Pilot Trial of an Implantable Microdevice for in vivo Drug Sensitivity Testing in Patients with Early Stage, Triple Negative Breast Cancer

PROTOCOL FACE PAGE FOR
MSK NON THERAPEUTIC PROTOCOL

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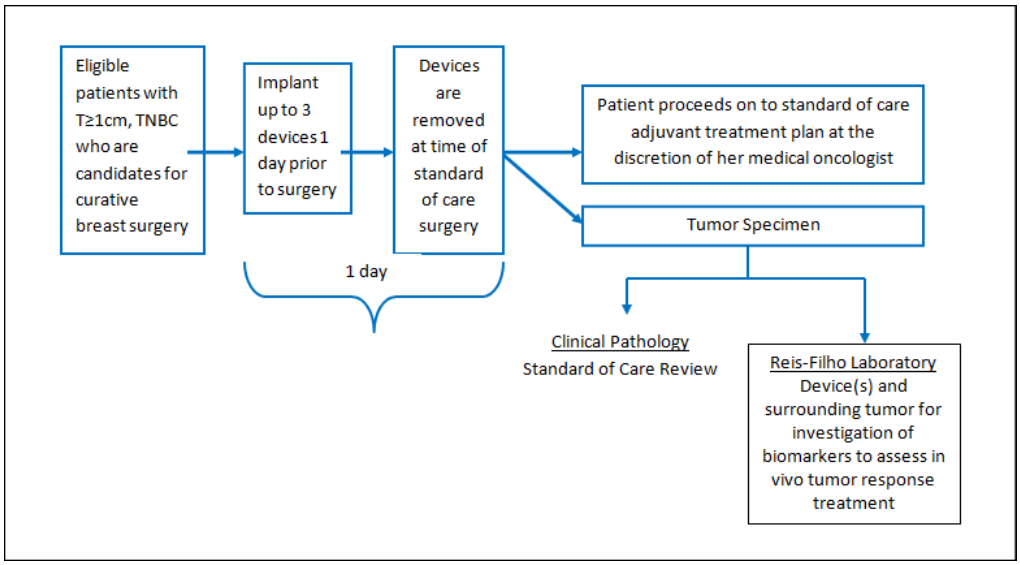
Table of Contents

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

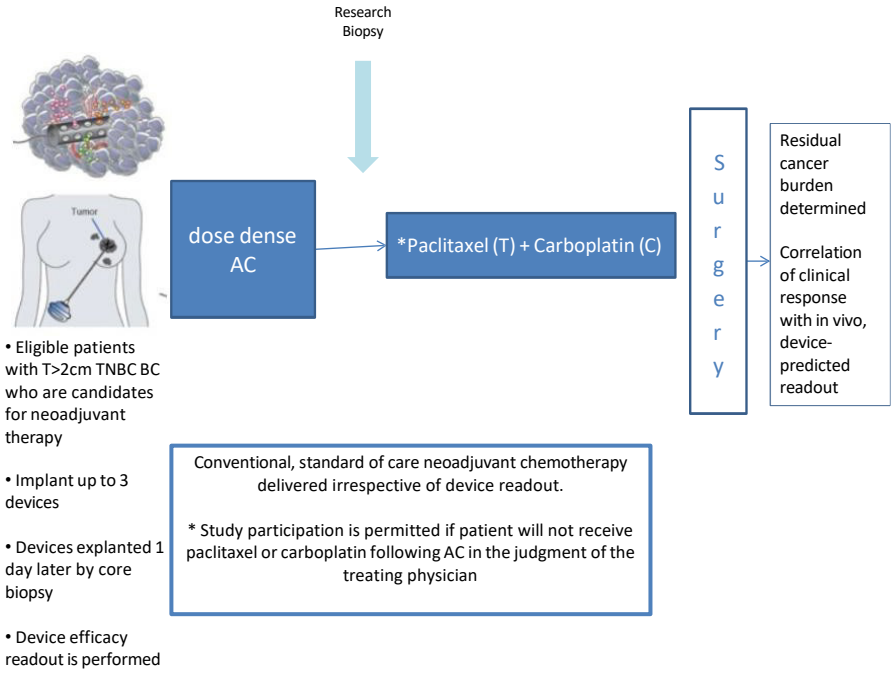
This pilot trial seeks to evaluate the feasibility of implanting and retrieving at least 1 implantable microdevice in patients with early stage Triple Negative Breast Cancer (TNBC) who are candidates for neoadjuvant therapy or upfront surgery. The overarching development plan, if proven feasible in this pilot, would be to test whether in vivo chemotherapy sensitivity assessed by an implantable microdevice (the “device”) correlates with response to standard of care, neoadjuvant chemotherapy for the treatment of patients with early stage TNBC. The study has been amended to include 2 cohorts. In Cohort 1, patients will have the microdevice placed prior to breast surgery and in the absence of neoadjuvant chemotherapy. Removal of the device with the entire tumor will be used to confirm our ability to pathologically correlate in vivo tumor response to the individual drug regimens included on the microdevice. In Cohort 2, patients will have the device placed, retrieved 1 day later by core biopsy and proceed on to standard of care neoadjuvant chemotherapy. In exploratory objectives, in vivo chemotherapy sensitivity to doxorubicin and cyclophosphamide (AC) as defined by a panel of prespecified biomarkers related to apoptosis, DNA damage and proliferation will be analyzed for correlation with pathologic complete response as defined by residual cancer burden (RCB 0/1) following a standard of care, neoadjuvant, AC-based chemotherapy regimen for patients in Cohort 2. Exploratory endpoints include the subanalyses to evaluate the device"s ability to correlate response from taxane/platinum therapy, to investigate the intervariability of the measurements by device and within a device and to evaluate pathologist interobserver variability. We will also evaluate the safety of implanting and retrieving the device. The protocol schema is shown in **Figure 1.**

Figure 1. Protocol schema

Cohort 1: Upfront Breast Surgery



Cohort 2: Neoadjuvant Therapy



2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objective:

- To determine the feasibility of implanting and retrieving at least 1 device in patients with early stage triple-negative breast cancer who are candidates for neoadjuvant chemotherapy or upfront surgery. Three devices will be implanted in the patient unless otherwise limited by tumor size, where fewer may be placed.

Secondary Objectives:

- To describe the safety and possible toxicity of implanting and retrieving at least 1 device in patients with early stage breast cancer who are candidates for neoadjuvant chemotherapy or upfront surgery.
- To preliminarily assess in vivo chemotherapy sensitivity to AC as predicted by the device correlates with residual cancer burden (RCB) 0/1 following standard of care, AC-based neoadjuvant chemotherapy regimen for patients with early stage TNBC who receive neoadjuvant therapy (Cohort 2).
- To evaluate whether in vivo chemotherapy sensitivity to paclitaxel and carboplatin as predicted by the device correlates with RCB 0/1 for patients with early stage TNBC receiving standard of care neoadjuvant chemotherapy with AC followed by paclitaxel + carboplatin (Cohort 2).
- To evaluate whether in vivo chemotherapy sensitivity to AC combination at baseline correlates with biomarkers of response in vivo following systemic administration of AC for those patients receiving neoadjuvant therapy.
- To evaluate the reproducibility of data from replicate drug/drug combinations in each device and across devices in the case where more than one device is retrieved.
- To define the inter-observer variability between pathologists for the assessment of phenotypic response in native tumor tissue
- To determine the accuracy by which tissue responses can be co-localized with the specific drug regimens on the microdevice, both in the setting of extracting the device surgically with the entire tumor, as well as extracting the device percutaneously.

