

Protocol For A Randomised, Multicentre, Double Blinded Phase III Study Of Perioperative Ketorolac In Women Of African Descent With Operable Breast Cancer

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Abstract

Cancer remains one of the leading causes of morbidity and mortality worldwide. Breast cancer is the most common form of malignancy occurring in women around the world. The aim of this present protocol is to outline the procedure for a randomised, multicentre, double blinded phase III study of perioperative Ketorolac in Women of African Descent with Operable Breast Cancer. The typical type of breast cancer in sub Saharan Africa is triple negative breast cancer (TNBC) and is usually considered the worst early breast cancer diagnosis since there are no known targeted therapies and patients often relapse and die early. sub-Saharan Africa seems the perfect place to conduct a randomized controlled double blinded clinical trial of perioperative NSAID Romano Demicheli 1, Erhabor Osaro 2, Michael Retsky 3 Forget Patrice 4. Vaidya Jayant S 5.

Scientific Directorate Fondazione IRCCS Istituto Nazionale Tumori di Milano Italy 1, Usmanu Danfodiyo University, Sokoto, Nigeria 2, Harvard TH Chan School of Public Health Boston MA USA 3, Department of Anesthesiology, Universite catholique de Louvain, St-Luc Hospital, Av. Hippocrate 10-1821, 1200 Brussels, Belgium 4, University College Hospital, London, UK 5. etorolac to potentially reduce early relapses in breast cancer. The primary and secondary objectives of this trial are to evaluate and compare the Disease Free Survival (DFS) and Overall Survival (OS) of operable breast cancer patients randomised to standard treatment versus standard treatment plus perioperative Ketorolac and to compare the safety and tolerability of the treatment as well as to identify pre- and post-operative factors with prognostic relevance and establish correlations with clinical outcomes. It is anticipated that trial will be successful and would turn TNBC from the worst prognosis to the best and potentially help improve the quality of life of African women suffering from breast cancer.

Keywords: Protocol; Randomised double blinded phase III study; perioperative; Ketorolac; Women; African; Breast Cancer.

Introduction

Background

Breast cancer is the most common form of malignancy occurring in women around the world. Each year, more than 200,000 women in the United States are diagnosed with breast cancer and 40,410 women died from the disease in 2006 [1]. In the European Community, an estimated 135,000 new cases per year and 58,000 recorded deaths per year are reported [2]. In sub-Saharan Africa the figures are 94,000 new cases per year and 48,000 deaths per year [3].

Surgery is the main modality of local treatment for breast cancer. Surgery and/or radiotherapy can control loco-regional disease in the majority of patients. However, rates of subsequent local recurrence is between 19–50% and a significant number will ultimately die due to distant recurrence of disease. Two types of systemic adjuvant therapy have been used increasingly over the last years to successfully reduce the rate of breast cancer recurrence and death. Adjuvant chemotherapy involves a combination of cytotoxic anticancer drugs; adjuvant hormone therapy deprives cancer cells of the hormone oestrogen, which some breast cancer cells need to grow. These therapeutic modalities are complementary and are often used in combination.

Concomitant to the achieved improvements in disease treatments, the paradigm explaining the natural history of breast cancer was refined and new important findings were reported. In particular investigations on local recurrences following mastectomy and on the dynamics of both local and distant recurrences established that the traditional concept of uninterrupted growth of metastases should be rejected and that an interrupted tumour growth model should be assumed, implying episodes of 'tumour dormancy' [4]. According to this biology, the development of metastases includes sequential passage through a few phases following tumour cell shedding from the primary: (a) single, mostly non-dividing, tumour cells (cellular dormancy), (b) non-angiogenic micrometastases (and angiogenic ones in the presence of antiangiogenic factors) that cannot grow more than the size of avascular foci (micrometastatic dormancy), and (c) vascularized growing metastases destined to reach the clinical level. This orderly process may be controlled by the primary tumour that can exert restraints on the transition between dormancy phases, thus retarding or inhibiting metastasis development. Therefore, surgical removal of primary tumour may disrupt tumour homeostasis, with sudden acceleration of the metastatic process, at least for some patients.

A most important finding of this paradigm is that something happens at or about the time of surgery to accelerate or induce metastatic activity that results in early relapses. Surgery-induced angiogenesis of dormant avascular micrometastases and surgery-induced activity of single malignant cells are im-

plicated. A fairly short time-window at the primary tumour surgical removal emerges as a critical phase apparently determining the disease course through the subsequent years.

