked potentials in patients treated with paclitaxel injection have suggested persistent optic nerve damage.
6. Arthralgia/Myalgia: Patients experienced arthralgia/myalgia; 8% experienced severe symptoms. The symptoms were usually transient, occurred two or three days after Abraxane® administration, and resolved within a few days.
7. Hepatic :Among patients with normal baseline liver function treated with Abraxane®, 7%, 36%, and 39% had elevations in bilirubin, alkaline phosphatase, and AST (SGOT), respectively. Grade 3 or 4 elevations in GGT were reported for 14% of patients treated with Abraxane®.
Rare reports of hepatic necrosis and hepatic encephalopathy leading to death have been received as part of the continuing surveillance of paclitaxel injection safety and may occur following Abraxane® treatment.
8. Renal: Overall 11% of patients experienced creatinine elevation, 1% severe. No discontinuations, dose reductions, or dose delays were caused by renal toxicities.
9. Gastrointestinal (GI): Nausea/vomiting, diarrhea, and mucositis were reported.
Rare reports of intestinal obstruction, intestinal perforation, pancreatitis, and ischemic colitis have been received as part of the continuing surveillance of paclitaxel injection safety and may occur following Abraxane® treatment. Rare reports of neutropenic enterocolitis (typhlitis), despite the

coadministration of G-CSF, were observed in patients treated with paclitaxel injection alone and in combination with other chemotherapeutic agents.
10. Injection Site Reaction: Injection site reactions have occurred infrequently with Abraxane® and were mild in the randomized clinical trial. Recurrence of skin reactions at a site of previous extravasation following administration of paclitaxel injection at a different site, i.e., "recall", has been reported rarely.
Rare reports of more severe events such as phlebitis, cellulitis, induration, skin exfoliation, necrosis, and fibrosis have been received as part of the continuing surveillance of paclitaxel injection safety. In some cases the onset of the injection site reaction in paclitaxel injection patients either occurred during a prolonged infusion or was delayed by a week to ten days. Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration.
Asthenia: Asthenia was reported. Asthenia included reports of asthenia, fatigue, weakness, lethargy and malaise.
11. Other Clinical Events
Rare cases of cardiac ischemia/infarction and thrombosis/embolism possibly related to Abraxane® treatment have been reported. Alopecia was observed in almost all of the patients. Nail changes (changes in pigmentation or discoloration of nail bed) were uncommon. Edema (fluid retention) was infrequent; no patients had severe edema. In clinical trials and during postmarketing surveillance of Abraxane®, dehydration was common and pyrexia was very common.
The following rare adverse events have been reported as part of the continuing surveillance of paclitaxel injection safety and may occur following Abraxane® treatment: skin abnormalities related to radiation recall as well as reports of Stevens-Johnson syndrome, toxic epidermal necrolysis, conjunctivitis, and increased lacrimation. As part of the continuing surveillance of Abraxane®, skin reactions including generalized or maculo-papular rash, erythema, and pruritis have been observed. Additionally, there have been case reports of photosensitivity reactions, radiation recall phenomenon, and in some patients previously exposed to capecitabine, reports of palmar-plantar erythrodysaesthesiae. Because these events have been reported during clinical practice, true estimates of frequency cannot be made and a causal relationship to the events has not been established.