3.0 BACKGROUND AND RATIONALE

Efforts to personalize treatment by in vitro tumor assays to predict chemotherapy sensitivity have been ongoing across solid tumors for many years. However, these methods have been limited in their usefulness to date. In fact, the American Society of Clinical Oncology issued a statement in 2011 which recommended against the use of chemotherapy sensitivity and resistance assays (CSRA) to select chemotherapeutic agents for individual patients outside of the clinical trial setting. [1]This practice guideline is based on the lack of evidence that any available CSRA demonstrated improved outcomes (i.e., overall survival, response to therapy, disease free survival, progression free survival, local tumor control or treatment toxicity) when used to make chemotherapy decisions rather than empiric choice based upon clinical trial literature.

However, it has been recognized that an in vitro strategy could have potential clinical importance and further development of models to individualize chemotherapy choice are a priority. Unfortunately, in vitro models are unable to recapitulate the exact microenvironment in which a tumor exists. Development of cell line and xenograft models take time that makes real-time decision making based upon results less practical. In addition, limited resources may result in difficulty bringing patient-derived xenograft models to practice on a larger scale.

There is an unmet need for an in vivo, chemosensitivity assay that could simply and quickly predict benefit from a particular treatment for patients with solid tumor malignancy. Kibur has invented an implantable microdevice which may serve as a rapid screen for multiple single agent or combination therapy regimens in vivo, reflecting the native tumor and patient microenvironment.

In this pilot first-in-human trial, we propose studying whether this device may be placed within a breast primary tumor, successfully retrieved (either through radiology-guided retrieval process prior to neoadjuvant chemotherapy or at the time of definitive breast surgery) and yield evaluable tumor tissue for readout of drug sensitivity. Secondary exploratory endpoints will evaluate whether the device has the ability to predict RCB 0/1 for patients receiving standard of care, neoadjuvant chemotherapy for early stage breast cancer in Cohort 2.

Neoadjuvant chemotherapy

The goal of systemic chemotherapy for the treatment of early stage breast cancer is to eradicate micrometastases and improve overall survival. In addition to this objective, the primary aim of neoadjuvant chemotherapy for nonmetastatic invasive breast cancer is to improve surgical outcomes in patients for whom a primary surgical approach is not feasible or for those patients who desire breast conservation. The standard, highly effective chemotherapy regimens typically used in the adjuvant, post-operative setting are instead administered preoperatively to facilitate surgery. To date, survival outcomes for chemotherapy given in the pre- or post-op setting have been comparable.

There is a trend towards increased usage of neoadjuvant therapy for patients with triple negative (TNBC) breast cancers, in part because these cancers are most likely to show good locoregional responses.

Triple Negative Breast Cancer

Two recent randomized phase 2 studies have demonstrated higher pCR rates with the addition of carboplatin to an anthracycline- and taxane-containing regimen for the neoadjuvant treatment of patients with early stage triple negative breast cancer. The GeparSixto study randomized a cohort of 315 patients with TNBC to weekly liposomal doxorubicin + paclitaxel with bevacizumab, with or without weekly carboplatin.[4] The addition of carboplatin was associated with a significantly higher pCR rate (57% vs. 43%, OR 1.94, 95% CI 1.24-3.04). These results were later confirmed by CALGB 40603, a randomized phase 2 trial which utilized a more conventional backbone chemotherapy regimen (weekly paclitaxel followed by dose dense AC) and randomized 443 women with TNBC to the addition of carboplatin AUC 6 every 3 weeks concurrently with paclitaxel.[5] Despite the slight variations in backbone chemotherapy, the addition of carboplatin was also associated with a higher pCR rate (54% vs. 41%, OR 1.71). Recently, the GeparSixto

trial reported that the addition of carboplatin improved disease free survival as compared to the non-platinum containing control arm (85.8% vs. 76.1%; HR 0.56 p=0.035) (von Minckwitz et al, Abstract S2-04 SABCS 2015). These results are supportive of the antitumor activity associated with platinum agents for the treatment of TNBC and the addition of carboplatin to weekly paclitaxel as part of a neoadjuvant regimen is a reasonable option for patients with stage II-III TNBC. However, platinum-based chemotherapy regimens have not replaced acceptable anthracycline- and taxane-based regimens for the treatment of early stage breast cancer, nor has neoadjuvant administration of chemotherapy become a standard for *all* triple-negative breast cancers. The incorporation of platinum to ddAC-T presents one additional reasonable option for treatment of early stage TNBC.

Neoadjuvant treatment allows for an early evaluation of the effectiveness of systemic therapy from clinical observation, radiographic findings and ultimately pathologic response evaluations at the time of definitive breast surgery (i.e., pathologic complete response). Additionally, in the HER2 and TNBC subtypes of breast cancer, pathologic complete response has been shown to correlate with survival outcomes. [6]These features make the neoadjuvant space an appealing setting for the conduct of clinical trials in which tissue acquisition is required to identify tumor- or patient-specific biomarkers.

An implantable microdevice to predict in vivo chemotherapy sensitivity

Kibur Medical has developed a rapid parallel *in vivo* assay that consists of an implantable microscale device that is placed inside the native tumor microenvironment. This device contains 16 reservoirs, each with a unique single agent or drug combination in microdose amounts of less than one millionth of a systemic patient dose. The device is implanted directly into the tumor during a biopsy procedure, and remains *in situ* for ~24 hours (**Figure 2**). Drugs from each reservoir are released during this time into distinct regions of tumor tissue, effectively creating *in vivo* micro-reactors for the interaction of tumor with a specific drug. Crosstalk between drugs from different reservoirs is eliminated by appropriate spatial separation of reservoirs and by drug formulation and other techniques. The microdevice is of cylindrical shape measuring 820μm in diameter, and is delivered through an 18 gauge biopsy needle.

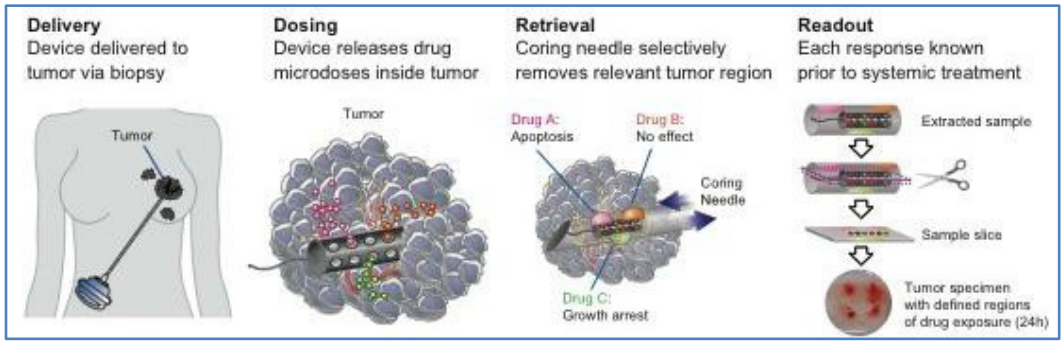


Figure 2: Concept for in-vivo drug sensitivity assay: Device is implanted directly into tissue. During implantation, drugs diffuse into confined regions of tumor. Each such region can be assayed independently to assess the tumor-specific response of a given drug. Follow ing incubation, a second biopsy procedure is administered in which a coring needle selectively retrieves a small column of tissue that immediately surrounds the device. This tissue contains the regions of drug diffusion and is sufficient for determination of efficacy of drugs.