Recently, a Belgian group reported data from a retrospective disease free survival study of 327 consecutive patients who were compared according to the perioperative analgesics administered (sufentanil, clonidine, Ketorolac and ketamine) [5]. As stated in the initial report, the sample size was limited by availability of medical records and to maintain oncologic treatment homogeneity. Indications for mastectomy with axillary clearance were defined according to international recommendations. Chemotherapy, radiotherapy and endocrine therapy were performed according to the international expert consensus (9th and 10th St-Gallen consensus). Follow-up in that initial report was average 27.3 months with range 13-44 months. Perioperative administration of the Non-Steroidal Anti-Inflammatory Drug (NSAID) Ketorolac was associated with significantly superior disease-free survival in the first few years after surgery. After 24 months the ketorolac group hazard rate pattern is indistinguishable from the corresponding pattern for the no-ketorolac group. The updated analysis confirmed that the benefit appears in the 9-18 month hazards and is of magnitude 4 – 6 fold, consistent with the early report [6]. This finding is in keeping with a number of recent reports suggesting that surgery may be less successful in patients with a pre-existing elevation of some inflammatory scores, such as the neutrophil/lymphocyte ratio (NLR), the prognostic value of which was observed as well in mastectomy as in conservative breast cancer surgery [7-8].

These results may be deciphered in the light of this model. Indeed, post-surgical transient systemic inflammation might be the precipitating factor and common denominator for early relapses. In particular, inflammation would be important for angiogenesis induction of avascular distant micrometastases. Several molecular processes could be involved, either as single mechanisms or concurrently. For example, inflammation induced upregulation and release of factors stimulating endothelial cells to proliferate could also induce endothelial cells to secrete specific cytokines that reciprocally support the regeneration of malignant stem cells within the metastatic niche. Or else, as the SDF-1/CXCR4 axis is a main regulator of normal and tumoral cell trafficking, it is reasonable to hypothesize that NSAIDs may interfere with SDF1 levels via the pathway COX-2 → PGE → SDF-1, thus resulting in impairment of processes underlying metastasis development. Another possible explanation involves inflammation induced platelet degranulation, with release of angiogenesis regulating factors including VEGF, which would be countered by Ketorolac. Lastly, tumour cells in circulation prior to surgery and tumour cells released as a result of surgery in the presence of transient systemic inflammation and capillary permeability could also account for succeeding metastatic development [9-10].

Number of Subjects

200 patients

Target Population

Women with primary breast cancer T1- 3, N0-2, M0

Treatment Arms

Treatment arm 1 - Ketorolac tromethamine 30 mg iv bolus before surgical incision and then orally every 8 hours for 13 times on days 1 to 4 post operation.

Treatment arm 2 -Standard or care as per predefined protocol.

Length of Study

Recruitment will last approximately 48 months. During the first year of follow up, patients will be followed every 3 months. Patients will be followed every 6 months during years 2 to 4 of follow up and annually thereafter, up to year 10 after randomization of the last patient.

Statistical Analysis

One formal interim analysis is planned approximately when the first assessable 120 patients will reach the median follow-up of 30 months. This interim analysis will occur approximately three years after study initiation.

Secondary Endpoints: Adverse events will be summarized descriptively according to grade. Differences between treatment groups will be tested for significance using methods for categorical data.

Trial Design

Parallel, two-arm, randomised, double blinded and multicentre phase III study in women with primary breast cancer

Rationale

Most relapses (50–80%) reside in this first peak, while the others are distributed mostly around the fifth to the sixth postoperative years. The hazard rate of early recurrence is greater in high risk patients identified with worse primary tumour characteristics (large size, high grade, lymph node involvement). Intraoperative NSAIDs administration is associated with a lower incidence of early detection of postoperative distant metastases. As a consequence, it could be assumed that the effect of ketorolac may be higher in the subgroup with the highest risk of early recurrence [11].

It is worthy to note that all the differential effects of factors

analysed so far on the recurrence risk seems to suggest that a balance between tumour and host traits influences the pace of the common pathway. The recurrence risk profile of a single patient apparently results from a specific mix of factors that are related to genetic, environmental, or behavioural traits, which may be affected by race-related factors [12]. For instance, triple negative breast cancer (TNBC) patients have frequency of 12% in US population, 25% among African Americans and even more in Sub-Saharan Africa populations [13]. TNBC is looked upon by clinicians as a “bad tumour” with high recurrence rate in spite of adjuvant chemotherapy. That pessimistic viewpoint seems justified since in USA TNBC has 12% incidence but accounts for approximately 20% of mortality in breast cancer. Recurrence dynamics of TNBC patients displays a dominant early peak that looks remarkably similar to the no-Ketorolac group in the Forget *et al.* study [8]. TNBC, therefore, appears to be an ideal study group with which to test the benefit of perioperative ketorolac in a clinical trial [14].