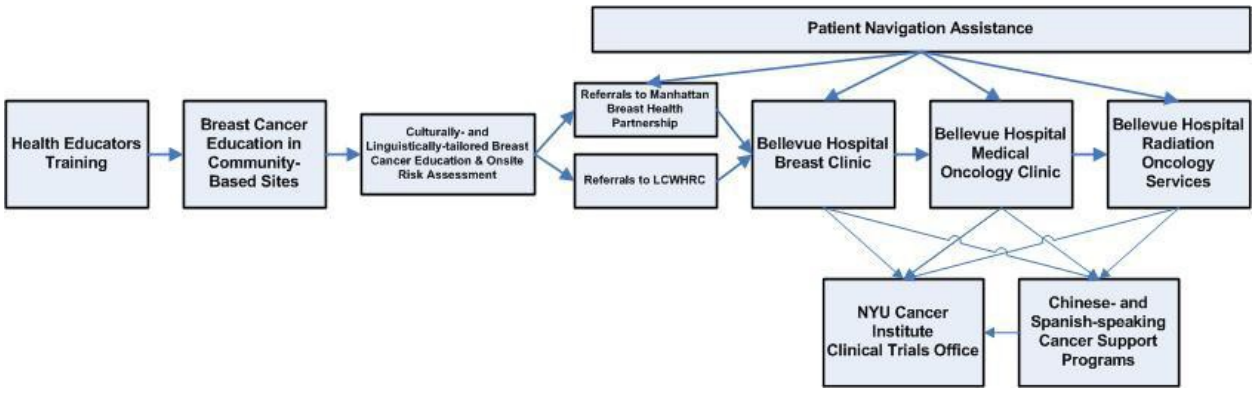
NURSING IMPLICATIONS
1. Monitor CBC and platelet count prior to drug administration.
2. Symptom management of expected nausea, vomiting, and stomatitis.
3. Monitor for and evaluate abdominal pain occurring after Abraxane® administration (especially in severely neutropenic patients and in those receiving G-CSF) due to the risk of ischemic and neutropenic enterocolitis.
4. Advise patients of possible hair loss.
5. Cardiac monitoring for assessment of arrhythmias in patients with serious conduction abnormalities.
6. Monitor liver function tests.
7. Advise patient of possible arthralgias and myalgias which may occur several days after treatment. Monitor for symptoms of peripheral neuropathy.
8. Monitor for signs and symptoms of hypersensitivity reactions..
9. Evaluate IV site regularly for signs of infiltration.

10. PATIENT RECRUITMENT AND REGISTRATION

10.1 Recruitment and Registration in New York City

10.1.1 Recruitment

Potential patients in New York City will be recruited through several outreach programs in addition to walk-ins to the Bellevue Hospital Clinic (see Figure 1). Bellevue Hospital Center (BHC) is an NYU School of Medicine teaching institution with a diverse patient population of which 74% are ethnic minorities. Approximately 60% of BHC's patients are Latino (predominantly Puerto Rican, Dominican, and Mexican), 20% are Chinese and the remainder comprises a mixture of South Asians, Africans, African-Americans (approximately 14%) and non-Hispanic whites. Many women with breast cancer are identified as walk-ins to the BHC clinic and will be recruited into this study if eligible by informed house staff. In addition, potential patients will be recruited to the BHC Breast Cancer clinic through Patient Navigation Assistance and Health Education programs supported by BHC and the Center for Immigrant Health (CIH) at New York University School of Medicine. The CIH has a highly regarded history of working with immigrant communities, bridging some of the City's most medically underserved populations with the health care system for the past 15 years. The CIH has established a large series of community based breast cancer education and referral services for women to the BHC. The CIH arranges for health care coverage for all women regardless of citizenship and ability to pay, and coordinates scheduling of mammograms with the BHC Patient Care Resources and the Rita Kaplan Breast Cancer Imaging Center. All women with suspected breast cancer are then guided to the BHC Breast Clinic, where in triage by consultation with a surgical and medical oncologist women are informed of the COE LABC protocol.



10.1.2 Registration at New York University and Bellevue Hospitals

Eligible patients will be identified by clinical staff at each site. Once eligibility is confirmed (see Patient Eligibility Sections 4.3 and 4.4) and Informed Consent is documented, the patient will be registered with the NYUCI Clinical Trials Office (CTO) registry which is available Monday through Friday from 8:00 AM to 5:00 PM, EST at (212)263-4210, Fax (212)263-4111. All patients will be consented (see Section 12, below) and the last page of the Informed Consent form will be faxed to the CTO registry.

10.2 Recruitment and Registration at South Africa Sites

Patients will be identified by clinical staff at each site for possible recruitment into the study. Registration for each patient will take place at the local clinical center. To complete registration of a patient accrued to trial from Tygerberg Hospital (South Africa), the study coordinator at each site will fax to the NYUCI Clinical Trials Office (CTO) the completed Eligibility form and Informed Consent (signed) and supporting source documentation according to the same registration process described above in Section 10.1.2.

Further details regarding these clinical centers (including Standard of Care and Patient Safety Assurances; as well as IRB committees and criteria) are included in Appendix 6.8.

11. PROTECTION OF HUMAN SUBJECTS

11.1 Benefits/Risks

11.1.1 Clinical procedures: specific benefits/risks associated with each clinical procedure were outlined in the TREATMENT Section 6. Potential toxicities associated with treatment were listed there as well.

