A Pilot Study of Pre-Operative, Single-Dose Ipilimumab, Nivolumab and Cryoablation in Early Stage/Resectable Breast Cancer

PROTOCOL FACE PAGE FOR
MSK THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

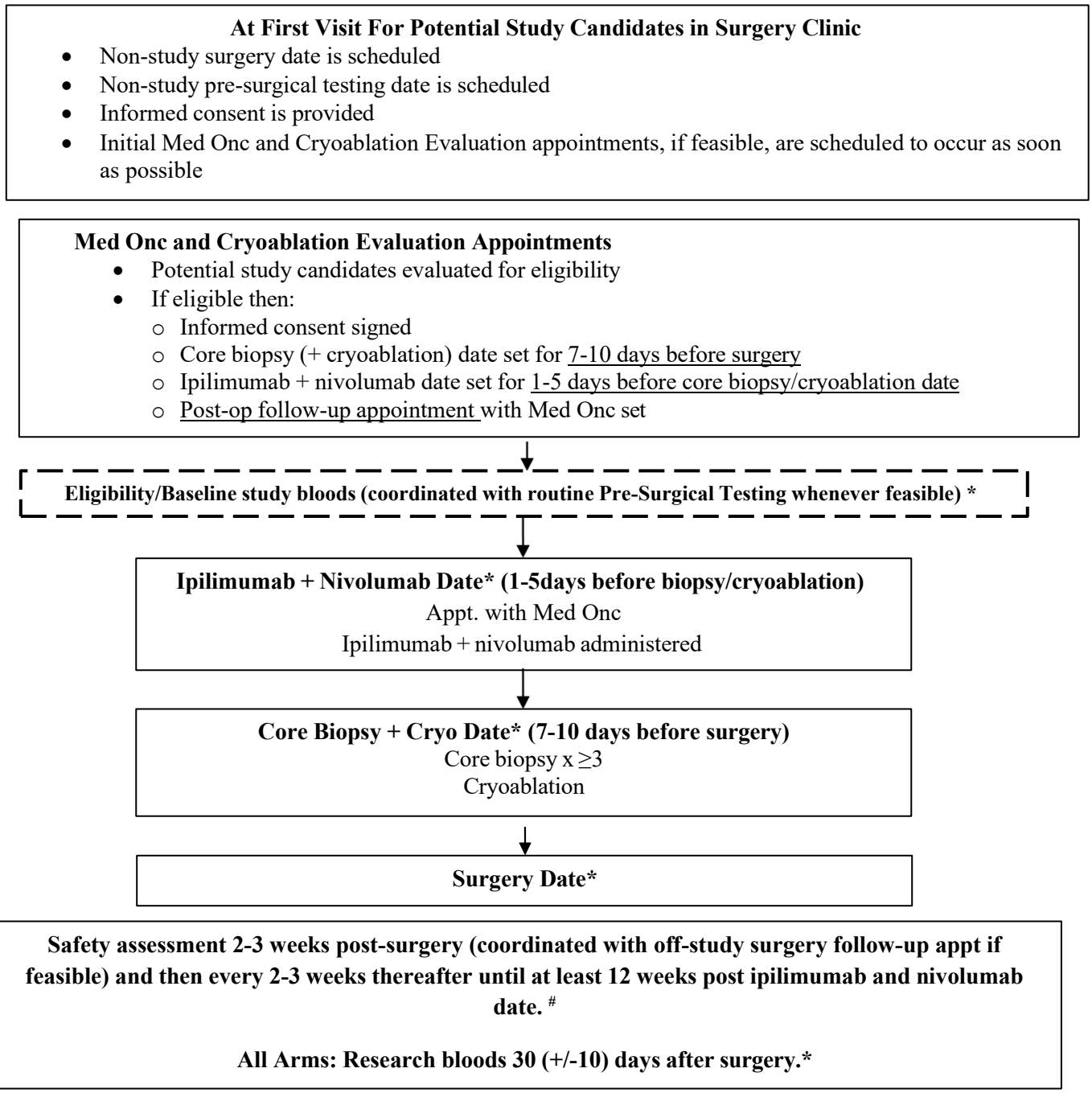
This is a pilot study evaluating the safety and tolerability of pre-operative, single-dose ipilimumab, nivolumab and cryoablation in patients with early stage/resectable breast cancer. The central idea for this project is to make a major advance for women with early stage breast cancer by augmenting an effected individual's immune response to their recently diagnosed, resectable breast cancer prior to definitive surgical management, thereby conferring long-term immunity to the primary breast tumor, and ultimately, cure.