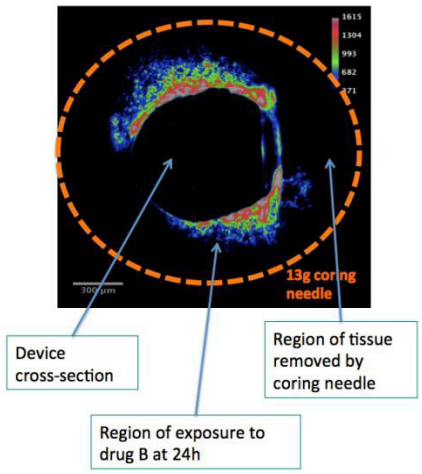


Figure 3: Removal of drug-exposed tumor region by a coring needle. The device and the region of drug distribution w as removed by a 13-gauge coring needle after 20-24 h for pure and formulated drug contents. More than 99% of drug was removed, as measured by autofluorescence of pure doxorubicin.

The device with surrounding tissue is excised using a larger coring biopsy needle (~7-12 gauge) which is inserted into the tumor and positioned concentric to the device using ultrasound imaging. The coring needle then protrudes over and beyond the device, capturing the device itself and a cylindrical column of tissue of 1.6mm thickness and 4-5mm length, including ~400μm thickness radially outward from the device along its entire length. This represents virtually the entire region of drug distribution over the incubation period of 1 day and is thus sufficient for examining drug effect. The drug amounts that were released from reservoirs into tissue are removed almost entirely during the retrieval procedure, along with the tumor tissue that was exposed to these compounds. In this manner, Kibur investigators were capable of gathering *in vivo* phenotypic data on the action of a large number of anti-cancer single agents or combinations within 1 day inside the tumor microenvironment, all without systemic or residual exposure to the drugs tested.

The local concentration of drug released into distinct regions of tumor tissue can be measured by drug autofluorescence (A-D) or mass spectrometry tissue imaging (E, F), and can be observed at different time points (J) as shown in **Figure 4**.

Figure 4: A-I: Anti-cancer drugs are delivered into confined regions of tumor. Detection by fluorescence: **(A)** Doxorubicin **(B)** Sunitinib **(C)** Lapatinib. **(D)** Cetuximab conjugated w ith Alexa488. Detection by MALDI mass spectrometry: **(E)** Gemcitabine and **(F)** Docetaxel. **(J):** Image compares the release of a microdose of sunitinib at 3 time points, demonstrating expanded but confined region of tissue distribution even at longer time points. Scale bar = 300 μm.

A small region of tissue is removed from the tumor and analyzed by multi-parameter immunohistochemistry (IHC) to determine anti-neoplastic effect for each treatment being evaluated on the device at the end of the device implantation period. Multiple biomarkers can be used to understand the specific effect of drug on tumor tissue.

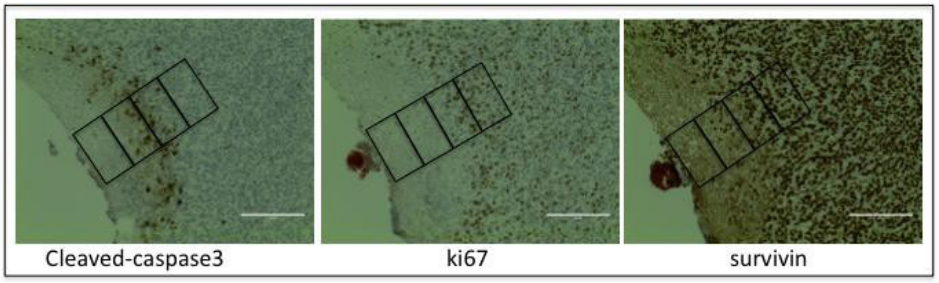


Figure 5: Device/tissue cross-sections stained for multiple IHC biomarkers, showing time and concentration dependent effects on proliferation and apoptosis of drug-exposed cells. Scale = 200μm.

Preclinically, Kibur Medical has validated the ability to use the local microdose effect as a predictor of drug efficacy across multiple drugs and tumor models. (**Figure 6**)

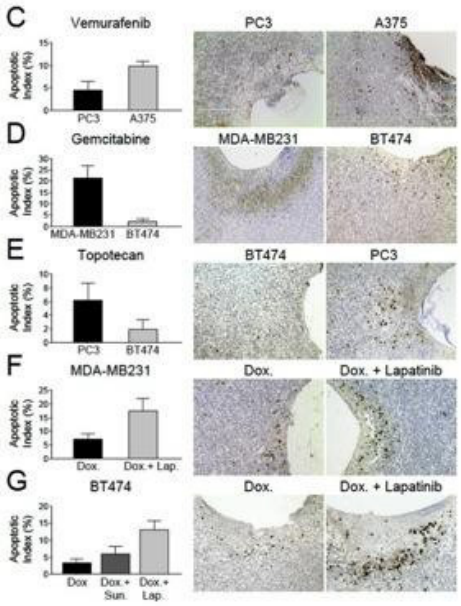


Figure 6: (A) Differential response of three tumor models to doxorubicin. TOP: Representative tumor sections IHC-stained for CC3: PC3 (left), BT474 (middle) and A375 (right). BOTTOM: Left: Automated counting of sections for CC3-positive cells. Right: Averages for each tumor model response are calculated from at least 18 unique reservoirs from 12 tumors. Scale = 250μm. **(B)** Apoptosis induction following systemic administration of doxorubicin in A375 and PC3 tumors. Representative sections of PC3 (top) and A375 (bottom) are shown at 8 mg/kg (left) and control (right). CC3-stained regions (brown) indicate induction of apoptosis in affected cells. Averages are calculated from 12 spatially separated sections from 3 distinct tumors. **(C-E)** Comparison of differential apoptotic response for vemurafenib, gemcitabine and topotecan, as assessed by CC3 expression after 24h in 12 spatially distinct tumor regions from at least 4 tumors each. Scale = 200μm. **(F-G)** Enhancement of apoptotic response by addition of targeted agents to doxorubicin in device reservoir. Lapatinib leads to an enhancement of CC3 expression in MDA-MB231, and a very significant apoptosis increase in BT474. Averages are calculated from 10 distinct reservoirs from 4 tumors. All sections taken at 20-24h post implantation. **(H)** Control sections for tumor models used, representing empty device reservoirs with no local drug treatment. Scale = 400μm. All error bars represent one standard deviation.

Modified Device:

We have studied our methodology and outcomes of first patient accruals on study and have noted that the microdevice, although successfully retrieved, would often become dislodged from surrounding tumor tissue during retrieval process. In response to this, in collaboration with Kibur, we have developed a modified microdevice that seeks to address the difficulties with tissue retention and biopsy retrieval incurred with the previous device design (**Figure 7**). There are 3 modifications featured on the new microdevice design:

- The back of the microdevice features a barb which restricts axial movement and keeps the device locked onto tissue more tightly.
- The diameter of the cylindrical section bearing the reservoirs is reduced from 820 μm to 720 μm.
- The tip of the microdevice is conical, allowing for better penetration into tissue and lower associated tissue damage. This also provides a tighter encapsulation of the device with tissue.

All methods of device insertion and specimen analysis remain unchanged and have been successfully piloted preclinically (Figure 8). Radiology has modified retrieval methodology to minimize disruption to device/tumor relationship as a result of the biopsy procedure and pathology has optimized specimen handling techniques. These modifications are not thought to alter the risk level of the protocol. While the design of the microdevice has been changed, it is composed from the same inert polymer that the original device was made.