11.1.2 Research procedures: each patient will be asked to donate a small part of the biopsy tissue that was collected to confirm her cancer diagnosis. In addition, she will be asked to donate a small amount of blood (2-3 tablespoons). The study biopsy is usually a well-tolerated procedure that involves only minor discomfort. Occasionally, biopsy sites may bleed or become infected. The study blood will be drawn in three occasions during a routine blood test so no additional needle stick is required. Occasionally, the site of the blood draw may bleed, become sore for a few days or become infected. The potential risks and discomforts associated with tissue sample, blood sample are explained in the Informed Consent document (Appendix, Section 16.5).

11.2 Privacy/Confidentiality

11.2.1 The medical, hospital and research records associated with this study are considered confidential. Members of the treating team and designated study assistants will have access to the records as required to administer treatment and comply with the protocol. Neither the name nor any other identifying information for an individual will be used for reporting or publication regarding this study. All laboratory and baseline data including Baseline Case Report forms and Qualitative Interviews will be de-identified and transferred via secure links to the BDM core at NYU School of Medicine. Patient records will be made available for inspection to auditing agencies to satisfy regulatory requirements.

11.3 Non-Health Related Risks

Participation in this research study could potentially expose the research subject to non-health related risks. These include the potential breach of medical

confidentiality and release of personal medical information to an insurer, employer, family members, or other persons outside the research study staff. The release of this information could result in denial of medical coverage, termination of existing medical insurance coverage, or loss of employment. The actual risk of these events is extremely low.

11.4 Serious Adverse Events (SAE) Reporting

SAEs that occur in this study must be promptly reported to the study P.I. (Dr. Nelly Huppert) as well as to the NYU IRB (1 Park Avenue, 6th Floor New York, NY 10016) and to the NYU Clinical Trials Office (462 First Avenue, New York, NY 10016) for reporting to the NYUCI Data Safety Monitoring Committee.

The IRB Serious Adverse Event Report Form (Appendix, Section 16.6) available electronically at http://www.med.nyu.edu/IRB/ should be used for all adverse events with an attached copy of information provided to the Sponsor. The requirements for the reporting of adverse events are delineated in the Adverse Event policy that is appended to document 16.6. Investigators should also be aware of local facility requirements for reporting adverse events associated with patients seen at the local facility. The NYU Clinical Trial Office will submit all summaries of safety data and the standardized SAE reports to the NYUCI Data and Safety Monitoring Committee for the regularly scheduled semi-annual review. Dr. Huppert will attend the DSMC meeting to present the trial and discuss the safety issues. DSMC will review the SAEs and the accuracy of the reporting to appropriate review committees annually from the date the first patient is enrolled and at pre-specified interim analysis time points defined in the protocol (Appendix 16.7 DSMC Procedures). Details of the interim safety monitoring rule for each site are provided below in Section 13.0.

The Medical Monitor is required to review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor should comments on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator. Expedited SAE reporting will utilize for this study the descriptions and grading scales as presented in Expected Toxicities and Treatment Modifications sections for Breast Irradiation (Sections 6.1.3-1.1.4) and Chemotherapy (Sections 6.2.4-6.2.5).

11.5 Medical Care for Research Related Injury

Consent Forms (Appendix 16.5) contain the following language to inform volunteers regarding medical care for research related injuries: –If you experience any research-related injury or side effect during the course of this study, please contact the Principal Investigator, Nelly Huppert, MD, at the following telephone number: 212-731-5003. In the event that you experience a side effect or injury as a result of the drugs or procedures related to this study, the study doctor will assist you in obtaining appropriate medical treatment but this study does not provide financial assistance for medical or other injury-related costs. You do not give up any rights to seek payment for personal injury by signing this form. All forms of medical (or mental health) diagnosis and treatment—whether routine or experimental—involve some risk of injury. In addition, there may be risks associated with this study that we do not know about. In spite of all precautions, you might develop medical complications from being in this study. If you have questions about this medical care, talk to the principal investigator for this study, Nelly Huppert, MD, at the following telephone number 212-731-5003. If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator.

12. INFORMED CONSENT

12.1 Procedure for obtaining Informed Consent

Patients or their legal guardians will be provided a comprehensive explanation of the proposed treatment including the type of therapy, the rationale for treatment on the protocol, alternative treatments that are available, any known adverse events, the investigational nature of the study and the potential risks and benefits of the treatment. The Informed Consent document will meet all requirements of Federal Regulations, as well as the Institutional Review Boards of the treating centers. An English and Spanish version of the Informed Consent form is included in the Appendix, Section 16.5. The physicians who may obtain informed consent are listed on the title page of this protocol for each participating site. The Informed Consent form will be signed by the participant and the registering physician in triplicate. One signed original will be given to the patient, one will be maintained with the patient's medical record and one will be kept on file at the CTO. A copy will be kept on file by the EDC Core.