A pilot study evaluating pre-operative, single-dose ipilimumab and/or cryoablation in patients with early stage/resectable breast cancer was recently completed at MSKCC and met the primary endpoint of safety/tolerability. In this study we are building on the success of this pilot study and the improvements observed in other tumor types with combined checkpoint blockade, to further evaluate the safety of a therapeutic strategy whereby an individual's immune system is stimulated to recognize and respond to the specific features of their own tumor. Our strategy combines two interventions: first we induce activation and maturation of dendritic cells and tumor-specific T cells by cross-presentation of tumor antigens via local destruction of tumor tissue by cryoablation. Second, we administer ipilimumab, a CTLA4 blocking antibody, and nivolumab, an antibody against programmed death receptor-1 (PD-1), that together enhance the magnitude and potency of the tumor specific T cell response.

One study arm of 6 patients will be enrolled. At the time of the initial surgical consultation, potentially eligible candidates will be identified by their surgeon and referred for an appointment with the Principal Investigator (Dr. Elizabeth Comen) or a consenting medical oncologist and an appointment with consenting professionals from radiology, if feasible, to evaluate eligibility for cryoablation. Prior to protocol intervention, consent will take place at the first available appointment with a consenting professional. Consenting, eligible women will then be registered to receive single-dose ipilimumab with nivolumab and cryoablation. All study interventions (including the cryoablation, drug administration, and imaging dates) will be defined by the pre-determined, non-study surgical date.

The purpose of the study is to determine the safety of pre-operative ipilimumab, nivolumab and cryoablation in patients with biopsy proven resectable breast cancer. If at least 5 of the 6 patients are without an adverse event (AE) necessitating delay in surgery, the regimen will be considered safe/tolerable for further study. Secondary aims of this study are to explore and characterize pre- and post-intervention peripheral and tumor responses to ipilimumab, nivolumab and cryoablation.

Med Onc and Cryoablation Evaluation Appointments



***** Indicates time points for obtaining Research blood samples. Baseline study bloods may be drawn with routine pre-surgical testing or on the day of ipilimumab injection (if applicable), prior to drug administration.
[#] Indicates time points for obtaining Safety blood Samples

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objective

- To assess the safety and tolerability of pre-operative ipilimumab, nivolumab and intratumoral cryoablation in patients with resectable breast cancer.

Exploratory Objectives:

To evaluate the effect of cryoablation and ipilimumab/nivolumab administration on intratumoral and serum lymphocyte phenotypes, intratumoral and serum dendritic cells (DCs), serum cytokines, disease related biomarkers, antibody responses to selected antigens, and humoral and cellular responses to tumor antigens and recall non-tumor antigens.

- To assess the effect of treatment on ratios of specific intratumoral and serum T cell populations including CD4+FOXP3+ T-regulatory cells (Tregs) and CD8+Effector cells.
- To explore T cell diversity and clonality changes by deep sequencing
- To assess the pharmacodynamic effects of ipilimumab, nivolumab and cryoablation on Absolute Lymphocyte Count (ALC) and absolute T cell count (see below).

3.0 BACKGROUND AND RATIONALE

Our goal is to make a major advance for persons with early stage breast cancer by augmenting the effected individual's immune response to their recently diagnosed, resectable breast cancer prior to definitive surgical management, thereby conferring long-term immunity to the primary breast tumor, and ultimately, cure.

Breast cancer is a global public health burden with more than 1 million new cases diagnosed worldwide and approximately 200,000 new cases diagnosed in the United States each year.[1, 2] For most patients in the developed world, breast cancer is diagnosed at an early stage disease. In fact, the proportion of early stage diagnoses is increasing, largely as a result of significant improvements in screening and public education programs.[3] However, despite ongoing therapeutic innovations, approximately one-third of women with early stage disease still develop distant metastases and ultimately die of metastatic breast cancer.[1]