Breast cancers diagnosed among African women [4] reportedly include a disproportionate number of poor prognosis tumours, including hormone receptor negative, triple negative, and core basal phenotype tumours. The average age of diagnosis of breast cancers among African women tends to be young, with estimates that a majority of cancers develop among women 50 years or younger a considerably younger age (about a decade) than seen in Caucasian populations. This trend is similarly detectable in USA where African-American women also tend to develop breast cancers at younger ages than Caucasian women. The majority of studies report a high frequency of poorly differentiated tumours, although there has been considerable variation across studies. Tumours tend to be large, with the vast majority being >2 cm. In addition, the majority of studies showed greater than 70% of patients had node positive or Stage III tumours. Consistent with the reported high prevalence of poorly differentiated and early-onset tumours, many of the tumours have been reported as hormone receptor negative. However, reported rates of both ER and PR negativity have varied substantially across studies, with the respective rates ranging from 36–79% and 30–87%. Fewer studies have reported on HER2 status, but tumours have largely been classified as not expressing this marker. As a result, the rates of triple negative cancers have been high, with a number of studies showing that the majority of African women are diagnosed with such tumours. Similar situation is reported for Nigeria as well [15].

It is estimated that by the year 2020, 70% of the twenty million new cancer cases will occur in countries that collectively have only five percent of the global resources for cancer control [16]. Globally the incidence of breast cancer is increasing and concomitantly the need for systemic anticancer agents will continue to increase over the next ten years. The pharmaceutical companies are developing increasingly expensive novel anticancer molecules with no indication that the rapid-

ly escalating cost of new treatments will ease in the future. It can be safely assumed that the cost of novel anticancer drugs will continue to expand as an insurmountable obstacle to care for an ever greater proportion of cancer patients in Low and Middle Income Countries (LMCs) where the majority of the world's population live. This cloudy outlook underscores the prominent importance of confirming the hypothesis that Ketorolac is effective in reducing breast cancer recurrence rate. If confirmed, this therapy could be an ideal inexpensive and non-toxic solution for a significant fraction of the early relapse problem in breast cancer, with enormous impact on breast cancer treatment of LMCs.

Randomized clinical trials are the bedrock of evidence-based decision making and are considered the gold standard for clinical research worldwide. Clinical trials generate data on safety and efficacy of therapy [17]. The goals of a randomized clinical trial are 2-fold; testing medical treatments to confirm that they work well enough (efficacy or effectiveness) and to investigate whether they are safe enough (safety) ensuring that the benefits of treatment outweigh the potential side effects. There are however several challenges associated with carrying out randomized clinical trials in developing countries; the bureaucracy and administrative bottle necks involved in obtaining ethical approval from the health authority/ ethical review board in the country/institution where approval of the therapy is sought, challenge of size, cost (non-affordability of required monitoring laboratory test and medication), cultural challenges, socioeconomic factors, effective and accurate data collection, lack of trained manpower, suboptimal laboratory and health infrastructure and suboptimal cold chain management of temperature-dependent reagents (due to power outages) [18]. In low-resource settings, structures such as the Regulatory Authorities (RA) and Institutional Review Boards (IRB) that play key roles in monitoring and approval of trials may be non-existent, non-functional or lack the skills to critically appraise a research protocol. There is often a lack of data on trial methods and operational challenges in low-income countries. A previous report advocates the need for researchers based in developing countries to form collaborations with scientists in the West to work effectively together and share evidenced-based best practices, experiences, methods and resources [19]

The cost of carrying out a clinical trial however tends to be less expensive in developing countries compared to developed countries. There are multi-factorial reasons for this; lower salary and overhead costs, less time required to enroll participants, less informed consent-related challenges, prevalence of larger treatment naive populations with higher incidence rates and more advanced stages and high prevalence of the diseased state in the population [20-21].

Provided the therapy being investigated and all the monitor-

ing laboratory investigations are going to be done at no cost to the subjects, recruiting adequate numbers of subjects is usually not a challenge. This is particularly true because in most developing countries, patients requiring medical attention are required to pay for the services. Healthcare delivery is only universal for those that can afford it. It makes moral and ethical sense for wealthier countries to help sponsor life-saving research in developing countries to bring maximum public health benefits to these communities [22].