Figure 7. New microdevice design.

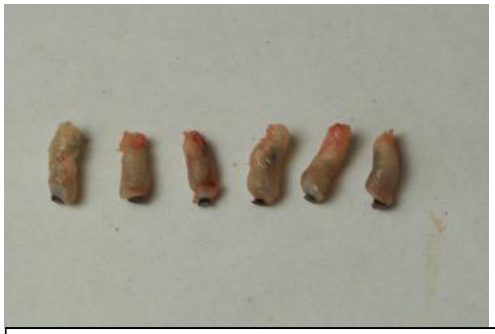


Figure 8. Retrieved specimens, using new microdevices and standard coring biopsy needle.

Rationale

This first in human pilot study will preliminarily assess the feasibility of implanting and retrieving at least 1 device from the breast in patients with early stage breast cancer and to assess whether the Kibur device and the in vivo measurements it produces correlate with pathologic response to chemotherapy. Pathologic response will be measured by residual cancer burden. The patient population includes patients with breast cancer receiving standard of care, neoadjuvant anthracycline- and taxane-based chemotherapy for T2 N any, triple negative breast cancer. The Kibur device measurements linked to the AC wells will be used to test our primary hypotheses. To date, there are no available methods for predicting an individual patient‟s or tumor‟s response to therapy which recapitulates the in vivo complexities of both the tumor and its microenvironment. Individual patient derived xenograft or cell line models are costly, time consuming and difficult to scale into clinical practice.

If the readout obtained from the Kibur device is shown to be feasible and correlated with chemotherapy sensitivity in vivo, this pilot trial will lead to a larger study to validate findings and investigate the use of the device in making individual treatment decisions.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This study will assess the feasibility of implanting and retrieving at least 1 device in patients with early stage breast cancer who are candidates for neoadjuvant chemotherapy or upfront surgery. We will also obtain preliminary data regarding the ability of the Kibur device, an in vivo indicator of chemotherapy sensitivity, to predict response to neoadjuvant AC in patients with early stage breast cancer receiving systemic, standard of care, pre-operative anthracycline- and taxane-based chemotherapy as defined by residual cancer burden (RCB) 0/1. RCB is an accepted measure of response to neoadjuvant chemotherapy which correlates with survival (**Appendix B**).[7] Twelve patients with early stage triple negative breast cancer, who are candidates for upfront surgery (Cohort 1) or neoadjuvant therapy (Cohort 2) will be accrued.

Patients with early stage TNBC with primary tumors greater than or equal to 1 cm in size are candidates for this protocol. Patients may be accrued to one of two Cohorts. Appropriateness for one cohort over the other will depend on whether patient will pursue a neoadjuvant chemotherapy approach (Cohort 2) or elect to proceed directly to surgery and defer chemotherapy to the post-operative setting (Cohort 1). This decision will be made in a multidisciplinary way with input from Breast Surgery and Breast Medical Oncology, taking into account patient preference as well. The infrastructure for this multidisciplinary approach is already in place within the Breast Disease Management Team.

Cohort 1: Upfront Surgery

In this cohort, patients will undergo placement of 3 devices within the tumor unless limited by tumor size, where fewer may be placed. In patients undergoing a lumpectomy the devices may be placed at the time of wire or seed localization. Each device has several wells loaded with single agent and combination therapy. The devices will be retrieved 1 day later at the time of definitive breast surgery (breast conservation or mastectomy per standard of care). The sample will be sent to pathology fresh and if a device is encountered intra-operatively, the device will be separately delivered to pathology. Study team will coordinate scheduling such that Dr. Elizabeth Morris is available for placing the device and Dr. Hannah Wen is available in the Pathology Department to help in tissue handling appropriately. Specimen radiograph to confirm removal of devices will be performed in pathology. Devices and a small amount of surrounding tumor will be sent to Reis-Filho‟s laboratory where tumor analyses will be performed to evaluate an apoptotic index defined by cleaved caspase 3, Ki67 labeling index, phospho-H2AX score, and RAD51 score to estimate response to therapy. The patient will then proceed on to adjuvant therapy at the discretion of the treating medical oncologist.

Cohort 2: Neoadjuvant Therapy

In this cohort, patients will undergo placement of 3 devices prior to systemic therapy, unless limited by tumor size, where fewer may be placed. Each device has several wells loaded with single agent and combination therapy. The devices will be retrieved 1 day later by core needle biopsy. The sample will be sent to pathology fresh and if a device is encountered intra-operatively, the device will be separately delivered to pathology. Study team will coordinate scheduling such that Dr. Elizabeth Morris is available for placing the device and Dr. Hannah Wen is available in the Pathology Department to help in tissue handling appropriately. Specimen radiograph to confirm removal of devices will be performed in pathology. Devices and a small amount of surrounding tumor will be sent to Reis-Filho‟s laboratory.

Tumor analyses will be performed to evaluate an apoptotic index defined by cleaved caspase 3, Ki67 labeling index, phospho-H2AX score, and RAD51 score to estimate response to therapy. Patients will go on to receive standard of care neoadjuvant therapy. A research biopsy will be performed following AC treatment. RCB will be determined by the study pathologist at the time of definitive surgery following neoadjuvant chemotherapy.

All analyses will be primarily descriptive and graphical in nature. The primary endpoint will be to evaluate the feasibility of the device. We have defined feasibility as the ability to implant and

retrieve at least one device. Numerous secondary and exploratory analyses are planned. We will evaluate the safety and toxicity of implanting and removing the device as well as our ability to determine tissue response to the agents incorporated in the device, both by core biopsy or at time of definitive surgery. We will explore whether in vivo chemotherapy sensitivity to AC as predicted by the device correlates with residual cancer burden (RCB) 0/1 following standard of care. In this patient population, we estimate that standard neoadjuvant regimens will achieve RCB 0/1 in 50% of patients. Similar analyses to evaluate the sensitivity of subtype-specific regimens will be done in the TNBC subsets. In addition, we will evaluate whether AC chemotherapy sensitivity in vivo at baseline (measured by apoptotic index defined by cleaved caspase 3, Ki67 labeling index, phospho-H2AX score and RAD51 score in the area closest to the AC wells of the device) is correlated with the same measures obtained from tumor biopsy after AC therapy. Exploratory endpoints will evaluate the reproducibility of each device and across devices, as well as interobserver variability.

4.2 Intervention

Patients will be offered participation in this trial by their Breast Surgeon or Breast Medical Oncologist once a determination of neoadjuvant or upfront surgery has been made. Eligible patients who give informed consent will be referred to Breast Radiology for implantation of 3 devices within the primary tumor unless limited by tumor size, where fewer may be placed. Each device has 16 wells; the 8 drug regimens shown below will be loaded in duplicate on each device.