Some of the most notable recent therapeutic innovations in breast cancer reflect improvements in the ability to recognize biologically distinct breast cancer subsets and ultimately, to tailor treatment recommendations to these features. Currently, biologic subsets are primarily defined by hormone receptor and HER2 status outside of clinical trials. However, the subtyping of breast cancer represents both an advance (better targeted therapies and improved patient selection) and a challenge (ever smaller subsets make drug development difficult). Therefore, a therapeutic strategy that simultaneously utilizes the individual (and possibly unique) aspects of each patient's tumor combined with greater generalizability (to the entire patient population) could represent a significant advance. Our goal is, therefore, to combine the biological promise of personalized therapy (via stimulation of the effected individual's immune system) with a standard therapeutic intervention (cryoablation with ipilimumab/nivolumab administration) thereby overcoming the issue of heterogeneity and potentially increasing the probability of cure.

To induce the desired immune response, we selected local thermal ablation of the tumor using cryoablation specifically. This approach has emerged as alternative to surgical resection to treat many types of inoperable tumors including kidney, liver, adrenal, lung, and others and is therefore clinically feasible.[5] Cryoablation involves the insertion of a probe into a tumor nodule in order to administer tissue ablative freezing temperatures.[6] This will lead to immediate disruption of the tissue structure and cellular damage. The necrosis observed in the ablated lesion is mediated by mechanical disruption from crystallization, osmotic changes, and vascular stasis. The necrotic tumor lesion remains within the body, and the release of the tumor antigens by the dying cells could activate a specific immune response through antigen presentation by DCs to T-cells. Several feasibility studies have indicated that thermal ablation can be performed safely in small breast cancers and effectively eradicate breast cancer cells.[7]

To further amplify the immune response, we plan to use two fully human monoclonal antibodies that blocks the normal host's attenuation of immunity. Ipilimumab specifically binds to human cytotoxic T lymphocyte antigen 4 (CTLA4) resulting in immuno-stimulation. Treatment with ipilimumab has resulted in clinically important and durable tumor responses in several solid malignancies, including prostate, bladder, and renal cell carcinoma. However, it has been most extensively studied in malignant melanoma with overall response rates of 10-20% demonstrated in multiple studies and significant survival benefits when compared with a gp100 vaccine.[11,12] The agent is generally well tolerated with few grade 3/4 adverse events.[8,9] Nivolumab, an anti-PD1 antibody, has demonstrated activity not only in melanoma but also in multiple solid tumors.[58-60] Furthermore, improved responses have been observed in untreated metastatic melanoma with the combination of ipilimumab and nivolumab when compared with either drug alone.[61]

The combination of tumor cryoablation and immunomodulation has been shown in animal models, by our colleagues, to generate such a systemic anti-tumor response.[4] Specifically, local tumor destruction with cryoablation can lead to exposure of DCs with sufficient quantities of tumor antigens to ultimately lead to DC maturation and activation. Recently, our collaborators (Dr. James Allison's group at MSKCC) showed that combination therapy with cryoablation and CTLA4 blockade successfully mediated rejection of metastatic prostate cancer lesions and prevented the growth of secondary tumors in preclinical murine models.[4] Furthermore, in a pilot study of single-dose pre-operative ipilimumab and/or cryoablation in women with early stage breast cancer completed at MSKCC, the combination of CTLA4 blockade with cryoablation was safe and tolerable Cryoablation and single dose ipilimumab at 10mg/kg were safe/well tolerated alone and in combination. The primary endpoint of this trial was reached, with all 18 patients receiving standard-of-care surgical mastectomy without delay. No treatment-associated grade III/IV adverse events, and one unrelated grade III/IV adverse event, were recorded on study. One subject from group C (cryo+ ipi) developed a grade 3maculopapular rash originating at the site of the mastectomy drain, which erupted hours after mastectomy and spread to involve the face, neck and chest. Based upon the timing, appearance, and distribution of the rash, the treating dermatologist concluded that the rash was related to peri-operative anti-septic wash and/or cephalexin administration, rather than the study intervention. Skin biopsy revealed mixed-pattern dermatitis with eosinophilic infiltrate, consistent with a hypersensitivity reaction. Combination therapy was also associated with sustained peripheral elevations in Th1-type cytokines, proliferating CD4+ and CD8+ T cells, and ICOS expression as well as post-treatment proliferation of T-effector cells relative to T-regulatory cells within the tumor.[56]

Given the safety of ablation techniques in the early stage breast cancer setting, the efficacy and tolerability of ipilimumab with nivolumab administration in other solid tumors, and the success of a pilot study evaluating the safety/tolerability of pre-operative ipilimumab at 10mg/kg with cryoablation in early stage breast cancer, we aim to confirm the safety/tolerability of cryoablation, ipilimumab and nivolumab using the model of the successfully completed pilot study.