Objectives

Primary

The primary objective will be to evaluate and compare Disease Free Survival (DFS) and Overall Survival (OS) of operable breast cancer patients randomised to standard treatment versus standard treatment plus perioperative Ketorolac. DFS time will be measured from the date of surgery to the date of first recurrence and OS time will be measured from the date of surgery to the date of death. Recurrences will be separated into local recurrence, regional recurrence and distant metastasis. Distant metastases will be categorized as soft tissue, bone and visceral metastases. The first evaluation and comparison of DFS values will be performed at 3 years and repeated at 5 years when the first evaluation and comparison of the OS values will be added.

Secondary

- To assess the safety and tolerability of Ketorolac administration
- To study inflammation related markers before and after primary tumour removal in both arms of the study and to establish correlations with clinical outcome

Study Design

This is a parallel group, two-arm, randomised, multi-centre, double blinded phase III study. Eligible patients must be women with primary breast cancer ≤ 2 to ≥ 5 cm diameter who have not undergone previous treatment for invasive breast cancer. The study will compare the efficacy and tolerability of perioperative Ketorolac versus placebo.

Treatment Assignment and Stratification Factors

Patients will be assigned to study treatment in accordance with the randomization schedule. The randomisation will be stratified according to the following four factors, each of which must be known prior to commencing randomisation:

- Tumour size [T1 (≤ 2 cm in diameter), T2 (2 to 5 cm in diameter), T3 (more than 5 cm in diameter)];

- Clinical nodal status [N0 versus N1/N2];

Subjects will be identified by a unique subject number that will remain constant for the duration of the study. Involved centres will contact the data management office to register patients and receive treatment assignments. A confirmation of randomisation will be registered by the investigator and the document must be retained with the case registration form (CRF). Patients will be randomised to one of two treatment arms and receive the following study treatments:

Treatment arm 1 - before the surgical incision, an i.v. bolus of 30 mg of Ketorolac tromethamine will be administered. The standardized anaesthetic protocol will include: bolus followed by a continuous infusion of propofol (as needed to maintain bi-spectral index value between 40 and 60 during the surgery), ketamine 0.3 mg/kg, clonidine as needed to maintain hemodynamic stability up to 4 µg/kg and sufentanil by boluses of 0.1 µg/kg as needed [23]. Airways will be instrumented by a laryngeal mask airway. Ketorolac tromethamine 30 mg iv will be administered every 8 hours from the first (preoperative) administration for 13 times on days 1 to 4 post operation. If needed, postoperative analgesia may include the administration of acetaminophen (3–4 g/day). Routine practice by Forget and colleagues [5], Ketorolac, when administered is used as follows: 20 mg pre-incisional in patients under 60 kg, and 30 mg in patients over 60 kg). However, for a clinical trial, a fixed dose of 30 mg pre-incisional is easier [23].

Treatment arm 2 - before the surgical incision, an i.v. bolus of NaCl 0.9% (3 mL) will be administered. The standardized anaesthetic protocol will include: bolus followed by a continuous infusion of propofol (as needed to maintain bi-spectral index value between 40 and 60 during the surgery), ketamine 0.3 mg/kg, clonidine as needed to maintain hemodynamic stability up to 4 µg/kg and sufentanil by boluses of 0.1 µg/kg as needed. Airways will be instrumented by a laryngeal mask airway. I.v. bolus of NaCl 0.9% (3 mL) will be administered every 8 hours from the first (preoperative) administration for 13 times on days 1 to 4 post operation. Postoperative analgesia could include the use of acetaminophen as needed (3–4 g/day), tramadol 50 mg and piritramide 10 mg IM/6 hour The placebo will be identically presented as the Ketorolac group to ensure double blinding.

Post-operative Treatments

In patients for whom chemotherapy and/or radiotherapy is appropriate, it will be performed according to the standard guidelines.

Study population

Inclusion Criteria

- 1) Female gender;
- 2) Age ≥ 18 years;
- 3) Performance Status- Eastern Cooperative Oncology Group (ECOG) 0-1 (See Appendix 2);
- 4) Histologically confirmed invasive breast cancer:
 - Primary tumour no more than 5 cm diameter, measured by mammography and echography,
 - Any axillary nodal status,
 - No evidence of metastasis (M0) (isolated supraclavicular node involvement not allowed);
- 5) Haematopoietic status:
 - Absolute neutrophil count $> 1,5 \times 10^9/L$,
 - Platelet count $> 100 \times 10^9/L$,
 - Haemoglobin at least 9 g/dl,
- 6) Hepatic status:
 - Bilirubin $\leq 2 \times$ upper limit of normal (ULN),
 - AST and ALT ≤ 2.5 times ULN,
 - Alkaline phosphatase ≤ 2.5 times ULN,
- 7) Renal status:
 - Creatinine ≤ 2.0 mg/dL,
- 8) Cardiovascular:
 - No signs or symptoms of disease,
- 9) Negative serum pregnancy test within 2-weeks (preferably 7 days) prior to randomization (For women of childbearing potential);
- 10) Signed informed consent form (ICF);
- 11) Patient accepts to make available tumour samples for submission to laboratory to conduct studies as part of this protocol;

12) The patient should be easily followed by clinicians involved in the trial (compliance with the follow up modalities and geographical accessibility).