Kibur Drug Wells
1. Doxorubicin and cyclophosphamide (AC)
2. Doxorubicin (A)
3. Paclitaxel (T)
4. Paclitaxel (T) and carboplatin (Pl)
5. Carboplatin (Pl)
6. AC + paclitaxel + carboplatin (AC-TPl)
7. Gemcitabine (G)
8. Eribulin (E)

The 3 devices will be implanted into the primary tumor by a Breast Radiologist trained in this method on Monday through Thursdays. Women accrued to the trial will be scheduled for appointments on two consecutive days; Cohort 1 will have device placement followed by definitive surgery the next day. Cohort 2 will have device insertion one day, followed by a removal the next day. Three devices will be implanted into each tumor. The technique involved with device insertion is equivalent to placement of a radioactive seed using ultrasound guidance or placement of a localizing clip after ultrasound guided biopsy, which is standard practice. On the day of implantation, the biopsy proven cancer will first be localized with real time sonography. The skin will be cleansed with Betadine and 8 cc of 1% lidocaine will be used for superficial and deep anesthesia. A skin nick may be made. There will be one insertion site for local anesthesia and efforts will be made to place all devices through the same needle insertion. The 18 gauge spinal needle will then be inserted through the skin and directed into the cancer where the device will be deployed. Each device will be loaded into an 18 gauge spinal needle. Three separate 18 gauge spinal needles will be used; each will insert one device into the tumor.

Cohort 1

Patients on Cohort 1 will proceed to surgery as previously scheduled, the day after device placement. The type of surgery, either a lumpectomy or mastectomy will be at the surgeon"s discretion. In the setting of a lumpectomy, non-palpable tumor will be localized with a radioactive seed the day prior to surgery at which point the devices will be placed. The surgery will proceed as routine except that the tumor will be sent to pathology freshly. A specimen radiograph of lumpectomies will be performed at the discretion of the surgeon to confirm retrieval of the radioactive seed or biopsy marker. If on this radiograph the devices are not evident, the additional shave margins that are taken as per routine will also be radio graphed in pathology to determine if the devices are present. If the devices are not accounted for, no additional tissue will be excised from the patient and a post excision mammogram will performed postoperatively when tolerable to the patient to confirm a retained device. In patients undergoing a mastectomy, the breast will be delivered to pathology where it will undergo X-ray to determine device presence. If a device is encountered intraoperatively it will be placed in a container and sent to pathology.

Cohort 2

Patients on Cohort 2 will return to Breast Radiology to have the devices retrieved 1 day following implantation. The device with surrounding tissue will be excised using a ~7-12 gauge core biopsy needle, which will be inserted into the tumor and positioned concentric to the device using stereotactic or ultrasound guidance. Study radiologists have undergone dedicated training for device removal under ultrasound guidance. Devices with empty reservoirs have been placed in a chicken breast to simulate the breast tissue. Each radiologist has successfully removed three devices from these models using a 7-12 vacuum needle on several occasions. Device removal under stereotactic guidance models standard practices for breast tumor biopsies. The decision about the method for device retrieval will be based on the visibility of the device within the tumor. Based on phantom studies, it is expected that ultrasound will be feasible in the majority of cases. Ultrasound has the advantage of real time visualization and the ability to line up the device with the opening of the biopsy needle. However in the event that the device is not able to be seen on ultrasound, stereotactic biopsy will be performed for device retrieval. All devices have markers that will be easily seen with x-ray and therefore can undergo stereotactic biopsy. It is expected that all devices can be removed with either of these methods.
For ultrasound guided device removal, the target will first be localized sonographically. The breast will be cleansed with Betadine, and 3 cc of 1% lidocaine and 10 cc of 1% lidocaine with epinephrine (unless contraindicated) will be used for deep anesthesia. A skin nick will be made with a scalpel. A vacuum assisted needle will be inserted and its accurate position confirmed with sonographic images. Multiple samples will be taken at each site in order to attempt removal of all three devices. A maximum of three insertions will be performed with procedure time not exceeding 30 minutes. The specimens will be delivered for analysis.

For device removal under stereotactic guidance, a preliminary grid localizing film will be obtained to localize and the skin marked. The patient will then be positioned on the stereotactic table or chair and the target localized with digital images. The breast will be cleansed with Betadine, and 3 cc of 1% lidocaine will be used for superficial anesthesia and 10 cc of 1% lidocaine (with epinephrine) will be used for deep anesthesia. A skin nick will be made with a scalpel. A 7-12

gauge probe will be inserted and its accurate position confirmed with images. Multiple samples will be taken in order to attempt removal of all three devices. If possible, the same incision will be used to retrieve all three devices. However, depending on the distance between three devices, separate skin incisions and biopsy devices may be required. The procedure time will not exceed 30 minutes.

Following the ultrasound or stereotactic guided procedure, pressure will be held on the site(s) until bleeding ceases. The area(s) will be cleansed and Steri-Strips applied. Post biopsy instructions will be given verbally and in writing.
Informed consent will be obtained prior to both device implantation and removal. Risks of either procedure include bleeding and infection.
In the event that a device cannot be retrieved, we do not expect side effects from prolonged incubation. The device consists of a polyaryletherketone (PEEK) polymer which has demonstrated long-term safety and biocompatibility in multiple biomedical applications, such as trauma, orthopedic and spinal implants.[8] A close comparable for the Kibur device is the “Cassi Beacon” tissue marker, an FDA-approved fiducial marker used for long-term localization of breast tumors (*http://scionmedtech.com/products/breast-biopsy/cassi-beacon/*). The Cassi Beacon is structurally very similar to the Kibur device (composed of PEKK) though it is more than 4 times larger by volume (cylindrical shape, 1.5mm in diameter and 5mm long). If devices cannot be retrieved 1 day following implantation, they will be removed from the patient along with the remainder of the tumor during surgical tumor resection which is maximally 6 months after implantation. In the rare circumstance where a patient did not have device retrieved on Day 2 and has unresectable disease at the time of completion of preoperative neoadjuvant chemotherapy, the study surgeon will discuss alternative options for device removal with the patient. It is possible that additional neoadjuvant therapy may be given to facilitate curative resection which may extend the time the device is in place to >6 months. It is also possible that the device is intentionally and knowingly left in situ if the risks of surgery were to outweigh the benefit.
Following device retrieval, or definitive surgery, the tumor-device specimens will be sent to the research laboratory of Dr. Reis-Filho. Specimens will be fixed in buffered formalin for 16 hours and subsequently processed and embedded in paraffin for histochemical and immunohistochemical analysis. Tissue sectioning will be performed essentially as described by Jonas et al., to ensure that at least 5 sections centered on each reservoir are cut. One section will be stained with hematoxylin-and-eosin and the remaining sections will be subjected to IHC or immunofluorescence using antibodies against cleaved caspase 3, Ki67, RAD51, γH2AX, and Geminin as previously described. (Jonas O et al, Sci Transl Med 2014)[9]

Quantification of the IHC and immunofluorescence results will be performed by two trained pathologists with experience and expertise in breast cancer. The regions in tissue directly perpendicular to the reservoirs will be quantified for the presence of morphologically unequivocal neoplastic cells expressing Caspase 3, Ki67, phospho-H2AX foci, RAD51 foci and Geminin.
To determine the impact of each therapeutic agent or drug combination on apoptosis, caspase 3 expression will be analyzed. Only cytoplasmic and/ or nuclear expression will be considered specific. The apoptotic index (AI) will be calculated as the number of Caspase 3-positive morphologically unequivocal cells/ 1000 morphologically unequivocal neoplastic cells (Jonas O et al, Sci Transl Med 2014). To define the impact of each therapeutic agent or drug combination on proliferation, the Ki67 labeling index in tumor tissue around each reservoir will be calculated,