This project directly translates the pre-clinical data developed at our institution demonstrating that cryoablation combined with CTLA4 blockade synergize to mediate tumor rejection into clinical practice, and the clinical data generated at our institution demonstrating the safety of single dose ipilimumab with cryoablation in the pre-operative setting. We hypothesize that by exploiting the biology of the tumor and the immune system of the effected individual, that this strategy will confer long-term immunity, and ultimately cure. Our immediate goal is to study the safety and tolerability of pre-operative cryoablation in combination with single dose ipilimumab and nivolumab. Because of toxicity observed with ipilimumab and nivolumab administered in combination in other settings, each drug will be administered at a conservatively low dose of 1 mg/kg in women with early stage breast cancer (notably, ipilimumab was previously administered at 10mg/kg in the pilot study).[61] Our longer-term goal is to develop this strategy as a curative treatment for women with early stage breast cancer

Principles of the Proposed Therapies

Thermal Ablation in Cancer:
Thermal ablation treatments such as cryoablation emerged as an alternative to surgical resection and as treatment for many inoperable tumors including breast, prostate, kidney, liver, adrenal and lung.[13] Cryoablation, or tissue destruction by freezing, involves percutaneous placement of a needle-like probe(s) through the skin and into the tumor under image guidance whereby the lesion is ablated *in situ*. The treatment success is dependent on achieving adequate freezing margin of the ice ball containing the tumor. The temperature necessary for reliable cryoablation induced cell death is -20^{o}C.[14]

In a series of *in vivo* animal experiments we compared the thermal map around a commercially available cryoprobe to the ice ball edge using thermocouple temperature probes. This revealed that reliable -20^{o}C temperatures for porcine tissue were achieved at approximately 10mm inside the ice ball edge. We also observed that the treatment effect using thermal mapping varies with different organs. Cryoablation zones were larger in kidney than lung and liver and felt to represent variations in vascular perfusion relative to the tissue density of these different organs producing the phenomenon commonly referred to as the "thermal sink" effect, described by the Pennes bioheat transfer equation.[15]

The procedure induced cell death is due to the immediate and direct damage to the cell membranes and cellular organelles through the formation of the ice crystals. Thereafter, thrombosis of small vessels is observed in the ablation bed within hours to days following the procedure. Cryoablation, unlike other ablative tools such as radiofrequency ablation (RFA), allows the operator to visualize the complete ice ball on intra-procedural images. This permits greater confidence that the tumor is confined within the treatment zone. Additionally, when comparing our institutional experience with cryotherapy versus RFA, it was noted that the procedural pain was significantly less with

cryotherapy.[19] This experience primarily reflects renal ablation models; however, it is reasonable to expect that these advantages also apply in the ablation of the breast.

The ice ball from cryoablation is well visualized on MR images as an area of low signal. This can allow intra-procedural monitoring of the treatment to ensure adequate coverage of the tumor and ensure avoidance of injury to nearby structures such as skin. Breast cancer lesions are also well seen on MR images allowing an ideal target for cryoablation needle placement. The same devices that are used in the MR environment to allow breast biopsy and localization will be available to guide cryoablation probe placement.

In addition to its effect on the localized tumor, it has been hypothesized that cryoablation can induce systemic anti tumor response that results in regression of distant metastatic lesions. Following ablation the necrotic tumor remains within the body, the vigorous inflammatory response to the necrotic tissue with the sustained release of tumor antigens from the dying cells, which are captured by the antigen presenting cells (APCs), subsequently this will lead to maturation activation and migration of the APCs to the draining lymph nodes (LNs) where eventually the activation of tumor specific T cells occurs.[16,17] The immunological effect of cryoablation has been documented in many preclinical and clinical models. It was first demonstrated by Shulman and colleagues who described the production of anti-epididymal spermatozoal antibodies in rabbits after freezing.[18] Also Levy *et al* showed that combining cryoablation with cyclophosphamide induces a potent anti tumor response in murine model of metastatic colon cancer.[19] Clinically, Ablin *et al*, described remission of metastatic lesions in patients with prostate cancer whose primary tumors were treated with cryotherapy.[20] More recently, Dr. James Allison's laboratory has demonstrated that the combination of cryoablation and immune modulation (such as with CTLA4 blockade) can lead to rejection of tumor in murine models of prostate cancer (see below section on the rationale for the combination).[4]