Exclusion Criteria

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

- 1) Any prior treatment for primary invasive breast cancer;
- 2) History of other malignancy. However, subjects with a past history of completely resected basal and squamous cell carcinoma of the skin or successfully treated in situ carcinoma of the cervix are eligible;
- 3) Diagnosis of inflammatory breast cancer;
- 4) Bilateral breast cancer;
- 5) Multi-focal cancer;
- 6) Known history of uncontrolled or symptomatic angina, clinically significant arrhythmias, congestive heart failure, uncontrolled hypertension ($\geq 180/110$), unstable diabetes mellitus, dyspnoea at rest, or chronic therapy with oxygen;
- 7) Concurrent disease or condition that would make the subject inappropriate for study participation or any serious medical disorder that would interfere with the subject's safety;
- 8) Active or uncontrolled infection;
- 9) Dementia, altered mental status, or any psychiatric condition that would prevent the understanding or rendering of informed consent;
- 10) Malabsorption syndrome, disease significantly affecting gastrointestinal function, or resection of the stomach or small bowel. Subjects with ulcerative colitis are also excluded;
- 11) Concurrent neo-adjuvant cancer therapy (chemotherapy, radiation therapy, immunotherapy, biologic therapy other than the trial therapies);
- 12) Concurrent treatment with an investigational agent or participation in another therapeutic clinical trial;
- 13) Known immediate or delayed hypersensitivity reaction or idiosyncrasy to drugs chemically related to Ketorolac or their excipients;
- 14) Pregnant or lactating women.

Study Assessments and Procedures

All baseline evaluations must be completed prior to initiating treatment with study medication. Medical and physical examinations should be performed by a qualified physician and should include a thorough review of all body systems. Relevant data will be captured in the Case Report Form. All other data will be recorded in source documents. A signed written Informed Consent Form must be obtained prior to screening procedures, and before any study specific assessments are initiated.

Survival information will be collected until 10 years after randomization of the last patient. Subjects will have the following assessments and procedures performed as described. Study assessments and procedures are outlined in Appendix 1 for timing of assessments.

Baseline and Screening Assessments

The following assessments and procedures should be performed within 2 weeks prior to first dose of targeted therapy:

- Signed Informed Consent Form;
- Date of birth and race;
- Menopausal status; for women of childbearing potential, serum pregnancy test would be useful (preferably 7 days prior to randomisation);
- Medical history including details of malignancy: date of diagnosis, primary tumour type characteristics (histology, grade, stage);
- Current and concomitant medications;
- Physical examination, ECOG Performance Status, vital signs (blood pressure and pulse rate, body temperature), height in centimetres, body weight in kilograms
- Haematology and blood chemistry;
- Breast palpation with tumour measurement and nodal status by calliper or ruler measurements;
- Bilateral mammography and breast echography with tumour measurements;
- CT scan (if available);
- Chest X-ray or CT-scan (if available);
- Cardiac monitoring (preferably within 7 days prior to rando-

misation);

- Feasibility of surgery and type of planned breast cancer surgery;
- Pre-treatment tumour biopsy (minimum 1 core formalin-fixed paraffin-embedded)
- Pre-treatment blood samples for further studies;
- Hormonal receptor determination (on biopsy material or on surgical specimen).

Surgical Procedures

Anaesthesiology measures (drugs and doses) will be registered.

For the purpose of this study, the types of breast surgery are: a) Breast conserving surgery or b) Mastectomy.

Patients with involved or close surgical margins after breast conserving surgery will undergo re-excision or mastectomy to obtain negative margins.

Surgical staging of the axilla should also be performed by:

1) Complete axillary dissection and pathological examination of the level I and II lymph nodes;

2) Sentinel lymph node (SLN) biopsy, limited to the following recommendations:

- Indications include women with T1 and T2 breast cancers;
- Contraindications include women with palpable axillary nodes, adverse reactions to vital dyes and inability for the patient to give consent;
- All patients should undergo level I and level II axillary dissections if the sentinel node(s) are reported positive for malignancy or if the surgeon is unable to identify a sentinel node. Feasibility and type of surgery as indicated by surgeon prior to study treatment will be recorded at baseline for each patient enrolled.