through the analysis of at least 1000 morphologically unequivocal cells, as previously described. [10-12]To determine the DNA damage induced by each therapeutic agent or drug combination, sections will be subjected to immunofluorescence with antibodies against phospho-H2AX as previously described [9]. We will capture representative images from tissue adjacent to each reservoir on a confocal microscope, and nuclei from 500 morphologically unequivocal cells will be analyzed per reservoir. A cell will be considered positive if at least one 1 discrete phospho-H2AX focus is identified. The phospho-H2AX index will be calculated as the number of phospho-H2AX-positive morphologically unequivocal neoplastic cells/ total number of morphologically unequivocal neoplastic cells analyzed. To determine whether each therapeutic agent or drug combination induce DNA damage that is corrected by homologous recombination DNA repair, RAD51 and Geminin expression will be analyzed by double-immunofluorescence, as previously described. For this, immunofluorescence images will be captured on a confocal microscope, and nuclei from 500 morphologically unequivocal cells will be analyzed per reservoir. A cell will be counted as being RAD51 positive if there is at least 1 distinct focus per nucleus as previously described. Given that homologous recombination DNA repair preferentially happens during S/ G2 phases of the cell cycle, the RAD51 score will be assessed as the percentage of Geminin-positive cells that are also positive for RAD51. Samples where the device reservoir was located within a highly necrotic tumor region (i.e. >50% of the tissue adjacent to the reservoir is necrotic) or a stroma-rich area (i.e. >50% of the cells in the tissue adjacent to the reservoir are stromal cells) will be considered not informative. For each reservoir, the AI, Ki67 labeling index, phospho-H2AX foci score and RAD51 score will be recorded.

These results will not be used to determine patient treatment and are for research purposes only.

The patient may undergo any necessary preparatory procedures or tests in anticipation of beginning standard of care neoadjuvant chemotherapy during this protocol period.

Adverse event data will be collected with respect to relatedness to the device implantation, incubation and retrieval.

Following retrieval, patients on Cohort 1 will proceed to receive standard of care adjuvant therapy at the discretion of their treating medical oncologist.

If treated on Cohort 2, patients will proceed to receive a standard of care, neoadjuvant anthracycline- based chemotherapy regimen as appropriate for their specific tumor histology, co-morbidities and pre-existing conditions. Pre-operative chemotherapy may be administered at local physician practices outside of MSK.

Options for neoadjuvant regimens include the following:

1) Dose dense AC (doxorubicin + cyclophosphamide) IV q2 weeks x4 followed by weekly paclitaxel x12 + carboplatin IV every 3 weeks x4.[5] Growth factor support will be used for dose dense AC per standard of care.

2) Dose dense AC (doxorubicin + cyclophosphamide) IV q2 weeks x4 followed by weekly paclitaxel x12 + carboplatin weekly x12. Growth factor support will be used for dose dense AC per standard of care.
3) Dose dense AC (doxorubicin + cyclophosphamide) IV q2 weeks x4 (for those patients unable to receive taxanes or platinums). Growth factor support will be used for dose dense AC per standard of care.

Dose adjustments and modifications will be guided by standard clinical practice. If a patient will not receive taxane or platinum in the judgment of the treating physician, they may have the opportunity to participate on this trial following discussion with and at the discretion of the study PI. In the case of allergy, substitution or discontinuation is permitted.

For patients on Cohort 2, a research core biopsy will be performed following AC chemotherapy and prior to beginning taxane-based treatment. This biopsy will be performed in the time period immediately following the final AC infusion and up to 2 days later.

Patients will proceed to definitive surgery at MSK within 4 weeks of their last neoadjuvant chemotherapy dose. The choice for breast conservation or mastectomy will be decided upon by the patient and her breast surgeon based upon clinical and radiographic findings and personal preference.

At the time of breast surgery, pathologic complete response will be determined by standard Dept of Pathology methods and a residual cancer burden score will be assigned by a study pathologist. Correlations between AC and residual cancer burden 0/1 will be evaluated for all evaluable patients.

Additional adjuvant chemotherapy, radiation therapy or endocrine therapy may be given at the discretion of the treating oncologist.

5.0 CRITERIA FOR SUBJECT ELIGIBILITY

5.1 Subject Inclusion Criteria

- Patients with histologically confirmed invasive breast cancer that is: Triple negative (ER<10%, PR<10%, and HER2 0/1+ or 2+/FISH not amplified)
- Tumor size 1cm or greater; N any; M0 (Cohort 1)
- Tumor size 2cm or greater; N any; M0 (Cohort 2)
- Candidate for curative breast cancer surgery (Cohort 1 or 2)
- Candidate for neoadjuvant chemotherapy with a standard of care, anthracycline-based regimen (Cohort 2 preferred over Cohort 1)
- Age >18 years of age
- ECOG performance status of ≤2
- Serum or urine pregnancy test negative within 2 weeks for women of childbearing potential.
- Willing and able to provide informed consent

5.2 Subject Exclusion Criteria

- Prior treatment including surgery, chemotherapy or radiation therapy for the current primary breast cancer.
- Severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational device administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this

6.0 RECRUITMENT PLAN

Women of all races and ethnic groups are eligible for this trial. Patients will be recruited for enrollment on this trial primarily through referrals from their primary oncologists and surgeons. The clinical trial will be listed on the clinicaltrials.gov website as well as on the MSKCC website. Patients will not be paid for participation in this study. Prior to enrollment on the study, the physician will discuss the study protocol in detail with the patient, including possible toxicities. An informed consent will be reviewed by the physician with the patient.

Potential research subjects will be identified by a member of the patient‟s treatment team, the protocol investigator, or research team at Memorial Sloan Kettering Cancer Center (MSKCC). If the investigator is a member of the treatment team, s/he will screen their patient‟s medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the investigator/research staff of the study.

The principal investigator may also screen the medical records of patients with whom they do not have a treatment relationship for the limited purpose of identifying patients who would be eligible to enroll in the study and to record appropriate contact information in order to approach these patients regarding the possibility of enrolling in the study.

During the initial conversation between the investigator/research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator/research staff may also review portions of their medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes.

In most cases, the initial contact with the prospective subject will be conducted either by the treatment team, investigator or the research staff working in consultation with the treatment team. The recruitment process outlined presents no more than minimal risk to the privacy of the patients who are screened and minimal PHI will be maintained as part of a screening log. For these reasons, we seek a (partial) limited waiver of authorization for the purposes of (1) reviewing medical records to identify potential research subjects and obtain information relevant to the enrollment process; (2) conversing with patients regarding possible enrollment; (3) handling of

PHI contained within those records and provided by the potential subjects; and (4) maintaining information in a screening log of patients approached (if applicable).

7.0 ASSESSMENT/EVALUATION PLAN

Screening:

The following must be completed within 28 days of study start:

- Signed protocol informed consent
- Medical history
- Medication list review
- Physical exam with a clinical tumor measurement
- ECOG performance status
- Pathology confirmed at MSKCC. Local assessment of hormone receptor and HER2 status acceptable for study participation.

The following must be completed within 14 days of study start:

- Serum or urine pregnancy test negative for women of childbearing potential.

Baseline:

- Physical exam with a clinical tumor measurement
- Medication list review
- Toxicity review

Please note: Screening assessments may be used for baseline assessments.

Cohort 1:
Day 1 (must be completed Mondays through Thursdays):

- Placement of up to 3 devices by study Breast Radiologist.
- Toxicity review

Day 2:

- Standard of Care breast surgery at which time device(s) will be removed with resection.
- Toxicity review

Post- Operative Surgery visit:

- Toxicity review

Cohort 2:
Day 1 (must be completed Mondays through Thursdays):

- Placement of up to 3 devices by study Breast Radiologist.
- Toxicity review

Day 2:

- Removal of device(s) by concentric core needle biopsy using core needle.
- Toxicity review

Day 2 to 30:

- Begin standard of care, neoadjuvant chemotherapy. Patients may begin neoadjuvant chemotherapy on same day as device removal and no later than Day 30.