12.2 Procedure for Obtaining Research Authorization

Before any protocol specific procedures can be carried out, investigators/staff will fully explain the details of the protocol, the study procedures and the aspects of patient privacy regarding research information. In addition to signing the IRB Informed Consent, all patients must sign the Research Authorization component of the Informed Consent form.

The original signed documents will be a part of the patient's medical records as described above.

12.3 Minimization of Coercion and Undue Influence

All subjects/patients are informed in the Consent that participation or refusal to participate in the research study will not affect any of the clinical treatment or services to which they would otherwise be entitled.

13. DATA ANALYSIS/STATISTICAL CONSIDERATIONS

13.1 Study Objectives

The primary objective of the study is to confirm the pathological response rate of 34% (pCR+pPR), reported for patients in the US Phase I-II trial[44].

The anticipated power to interpret the results of the international clinical trial versus the US trial: An overall difference in pathologic response rates between and South Aftrica combined of +/- 24% compared to the overall NYU cohort will be detectable with power 80% (150 total, 100 at NYU) and significance level ≤5%, 2-sided binomial comparison. (The inclusion of 20 additional patients from India will yield a detectable difference of +/- 22%.). For the comparison of South Africa individually to the NYU cohort, with 25 patients/site, a difference of +/-34% is detectable. Each of the cohorts will be analyzed separately.

The classical chi-square test will be used to compare the three pathological response rates. If the test finds the rates differ as expected, we will identify the cohorts with different rates using multiple comparison tests and report confidence intervals for the response rates within and across the cohorts, and for the magnitude of related differences and odds ratios. If there are no differences between the cohorts with respect to overall response rates, with a total of 150 patients, we can estimate the pathologic response rate with 95% confidence limits of +/-8%, if the expected overall rate is 34% in this confirmatory study.

Across all cohorts, logistic regression models will be used to identify predictors of pathologic response. If the proportion of patients with LABC with the predictor ranges from 0.05 to 0.1 to 0.25 to 0.5, the detectable odds ratio at 0.05 level (2-sided) and 80% power ranges from 8.7 to 4.6 to 2.8 to 2.5 for the overall cohort of 150 patients (assuming path response rate of 34%).

If the cohorts are similar with respect to pathologic response rates, then they can be combined for further analysis of predictors of progression and survival. Progression free survival and survival will also be estimated in each cohort individually and across cohorts. All patients will have completed at least one year of follow-up on study after diagnosis and be evaluable for the analysis of the primary endpoint of pathologic response to treatment in year 5 of the study. Follow-up of all patients for recurrence, metastases, and death will continue beyond the 5 years of the study. Methods of censored data analysis that incorporate all patients taking into account variable lengths of follow-up will be used for the analysis of these other endpoints in study year 5 and again when more complete long term follow-up data are available.

Interim monitoring for safety will be conducted at the NYU/Bellevue site when 25 patients have completed the evaluation for pathologic response and when 50 patients have completed the evaluation for pathologic response. Toxicity/failure is defined as having at least one of the following during the chemoradiation phase of this study: grade 3-4 non skin *positively (possibly, probably, definitely)* related toxicity *(excluding Lymphopenia)*), disease progression requiring change in treatment, or recurrent toxicity requiring greater than a 50% reduction in chemotherapy dose. The study will discontinue enrollment at an individual site if the observed toxicity/failure rate is greater than the upper edge of the 95% confidence interval (assuming a true underlying toxicity/failure rate of 20%) as in the table below at each of the two early looks. If the NYU/Bellevue site continues past the 50 patient enrollment, enrollment will continue to the end of the planned 100 patients. For the other two sites combined, interim monitoring will occur when 15 patients and when 25 patients have completed the evaluation for pathologic response. The criteria for discontinuation of enrollment are provided in Table

A. Stopping rule for interim monitoring NYU/Bellevue

Interim Look	Number of Patients	Upper Edge of Exact* 95% Confidence Interval for Failure Rate
1	25	41%
2	50	34%

B. Stopping rule for interim monitoring (S. Africa. combined)

Interim Look	Number of Patients	Upper Edge of Exact* 95% Confidence Interval for Failure Rate
1	15	48%
2	25	41%

* Clopper Pearson exact 95% confidence interval; calculations from PASS 2008, NCSS, Hintze, J., Kaysville, Ut.