Registered descriptions of anaesthesiology and surgical procedures will be conserved.

Post-Surgical Assessments

Usual anatomic-pathological procedures aimed to assess the pathological stage will be performed. SBR grading, ER and PgR content and proliferation index will be assessed.

Tumour tissue for further studies from surgical specimen (equivalent to minimum 1 core formalin-fixed paraffin-embedded) will be stored.

Follow-Up Assessments

After surgery, patients will be assessed for follow-up: every 3 months during first year, every 6 months in years 2 to 4 inclusive, and thereafter annually up to year 10 after randomization of the last patient.

The following assessments will be carried out during the follow-up period:

- Physical examination (including thorax wall and axilla examination); ECOG Performance Status, vital signs (blood pressure and pulse rate); every 3 months during the first year of follow-up, then every 6 months up to year 4 included and yearly thereafter until year 10 after randomization of the last patient;

- Record any Adverse Event(s) and Serious Adverse Event(s) and assign appropriate adverse events grade (NCI Common Terminology Criteria for Adverse Events) up to 28 days after the last dose of investigational therapy. Thereafter, only Serious Adverse Event(s) that are related to study drug events should be reported for up to the end of year 10 after randomization of the last patient;

- Haematology and blood chemistry; every 6 months the first year of follow-up (month 6 and 12) and yearly thereafter until year 10 after randomization of the last patient;

- Chest x-ray or CT-scan and mammography, yearly until year 10 after randomization. Bone scan (bone x-ray in case of hot spots suspicious of metastasis or CT scan or MRI in case of suspicion of vertebral metastasis) and liver imaging are mandatory only if symptoms or clinical suspicion of bone and/or liver metastasis are present. The radiological tests should also be performed in addition to the scheduled reviews when symptoms suggest recurrence of disease or a second primary cancer.

NOTE: It is essential to obtain the best objective evaluation of the time of recurrence (see in the following).

Laboratory Assessments

Blood samples will be taken for haematological and serum chemistry monitoring. Analysis will be performed by the local laboratory.

Haematology – Haemoglobin, platelet count, white blood cell (WBC) count including differential (absolute neutrophil count) will be checked during treatment as reported in the Times and Events Schedule Table.

Serum Chemistry – Blood Urea Nitrogen, creatinine, alkaline phosphatase, AST and ALT, total bilirubin, and will be measured at screening only and during study treatment period as outlined in the Times and Events Schedule Table.

Due to the focus on the potential role of inflammation in breast cancer prognosis, the following factors will be assessed: C - reactive protein (CRP), Neutrophil to Lymphocyte Ratio (NLR), Platelet to Lymphocyte Ratio (PLR), Red cell Distribution Width (RDW) and Interleukin-6 (IL-6) . The timing of assessment is reported in the table “Data collection timing [24].

Event Definitions

The diagnosis of a first breast cancer relapse or second primary cancer can be made only when clinical, radiological and laboratory findings meet specific ‘acceptable’ criteria as defined below. Suspicious findings do not usually constitute criteria for breast cancer recurrence. In cases of diagnostic doubt (e.g. ill-defined, palpable mass in an irradiated breast), histological or cytological confirmation of recurrence should be obtained whenever possible. Some patients may develop a suspicious recurrence that leads to death quite quickly without having the possibility to confirm relapse of disease. Efforts should be made to obtain an autopsy report in such patients.

The earliest date of diagnosis of recurrent disease should be used and recorded. This should be based on clinical (in selected cases), radiological, histological or cytological evidence. The relapse of disease has to be backdated to the date of the first diagnosis of lesion (i.e., an objective finding), not to the date of occurrence of the first symptom. For example, a patient presenting with abdominal pain is found to have a possible lesion on liver CT scan of uncertain significance. If a subsequent CT scan confirms disease progression, the date of the first diagnostic CT scan should be taken as the date of recurrence (not the date of presentation with abdominal pain). Thus, the actual date of relapse of disease is the time of first appearance of a suspicious lesion (in a radiological procedure in this case), later proven to be a definitive recurrence or metastasis. Recurrent disease includes: local, regional, distant recurrence, contralateral breast, and second (non-breast) malignancy.

NOTE: Types of recurrent disease are listed below, along with acceptable methods of confirmation of recurrence.

Local Recurrence

a) In the ipsilateral breast after lumpectomy: Defined as evidence of invasive tumour in the ipsilateral breast after lumpectomy. Patients who develop clinical evidence of tumour recurrence in the remainder of the ipsilateral breast should have a biopsy of the suspicious lesion to confirm the diagnosis.