Cryoablation in Breast cancer

With recent improvements in breast cancer screening and diagnosis, most newly diagnosed breast cancers in the developed world are small and non-palpable. Consequently, a number of lumpectomy alternatives, including mini-invasive ablation techniques, have been explored. For example, methods such as RFA and cryosurgery for the *in situ* ablation of breast cancer, indicate that these procedures are safe and well tolerated procedures.[21]

Preclinical data from mouse models of mammary carcinoma showed that adoptive transfer of T cells from cryo-ablated tumor draining lymph nodes generated a significant increase in the fraction of tumor specific T-cells and anti-tumor activity. This increased activity ultimately translated into significant reduction of pulmonary metastases when compared to adoptive transfer of T-cells from mouse lymph nodes after surgical tumor excision or no treatment. These data demonstrate the effectiveness of the "cryo-immunotherapy" approach in the adjuvant treatment in breast cancer[17] Utilizing more modern techniques, researchers in Japan demonstrated the ability to manage locally advanced breast cancer with cryosurgery. In 49 patients with advanced or recurrent breast cancer treated with cryosurgery, alleviation of pain, control of hemorrhage, and reduction of tumor bulk were reported along with impressive survival benefits.[22] Cryoablation is also used safely in treating benign breast tumors such as fibroadenomas.[23]

More recently, Sabel *et al* conducted a phase I multi institutional study to determine the safety and feasibility of cryoablation in patients with early stage breast cancer.[24] Twenty-nine patients with tumors ≤ 2 cm treated with cryoablation followed by 1 to 4 weeks later by surgical resection. Cryoablation was successfully performed in an office-based setting with only local anesthesia. There were no complications to the procedure or post-procedural pain requiring narcotic pain medications. Cryoablation successfully destroyed 100% of cancers <1.0 cm. For tumors between 1.0 and 1.5 cm, this success rate was achieved only in patients with invasive ductal carcinoma without a significant ductal carcinoma-in-situ (DCIS) component. Morin *et al* explored the use of MRI-guided cryoablation in 25 patients.[25] Complete pathologic ablation was achieved in only 13 of 25 patients; however, this study included much larger tumors, ranging from 1.2cm to 6cm (mean = 2.8cm). The authors were able to accurately predict success using a combination of MR images and post-procedure scinti-mammography and no delays in scheduled mastectomies where reported. Both of the aforementioned studies concluded that cryoablation is safe and well-tolerated in early stage breast cancer.

Rationale for Immune Therapy with Ipilimumab and Nivolumab

Full activation of naive T cells requires not only stimulation of the antigen receptor by peptide/major histocompatibility complexes, but also by co-stimulatory signals.[27] These signals are provided by the engagement of CD28, which is constitutively expressed on T-cell surfaces, with CD80 and CD86 molecules, which are present on APC. CD28-B7 co-stimulatory signals are critical for the induction of T-cell proliferation, cytokine secretion, and effector functions which ultimately translate into clinical effects (Kearney 1995). CTLA4 is an activation-induced T-cell surface molecule that binds CD80 and CD86 with greater avidity than CD28. CTLA4 ligation down-regulates T-cell responses, which results in an abrogation of the clinical effects provided by T-cell activation.[29] Blockade of CTLA4/B7 interactions results in an increase in T-cell activation.

Perhaps the most convincing demonstration of the down-regulatory role of CTLA4 came from examination of mice with a null mutation.[30] These CTLA4 knockout mice appear to have spontaneously activated T cells evident at approximately 1 week after birth, followed by rampant lymphoproliferation and lymphadenopathy.[31] These mice die at approximately 3 weeks of age, either as a result of polyclonal T cell expansion and tissue destruction or as a result of toxic shock resulting from lymphokine production by the T cells. Since thymocyte differentiation and selection proceed normally in CTLA4-deficient mice, the rampant T cell expansion that occurs in the mice indicates that CTLA4 plays a critical role in down-regulating T cell responses in the periphery.[32] Many preclinical studies demonstrated that treatment with anti-murine CTLA4 monoclonal antibody resulted in tumor rejections, for example CTLA4 blockade led to rejection of immunogenic colon carcinoma and fibrosarcoma murine models.[33] However, rejection of less immunogenic tumors, such as murine melanoma B16, was achieved only when CTLA4 blockade was combined with vaccines or radiation therapy.