This project is part of the LABC Center of Excellence and patients will be included in the various projects to study genomics, biomarkers and hormonal markers. A common random sample of 75 patients, stratified by site, will be selected from the complete cohort of 150 patients enrolled in the treatment protocol in Project 1 for study in Projects 2 (functional genomic), 3 (molecular and biochemical alterations), 4 (mutation analysis), 7 (p53 polymorphisms) to allow a complete set of data to be generated for the same subset of patients. Similarly, the study of cultural norms (Project 6) will be based on a stratified random sample. The approach to using a common sample of patients for each of the research projects that will not be conducted on all 300 patients will facilitate the identification of characteristics that can be used to predict pathological response and progression and survival from the multi-ethnic cohorts under study and the identification of hypotheses for the generation of further studies.

The general strategy for these statistical analyses follows here. In the first stage of these analyses, we will use descriptive (numerical and graphical) statistics to summarize the baseline clinical and disease variables for each cohort, including pathological

response, disease progression and survival of patients enrolled on the clinical protocol. We will also use similar descriptive statistics to compare the data on the incomplete sample of 75 patients to the entire cohort. Next, we provide nonparametric analyses including confidence intervals for various proportions (e.g. response rates) within each cohort and differences in proportions between cohorts. For each project, variable selection strategies will be used. Once the variable selection within the project is completed, we will fit logistic regression models to study the relationships between the predictors (gene profiles, etc) and pathologic response to treatment (complete vs not) using variable selection tools. For time to progression and survival, we will develop proportional hazard models. Finally, we will describe approaches to analysis of data with partially observed covariates. The 3 steps in these analyses include: *1*. Use all data available in each individual project to select variables predictive of the outcomes (pathologic response to treatment, etc) in that project. For projects 1, 3-6 this will be accomplished using logistic regression with a variable selection tool such as Lasso. For project 2, we will use a classification method specific to microarray data to select variables (i.e. genes) predictive of the outcome [53]. We will then pool the sets of variables together to include the complete data model in step 2. Use the variables identified as predictive of pathologic response from each individual project as explanatory variables. Use complete data (75 patient sample) to fit a logistic regression model with variable selection procedure such as Lasso to identify the variables predictive of pathologic response. *3*. Use all available data on 150 patients with variables selected in Step 2 (some of which are only partially observed) and use maximum likelihood methods and the EM algorithm to fit the final model.

Some specific methods to be used are described here:
Logistic Regression: Multiple logistic regression models [54] will be used to select predictive variables for the binary outcome variables such as pathologic response (e.g., 1 for response and 0 for non-response) to the treatment of LABC characterized in Project 1. Other variables from the studied subjects will be used as explanatory variables, including gene signatures described in Project 2, molecular and biochemical markers from Project 3, immunological factors of Project 4, mutational factors from the selected patients of Project 5, estrogen metabolites from Project 6, genotypes from Project 7 and other demographic factors (age, behavioral-cultural, socioeconomic status, etc).

Variable Selection Methods: Multiple logistic regression with variable selection procedure such as Lasso [55] and Least Angle Regression [56] will be used to identify a subset of the predictors among the many factors to pathologic response, progression to local recurrence or metastatic disease, and survival, respectively. With the large amount of data obtained from this study concerning multi-ethnic cohorts and multi-disciplinary collaborative research projects, we can attempt to characterize sets of predictors that are common across cohorts as well as those specific to each individual cohort, ranging from genetic, molecular to immunological factors. To assess the performance of these models, we will use cross-validation methods.

Interactions: It is natural to expect that there might be interactions among the outcomes (pathologic response, progression, and survival), and biological factors (e.g. gene

profiles, Project 2, and genomic mutations, Project 5) or genotypes, Project 7) and other groups of variables (e.g. behavioral and cultural). We will employ statistical models to investigate these potential predictors concurrently, and the interpretation of these predictors and findings will be done in collaboration with COE investigators. For example, ethnic differences are documented in outcomes of LABC patients [3-5, 57] and, therefore, it is of interest to see whether the ethnicity remains important in the logistic regression model after adjustment for other factors in the model.