Acceptable: positive histology or cytology

b) Local recurrence other than ipsilateral breast after lumpectomy: Defined as evidence of tumour in any soft tissue or skin of the ipsilateral chest wall after mastectomy. This includes the area bounded by the midline of the sternum, extending superiorly to the clavicle, and inferiorly to the costal margin. Soft tissue recurrences in this area extending into the bony chest wall or across the midline will be considered as evidence of local recurrence.

Acceptable: – Positive histology or cytology

Regional Recurrence

Defined as the development of tumour in the ipsilateral internal mammary and/or ipsilateral axillary lymph nodes, as well as extra nodal soft tissue of the ipsilateral axilla, regional recurrence does not include supraclavicular lymph nodes or tumour in the opposite breast.

Acceptable: – Positive histology or cytology, or Chest-x-ray, CT-scan or MRI (especially in case of internal mammary lymph nodes if no biopsy was performed)

Distant Recurrence

Defined as evidence of tumour in all areas, with the exception of those described above, distant recurrence will be labelled as “soft tissue”, “bone”, “visceral” distant recurrence. Only the first distant recurrence must be reported at any time during the study. The following criteria apply:

a) Soft tissue distant recurrence: Skin, subcutaneous tissue, and lymph nodes (other than local or regional)

Acceptable: – Positive cytology, aspirate or biopsy, or Radiological (by CT scan or MRI or ultrasound) evidence of metastatic disease.

b) Bone distant recurrence

Acceptable: X-ray, CT scan, or MRI evidence of lytic or blastic lesions consistent with bone metastasis, or Bone scan (requires additional radiological investigation, alone not acceptable in case of diagnostic doubt), or Biopsy proof of bone metastases or cytology.

c) Visceral distant recurrence

• Lung

Acceptable: Radiologic evidence of multiple pulmonary nodules consistent with pulmonary metastases; or Positive cytology or histology (solitary nodules); Proof of neoplastic pleural effusions should be established by cytology or pleural biopsy.

- Liver

Acceptable: Abdominal CT scan, liver scan, ultrasound, or MRI consistent with liver metastases, or Liver biopsy or fine needle aspiration. NOTE: If radiological findings are not definitive (especially with solitary liver nodules) a liver biopsy is recommended; however, if a biopsy is not performed, serial scans should be obtained if possible to document stability or progression.

- Central nervous system

Acceptable: Positive MRI or CT scan, usually in a patient with neurological symptoms, or Biopsy or cytology (e.g., for a diagnosis of meningeal involvement) OR radiologic evidence (brain CT or MRI scan) consistent with metastatic disease. However, meningeal involvement may also be diagnosed by CT scan or MRI and depending from the general status of the patient additional investigations (including cytology of the cerebrospinal fluid).

Contralateral Invasive Breast Cancer

Acceptable: - Positive cytology or histology.

Second Primary Malignancy (Breast Or Other Cancer)

Any positive diagnosis of a second primary cancer other than basal or squamous cell carcinoma of the skin, or carcinoma in situ of the cervix will be considered an event in the analysis of DFS.

Lobular carcinoma in situ of the breast (LCIS), ductal carcinoma in situ of the breast (DCIS), and myelodysplastic syndrome are not considered an event, although such occurrences should be recorded. The diagnosis of a second primary cancer must be confirmed histologically. All second primary malignancies are to be reported whenever they occur during the study.

NOTE: Patients diagnosed with a second primary malignancy but with no evidence of breast cancer recurrence will remain on study.

Death without Recurrence

Any death related to causes other than breast cancer or second (non-breast) primary is considered an event.

Data Analysis and Statistical Considerations

Primary Comparisons and Sample Size Determination

The sample size considerations are based upon achieving an 80% power to detect a hazard ratio of 0.56 between the

Ketorolac group and control group assumed to have an overall DFS probability of 60% at five years follow-up, using a 0.05 two-sided significance level test. Enrolment of 100 patients in each of the two treatment groups is required (total enrolment = 200 patients). Calculations were performed using the PASS 13 Power Analysis and Sample Size Software (2014). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass software program.

Accrual Rate and Study Duration

The primary population for analyses in this trial will be the intent to treat (ITT) population, which includes all randomised subjects who receive any study treatment. In the ITT population, patients will be included in data analyses according to their randomised treatment assignment, irrespective of the treatment actually received. The total number of patients randomised and reasons why patients do not start study treatment will be reported. Approximately 50 patients per year are usually treated in the 5 involved Centres. If this rate can be achieved in this study, recruitment will be completed in approximately 4 years and final results will be available in approximately five years. The trigger for the final analysis will be the time of definitive surgery of the 200th patient.