Cycle #4 of AC:

- Research biopsy of tumor following final AC and prior to receiving first dose of taxane-based therapy. This biopsy will occur in the time period ranging from immediately following infusion of final AC to 2 days following final infusion. Radiology will provide a clinical estimate of tumor size at time of biopsy.

After all planned neoadjuvant chemotherapy:
Proceed to definitive surgery within 4 weeks of last dose of chemotherapy.

Post- Operative Surgery visit:

- Toxicity review

8.0 TOXICITIES/SIDE EFFECTS

Anticipated adverse events are those that may be expected from tissue biopsy. This mKiay include pain, bleeding, bruising, and infection or wound healing complications. We do not expect side effects from the device or from prolonged incubation in the event that it is not successfully retrieved the day following implantation. The device consists of a polyaryletherketone (PEEK) polymer which has demonstrated long-term safety and biocompatibility in multiple biomedical applications, such as trauma, orthopedic and spinal implants.[8] A close comparable for the Kibur device is the “Cassi Beacon” tissue marker, an FDA-approved fiducial marker used for long-term localization of breast tumors. (*http://scionmedtech.com/products/breast-biopsy/cassi-beacon/*) The Cassi Beacon is structurally very similar to the Kibur device (composed of PEKK) though it is more than 4 times larger by volume (cylindrical shape, 1.5mm in diameter and 5mm long). If devices cannot be retrieved, they will be removed from patient along with the remainder of the tumor during surgical tumor resection which is maximally 6 months after implantation. The device is not expected to interfere with neoadjuvant systemic chemotherapy.

9.0 PRIMARY OUTCOMES

	Screening[1]	**Baseline**	**Day 1**	**Day 2**	**Day 2-30**	**Post-AC**	**After all planned chemo**
Signed informed consent	X						
Medical history	X	X					
Medication Review	X	X					
ECOG PS	X						
Physical examination with tumor measurement	X	X					
Pathology confirmed at MSK	X						
Placement of 3 devices unless limited by tumor size			X				
Tumor analysis for apoptotic index[5]							X
Toxicity review[6]			X	X			X
COHORT 1 Specific:							
Standard of Care Breast Surgery				X			

COHORT 2 Specific:							
Removal of device(s)				X			
Begin standard of care neoadjuvant chemotherapy[2]					X		
Research breast tumor biopsy[3]						X	
Definitive breast cancer surgery[4]							X

[1] Screening assessments may be used for baseline.

[2] Neoadjuvant chemotherapy is to begin between Days 2 and 30.

[3] A research core biopsy of breast tumor will be performed in the time period between completion of final AC infusion and up to 2 days after final infusion. Tumor measurement will be documented at this time.

[4] Proceed to definitive breast cancer surgery within 4 weeks of last dose of chemotherapy if cancer is resectable.

[5] Tumor analysis of apoptotic index from tissue collected on Day 2 and Post-AC will not necessarily occur in real time. Data derived from the device will not be used for treatment decision-making. RCB will be determined by the study pathologist at the time of definitive cancer surgery following neoadjuvant chemotherapy.

[6] Toxicity review will be completed at post operative surgery visit.

10.0 CRITERIA FOR REMOVAL FROM STUDY

The following events may result in the removal of patients from the study:

- Inability to comply with protocol requirements
- Patients whose health would be jeopardized by continued participation
- Withdrawal of informed consent (subject"s decision to withdraw for any reason)
- Discretion of the Investigator

If a patient is removed based on the criteria above, we will replace the patient.

11.0 BIOSTATISTICS

This is a pilot study in 12 patients with early stage triple negative breast cancer to examine the feasibility of using the Kibur device that aims to test for in vivo drug sensitivity. With results from this study, we plan to open a larger trial in order to examine whether data obtained from the Kibur device is correlated with clinical outcome as defined by pathologic complete response measured by residual cancer burden (RCB 0/1).

Patients in this trial will be a mix of TNBC patients who 1) will have the microdevice implanted prior to breast surgery, in the absence of neoadjuvant chemotherapy and patients who 2) will have the device placed and retrieved on a core biopsy the following day, then receive neoadjuvant chemotherapy prior to definitive breast surgery. For the first type of patient, the device will be retrieved from the surgical tumor specimen. For the second type of patient, the device will be retrieved via core biopsy. To date, we have recruited 4 patients where the device has been retrieved by core. The sample size for each type of patient is not explicitly delineated. Rather, recruitment will continue until we have 12 patients of any of the two types. Analyses on feasibility will be done on all 12 patients together.

Our decision to deem this pilot study feasible will be based on the ability to implant, retrieve at least one device, and collect evaluable tumor tissue for readout on drug sensitivity. If we are able to collect data from at least 6 out of the 12 patients, we will deem this study feasible to conduct in

a larger cohort of patients. There is a .62 probability that we will be able to collect data from at least 6 patients assuming a .50 binomial probability. The below table shows the probability of having a successful trial under higher assumed probabilities of .60, .70, and .80.

True underlying rate of success	Probability of observing 6 or more successes
.60	.84
.70	.96
.80	~1.0

The sample size for this pilot is limited due to practical and budgetary constraints. As such, our analyses will be primarily descriptive and graphical in nature. All primary and secondary analyses will be based on the TNBC patients enrolled (n=12), unless other noted below.

We will summarize the safety and toxicity of implanting and retrieving the device(s).

In the second group of patients (candidates for neoadjuvant therapy) AC chemotherapy sensitivity in vivo (measured by apoptotic index defined by cleaved caspase 3, Ki67 labeling index, phospho-H2AX score and RAD51 score in the area closest to the AC wells of the device) is correlated with RCB 0/1 using graphical methods, such as boxplots. In this second group of patients, we will also evaluate the correlation between AC chemotherapy sensitivity (as measured by the apoptosis index, Ki67 labeling index, phospho-H2AX score and RAD51 score) assessed prior to treatment (in vivo) and assessed following AC treatment (the same 4 measures assessed in the research biopsy specimen). This also will be done graphically.

There is likely to be more than one set of values obtained for each patient. This is because each well is in duplicate and there can be up to 3 devices retrieved per patient, thereby creating up to 6 data points per biomarker per patient. In addition, two pathologists will review the readout from each device, bringing the maximum number of replicates possible up to 12 per biomarker per patient. For the main analyses, we will use the average value. However, we will also investigate the intervariability of the measurements by device and within a device. We will also evaluate pathologist interobserver variability. This will be done graphically and if warranted, by calculating the concordance correlation coefficient. These studies of variability will be especially valuable when designing a larger trial. In addition to our exploratory studies on intervariability, we will assess the quality of data in terms of the ability to successfully obtain the four measurements of interest.

There are several exploratory hypotheses. We will correlate device readouts at the paclitaxel and carboplatin wells with RCB 0/1 in TNBC patients who receive AC followed by paclitaxel and carboplatin. We will evaluate this graphically and if warranted, using a Wilcoxon rank sum test.

We expect to complete accrual of 12 patients in the next 12 months.

Note: In this amendment (September 2016) we are opening recruitment to include TNBC patients who will have the microdevice implanted prior to breast surgery. The biostatistics text above has been edited to reflect this change.

12.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

12.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

12.2 Randomization

Not applicable

13.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The data collected for this study will be entered into the CRDB, a secure database. Source documentation will be available to support the computerized patient record.