Treatment of Data with Partially Observed Covariates: Since several of the projects (2, 4, 5, 8) will be performed using a randomly selected subset of the subjects, we will have complete data available for these 75 selected subjects; data on the remaining 150 patients will have partially observed covariates. One approach for the analysis of pathologic response is to treat the covariates with incomplete observations as categorical and to fit a logistic regression model using maximum likelihood methods and the EM algorithm [58-59]. We will use 75 observations with complete data to fit proportional hazards models for progression free survival and survival. We will use Lasso penalty for variable selection [55]. The model will then be calibrated using the remaining data with incomplete observations on some covariates. While we expect that the models built on 75 patients with complete data and the model built on the full dataset of 150 patients with incomplete observations will be equivalent, we will compare the survival curves for patients included in the complete and incomplete data sets to verify this assumption.

13.2 Disposition of Data - Data Management and Monitoring

13.2.1 *Data-Sharing Agreement with Amrita Institute of Medical Sciences (Cochin India)*

Investigators at the Amrita Institute of Medical Sciences (AIMS) in Cochin India and NYUSOM agree to participate in a data-sharing arrangement for the investigation of outcomes yielded from this study. Specifically, patient demographic and clinical treatment data, toxicity data, and pathological outcomes are to be collected and stored locally at AIMS, and subsequently de-identified and shared with investigators at NYUSOM for the purpose of joint analysis and reporting in co-authored presentations and scientific papers.

13.2.2 *Database Properties*

Data will be entered into the Oracle Clinical database and maintained at NYUSOM under the direction of staff in the BDM core. De-identified data to be shared from the AIMS site will be kept in a distinct, parallel Oracle Clinical database maintained at NYUSOM, separate from the NYUSOM generated data. This will allow for comparison.

The Oracle system provides audit trails that track creation and modification of records that include userid and timestamp. Once entered, the data is subjected to validation procedures that are executed either immediately or upon saving the eCRF page or during the batch validation process. Validation failures that are identified before the page is saved can be corrected immediately. Validation failures during saving of the eCRF page and during batch validation processes will generate a discrepancy. Depending on the database account privileges, the data managers may be able to correct a discrepancy or if not, route it to the project

data manager at NYU who can take appropriate action to correct the problem. Data clarification forms can also be printed out when necessary to be sent to the project data manager at NYU. Once the discrepancy is closed, by marking ―resolved‖ or ―irresolvable‖, the data is marked clean and an audit trail is generated by the system.

All key end points will be source verified by a second person at each site and errors will be corrected. Once the data is verified and all discrepancies are closed, the data can be locked/frozen. Locking and freezing can be done at different granular levels and will follow institutional SOPs and any specific requirements for the project.

Security measures that will be taken in order to protect patient data will include firewall technology and database level security which will be achieved by assigning roles and privileges to different levels of users and by requiring that the users authenticate themselves using userid and password. Additional security for data transfer between remote clients and servers will be achieved by using digital certificates/SSL. All data will be backed-up to tape periodically according to the Institutional SOPs. All data will be stored for at least 5 years following the termination of this study.

13.3 Microarray data

The data generated from microarray experiments will be stored using GeneTraffic, a client-server relational database currently in use by NYU Bioinformatics Group. GeneTraffic can also store all microarray files generated by the Affymetrix system and by two-color microarray systems. It can store TIFF images as well as output from all of the common image analysis programs. The data can be stored as raw images and tables of signal intensities for each probe and gene. GeneTraffic also provides advanced algorithms for data normalization and analysis at the level of individual probes. Additionally, GeneTraffic software incorporates MIAME guidelines during data annotation and has project tracking features, data searching algorithms, data exploration and visualization.

The database is accessible over the Internet from other institutions, making it simple for members of the COE to collaborate on microarray projects. Data can be uploaded and analyzed from any workstation via a web interface, onto a server that is maintained in a secure data center and backed up automatically to tape. With the appropriate permissions (userid and password), users can directly upload data for a project, or view data that has been loaded and analyzed at NYU. Data will be stored for no less than 5 years following completion of this study.

14. DEVIATION FROM THE PROTOCOL

No modifications to the protocol should be made without the approval of the PI, the NYUCI IRB.. Changes that affect the study design including numbers of patients, safety of patients, the scope of the investigation, or the scientific quality or efficacy of the study will require IRB notification prior to implementation, except where the modification is necessary to eliminate an immediate and apparent hazard to patients. If an immediate departure from the protocol is necessary, the Investigator will contact the

PI to discuss the planned course of action. If possible, contact should be made prior to the implementation of any changes. Any departures from protocol must be fully documented in the Case Report Form and source documentation. .

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