Interim Analyses

One formal interim analysis is planned approximately when the first assessable 120 patients will reach the median follow-up of 24 months. This interim analysis will occur approximately three years after study initiation.

Power analysis

The impact of NSAIDs on cancer recurrence rate was very high in the retrospectively studied patients. For subsequent calculation, however, a conservative approach could be assuming 37% as a detectable reduction of relapse risk. This magnitude of effect (reduction of the risk of relapse by 33%) has been considered as achievable by other investigators in similar trials. For a power of 0.8 and an alpha of 0.05, 100 patients per group should then be needed to detect an increase of the disease-free survival from 60% to 75%. Therefore, at least 100 patients per arm should be included in the final trial to detect an effect of Ketorolac with a sufficient power.

Recruitment of breast cancer patients: the involved centres usually treat about 50 patients who have the characteristics needed to be recruited in the study. Therefore, the time for patient accrual may be expected in the order of 4 years.

Study Conduct Considerations

Regulatory and Ethical Considerations, Including The Informed Consent Process

The Principal Investigator will obtain favourable opinion/approval to conduct the study from the appropriate regulatory agency in accordance with any applicable country-specific regulatory requirements prior to a site initiating the study in that country. The study will be conducted in accordance with all applicable regulatory requirements. The study will also be conducted in accordance with “good clinical practice”, all applicable subject privacy requirements, and, the guiding principles of the Declaration of Helsinki.

This includes, but is not limited to, the following:

- Institutional Review Board/Institutional Ethic Committee review and favourable opinion/approval to conduct the study and of any subsequent relevant amended documents;
- Subject informed consent;
- Investigator reporting requirements.

Written informed consent will be obtained for each subject before she can participate in the study.

Quality Control (Study Monitoring)

The study will be carried out in some cooperating centres. Study monitors will contact the sites of candidate institutions prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory and ethical requirements.

Monitors will monitor the study consistent with the demands of the study and site activity to verify that the:

- Data are authentic, accurate, and complete;
- Safety and rights of subjects are being protected;
- Study is conducted in accordance with the currently approved protocol and all applicable regulatory requirements.

The cooperating investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

Quality Assurance

Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the in-

vestigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

Study and Site Closure

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations and procedures and any contracts governing the study. In addition, the Study Steering Committee, reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. This can occur at one or more or at all sites. The monitor will promptly inform all other investigators or the head of the medical institution (where applicable), and/or institutions conducting the study if the study is suspended or terminated for safety reasons.

Records Retention

Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records, except for those required by local regulations to be maintained by someone else, in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must assure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations; otherwise, the retention period will default to 15 years after the completion of the study and/or 2 years after relevant Health Authorities, whichever is longer.

Data Management

The data collection tool for this study will be pre-defined case report forms (CRFs). Data necessary for analysis and reporting will be entered into a validated database or data system.

Original CRFs will be retained by the leading centre, while each investigator will retain a copy.

Appendix 1

Data Collection Timing

	Within 2 weeks prior to randomization	Day of Surgery	Post Op day 1	Post Op day 2	Post Op day 3	Post Op day 4	Post Op day 7-10
Informed consent	X						
Date of birth and race	X						
Medical history	X						
Physical examination	X		X			X	X
Height, weight, P.S.	X						
Menstrual status/pregnancy test	X						
Haematology	X	X	X	X	X	X	X
Blood chemistry	X						
Inflammation parameters	X	X		X			X
Cardiac monitoring	X						
Tumour traits (TNM, receptors etc.)	X						
Staging procedures	X						

Appendix 2

ECOG Performance Status

Grade ECOG

0 Fully active, able to carry on all pre-disease performance without restriction

1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g, light house work, office work

2 Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours

3 Capable of only limited self-care, confined to bed or chair more than 50% of waking hours

4 Completely disabled cannot carry on any self-care. Totally confined to bed or chair

5 Dead

** Menopausal Status Criteria

a) Premenopausal status: less than 6 months since last menstrual period AND no prior bilateral ovariectomy AND not on oestrogen replacement; OR biochemical evidence of Premenopausal status, according to local policies;

b) Postmenopausal status: prior bilateral ovariectomy OR more than 12 months since last menstrual period with no prior hysterectomy; OR biochemical evidence of postmenopausal status, according to local policies;

c) Above categories not applicable and age < 50 years;

d) Above categories not applicable and age ≥ 50 years.

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