Ipilimumab is human monoclonal antibody specific to human cytotoxic T lymphocyte antigen 4 (CTLA4) with immuno-stimulating properties. Treatment with ipilimumab resulted in clinically important and durable tumor responses in several malignancies, including melanoma, prostate cancer, and renal cell carcinoma but most extensively studied tumor type has been malignant melanoma and result of many clinical trials demonstrating an overall response rate of 10-20%.[10] More recently, a

phase 3 study showed that ipilimumab, either alone or with gp100 vaccine, improved overall survival compared with gp100 alone in previously treated metastatic melanoma.[11] Specifically, a 3.8 month increase in overall survival and a doubling of the 1 year survival rate was demonstrated in favor of the ipilimumab-treated group.

PD-1 (or CD279) is a member of the CD28 family of T-cell costimulatory receptors that include immunoglobulin super family members CD28, CTLA-4, inducible co-stimulator (ICOS), and B and T-lymphocyte attenuator (BTLA). PD-1 is highly expressed on activated T cells and B cells. PD-1 expression can also be detected on memory T-cell subsets with variable levels of expression. Two ligands specific for PD-1 have been identified: PD-L1 (also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273). PD-L1 and PD-L2 have been shown to down-regulate T-cell activation upon binding to PD-1 in both murine and human systems.[69] The interaction of PD-1 with its ligands, PD-L1 and PD-L2, that are expressed on antigen-presenting cells (APC) and dendritic cells (DC), transmits negative regulatory stimuli to down-modulate the activated T-cell immune response. The absence or inhibition of PD-1 in murine models has resulted in the development of various autoimmune phenotypes and autoimmune diseases. Taken together, these results suggests that inhibition of PD-1 binding to its ligands has the potential to activate T-cell responses. Since these responses are variable and dependent upon various host genetic factors, PD-1 deficiency or inhibition is not accompanied by a universal loss of tolerance to self antigens.

Tumors can express tumor-specific antigens as a result of mutational burden and ongoing immune surveillance is believed to control the development of many tumors. Tumor progression may depend upon acquisition of mechanisms which permit them to evade an effective immune response. One such mechanism of evasion may be the expression of ligands which engage inhibitory receptor(s) on anti-tumor T-cells of many tumors. PD-L1 expression has been found on a number of tumors including breast cancer, and may be a mechanism by which tumors can directly engage PD-1 to evade an effective anti-tumor immune response.[70] Expression of INF-γ by T-cells is known to induce PD-L1 expression in tumors. PD-L1 expression has been associated with poor prognoses multiple cancers, and binding of PD-L1 to PD-1 on T-cells may limit effective immune responses. Co-localization of lymphoid cell infiltrates and PD-L1 staining has been observed in human melanoma.[71]

Studies in multiple tumor models using a chimeric murine anti-mouse PD-1 antibody showed that PD-1 blockade has anti-tumor activity. Blocking PD-1 in PD-L1+ tumors may reverse the inactivation of tumor-specific effector T-cells at the tumor site as well activate anti-tumor responses that are limited by PD-L1 expression on “host” DC or APC. The anti-tumor effects of anti-PD-1 observed in several murine models suggest that both PD-L1+ and PD-L1- tumors may be targeted using this approach. In addition, in several tumor models in which anti-PD-1 has proved ineffective, PD-1 blockade can be combined with vaccines, tumor-destructive modalities, or other immunomodulatory antibodies for improved therapeutic efficacy.[72,73]

Nivolumab, an anti-programmed death-1 (PD1) antibody has demonstrated activity not only in melanoma but also in multiple solid tumors.[58-60] Furthermore, improved responses have been observed in untreated metastatic melanoma with the combination of ipilimumab and nivolumab when compared with either drug alone.[61]

Possible toxicities with ipilimumab may include:

- **Infusion reactions:** Fever, chills, sweating, shakes, itching, rash, hyper- or hypotension, difficulty breathing, flushing, nausea, vomiting. It is likely that most infusion-related adverse events will occur within the first 24 hours after beginning the infusion, and may be treated by slowing or interruption of the infusion, or with supportive treatment as indicated in Section 11.3.