13.1 Quality Assurance

Regular registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

13.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled —Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials‖ which can be found at: **http://cancertrials.nci.nih.gov/researchers/dsm/index.html**. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: **http://mskweb2.mskcc.org/irb/index.html**.

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center„s Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

14.0 PROTECTION OF HUMAN SUBJECTS

Prior to the enrollment of each patient, the risks, benefits and objectives of the study will be reviewed with the participant, including a discussion of the possible side effects. Alternative options will be reviewed, including standard therapy outside of a clinical trial, as appropriate. Financial costs and burdens of the trial will be reviewed, including a detailed discussion of the tests that will be the financial responsibility of the study, and the tests which will be the financial responsibility of the patient.

14.1 Privacy

MSK‟s Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

14.2 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant's last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 „Reporting of Serious Adverse Events", the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report is submitted electronically to the SAE Office as follows:

Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to sae@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject's initials
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

The PI's signature and the date it was signed are required on the completed report.

14.2.1

Not applicable

15.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

16.0 REFERENCES

1. Burstein, H.J., et al., *American Society of Clinical Oncology clinical practice guideline update on the use of chemotherapy sensitivity and resistance assays.* J Clin Oncol, 2011. **29**(24): p. 3328-30.
2. Gianni, L., et al., *Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial.* Lancet Oncol, 2012. **13**(1): p. 25-32.
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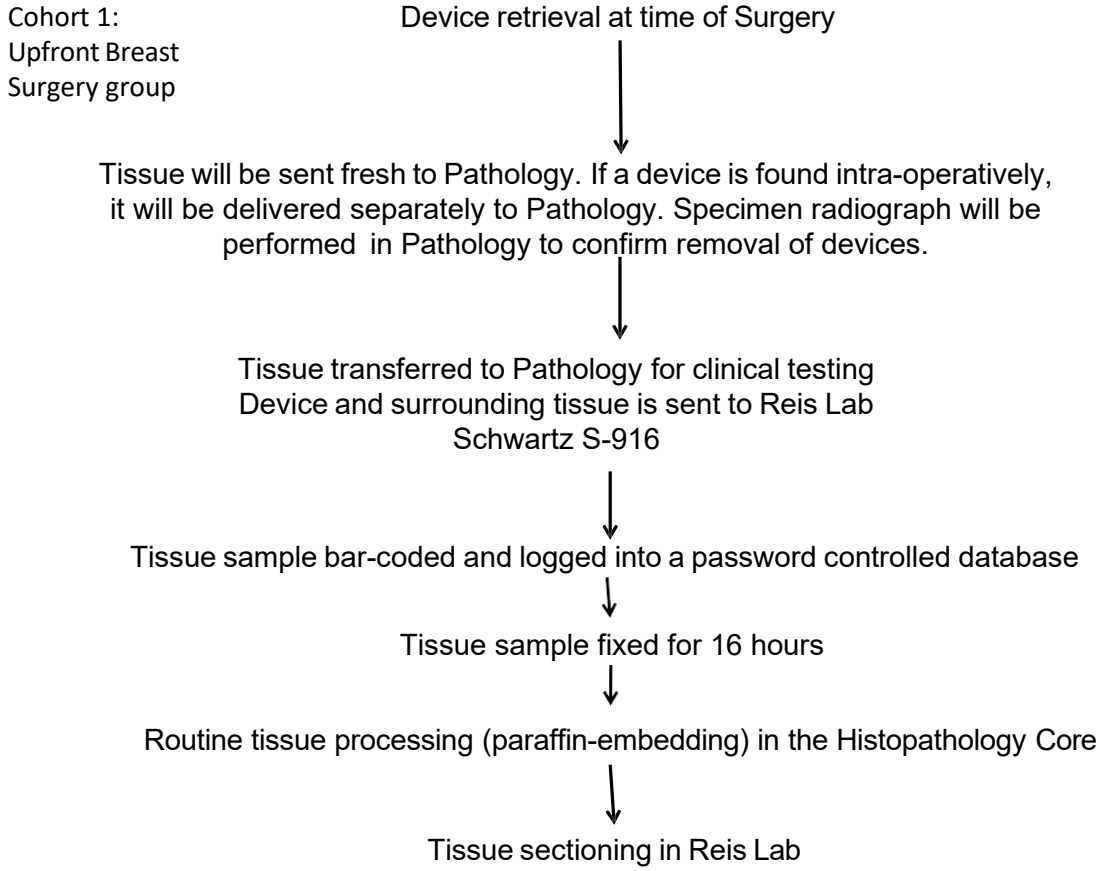
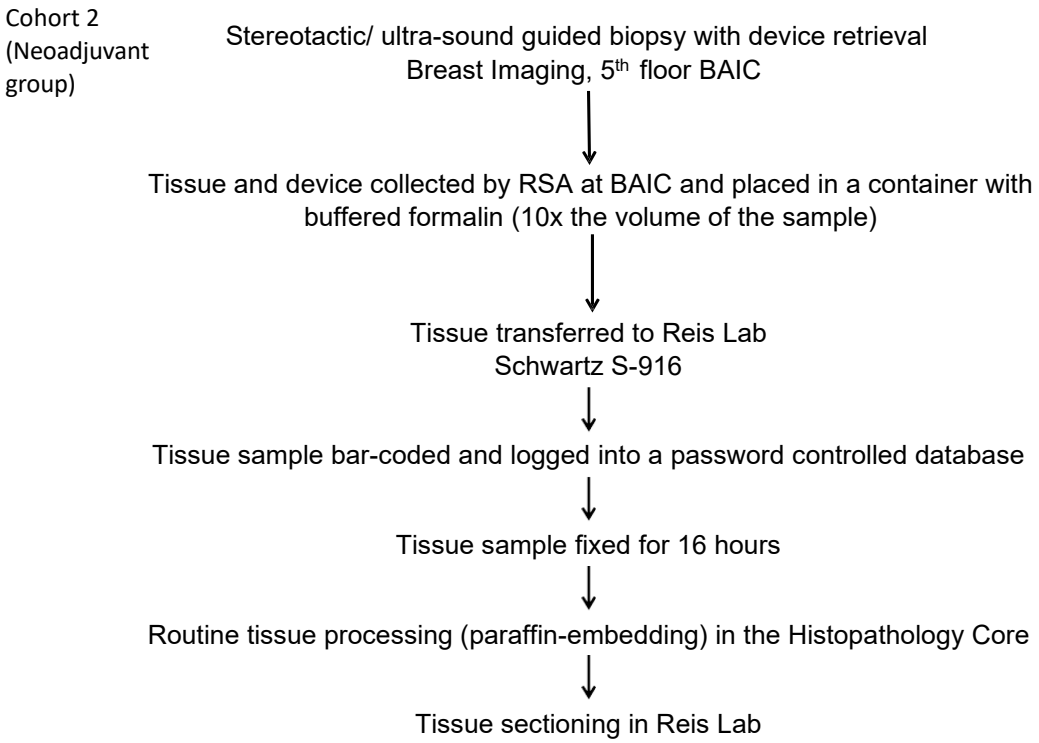
17.0 APPENDICES

Appendix A: Biospecimen Processing and Handling





Appendix B: Residual Cancer Burden Determination

Symmans WF et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol. 2007 Oct 1;25(28):4414-22

http://jco.ascopubs.org/content/25/28/4414.full.pdf+html

Appendix C: Manuscript Regarding Kibur Microdevice

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