- **Immune-related Adverse Events (irAE):** Many of the adverse events considered related to ipilimumab are immune in nature and presumably a consequence of the intrinsic biological activity of ipilimumab. In prior ipilimumab clinical studies, an irAE was defined as any adverse event associated with drug exposure and consistent with an immune-mediated event. Disease progression, infections and other etiologic causes were ruled out or deemed unlikely as contributing to the event. Supportive data, such as autoimmune serology tests or biopsies, were considered to be helpful but not necessary to categorize an event as an irAE. Events of unclear etiology which were plausibly immune mediated were conservatively categorized as irAEs even if serologic or histopathology data were absent. These irAEs likely reflect a loss of tolerance to some self-antigens or an unchecked immune response to gut or skin flora. Some breakthrough of immunity may be inseparably linked to the clinical antitumor activity of ipilimumab. Immune-related adverse events predominately involve the GI tract, endocrine glands, liver or skin. Experience with ipilimumab indicates that irAEs are typically low grade and self-limited, with few exceptions; irAEs were clinically manageable and reversible with supportive care or corticosteroids (see treatment algorithm Appendix 2-6). Notably, IrAE typically occur after multiple doses of ipilimumab. In this study a single 1mg/kg dose of ipilimumab is being administered, which make irAE unlikely. This is supported by a recently reported study wherein presurgical ipilimumab administration in patients with bladder cancer was tolerable and safe. [12]

- **Gastrointestinal irAEs:** The most common grade 3 or greater irAE involved the lower GI tract and clinically manifested as diarrhea or hematochezia. Diarrhea resulting from treatment with ipilimumab ranged from mild to severe and was life-threatening in some cases. Some cases of diarrhea began as mild and became very severe (Beck 2006). Serious GI irAEs mostly involved diarrhea or colitis (e.g. hemorrhagic colitis that was unresponsive to medical management and necessitated colectomy, bowel wall perforations associated with and without ipilimumab-induced colitis). Diarrhea/colitis is one of the most serious AEs and must be evaluated in a timely fashion (see diarrhea management algorithm in Appendix 4). All patients with diarrhea will be seen by physician and evaluated with a CT Scan/colonoscopy if necessary.

- The first onset of GI irAEs generally occurred within the first 12 weeks of treatment and was generally reversible. Bowel wall biopsies demonstrated a pleomorphic infiltrate, including many lymphocytes, consistent with colitis due to an autoimmune process. In addition, upper GI tract involvement including ileitis, duodenitis, and esophagitis have been reported.

- **Gastrointestinal perforation in association with narcotic use:** Isolated cases of intestinal perforation in association with narcotic use have been reported. The use of narcotics may potentially mask GI symptoms or delay detection of their relevant complications, such as bowel perforation.

- **Inflammatory Hepatotoxicity:** Immune-related hepatic dysfunction, including hepatitis or abnormal liver function tests (LFTs) attributed to ipilimumab therapy, has been reported. Subjects may develop elevations in LFTs in the absence of clinical symptoms. Inflammatory hepatotoxicity includes non-infectious hepatitis (e.g., autoimmune hepatitis). Hepatic irAEs generally occur in the first 12 weeks of treatment and are primarily reversible (see hepatotoxicity management algorithm in Appendix 5).

- **Hypophysitis/Hypopituitarism and Other Endocrine Conditions:**
  Hypophysitis/hypopituitarism, clinically manifested by fatigue, has been reported. Most subjects with hypopituitarism presented with nonspecific complaints such as fatigue, confusion, visual disturbance, or impotence. Some had headache as the predominant presentation. The majority of subjects with hypopituitarism demonstrated enlarged pituitary glands based on brain magnetic resonance imaging. Low adrenocorticotropic hormone and cortisol were the most common biochemical abnormality reported; low thyroid stimulating hormone, testosterone, or prolactin was also reported in some subjects.[50]
  The first onset of endocrine irAEs typically occurred between weeks 6 and 12 of treatment. Hypophysitis/hypopituitarism was controlled with appropriate hormone replacement therapy and may be dose related. Other endocrine glands may be targets of ipilimumab-related toxicity, and rare cases of primary adrenal insufficiency, hyperthyroidism and hypothyroidism were reported (see endocrinopathy management algorithm in Appendix 6).

- **Rash and Other Skin Conditions:** Rash was one of the most common irAEs, and most cases were Grade 1 or 2 in intensity; pruritus has also been reported.[51]
  When biopsied, pleomorphic infiltrates were noted in the skin. Skin irAEs were generally reversible. Some subjects reported vitiligo associated with ipilimumab administration.

- **Ocular Inflammation:** Ocular inflammation, manifested as Grade 2 or Grade 3 episcleritis or uveitis, was associated with concomitant diarrhea in a few subjects and occasionally occurred in the absence of clinically apparent GI symptoms.[52] Most of the subjects with ocular inflammation and known outcome recovered or improved with or without corticosteroid therapy with a median duration of approximately 6 days (range: 5 to 23 days).

Possible toxicities with Nivolumab may include:

CA209003 is a Phase 1 open label, multiple dose escalation study in 306 subjects with select previously treated advanced solid tumors, including melanoma, RCC, NSCLC, colorectal cancer, and hormone-refractory prostate cancer. Subjects received nivolumab at doses of 0.1, 0.3, 1, 3 or 10 mg/kg intravenously every 2 weeks, up to a maximum of 2 years of total therapy. As of 18-Mar-2013, a total of 306 melanoma subjects were treated with nivolumab in the dose range of 0.1 - 10 mg/kg.

No maximal tolerated dose was identified in CA209003. The incidence, severity and relationship of AEs were generally similar across dose levels and tumor types. Nivolumab related AEs of any grade occurred in 75.2% of subjects. Of the 306 treated subjects, 303 (99.0%) subjects have at least 1 reported AE regardless of causality. The most frequently reported AEs were fatigue (54.9%), decreased appetite (35.0%), diarrhea (34.3%), nausea (30.1%), and cough (29.4%).

Treatment-related AEs were reported in 230 (75.2%) of the 306 subjects. The most frequently reported treatment-related AEs were fatigue (28.1%), rash (14.7%), diarrhea (13.4%), and pruritus (10.5%). Most treatment-related AEs were low grade. Treatment-related high grade (Grade 3-4) AEs were reported in 52 (17.0%) of subjects. The most common treatment-related high grade AEs were fatigue (2.3%) and diarrhea (1%).

Drug-related SAEs occurred in 11.5% of subjects. Grade 3-4 drug-related SAEs reported in at least 2 subjects included: diarrhea (3 subjects, 1.0%), pneumonitis (3 subjects, 1.0%), pneumonia (2 subjects, 0.7%) and lipase increased (2 subjects, 0.7%). Select AE categories (events with a potential inflammatory mechanism requiring more frequent monitoring and/or unique intervention such as immunosuppressants and/or endocrine replacement therapy) include: GI AEs, pulmonary AEs, renal AEs, hepatic AEs, skin AEs, and endocrinopathies. In addition, Select AEs include a category for infusion reactions.

Each category is composed of a discrete set of preferred terms, including those of greatest clinical relevance. These Select AEs are considered events of interest based on the mechanism of action and were previously referred to as immune-related AEs or immune-mediated AEs. The 10 mg/kg cohort had numerically greater frequency of high-grade select AEs including the subcategories of endocrinopathies, GI, pulmonary, and infusion reactions (Table 11.1.3-1) Most high grade events resolved following the treatment guidelines for the treatment of pulmonary events, GI events, hepatic events, renal events, and endocrine events, respectively.

Treatment-related AEs leading to discontinuation were reported in 32 (10.5%) of the 306 treated subjects on CA209003. The most frequent of these were pneumonitis (8 subjects; 2.6%) and colitis (3 subjects; 1.0%).There were 3 (1%) drug related deaths; each occurred after development of pneumonitis.

The phase 3 dose for nivolumab monotherapy is 3 mg/kg. Additional details on the safety profile of nivolumab, including results from other clinical studies, are also available in the BMS-936558 (nivolumab) IB.

Possible toxicities for Ipilimumab and nivolumab in combination may include:

As of 15-Feb-2013, in the Phase 1 study CA209004 of 86 subjects with unresectable or metastatic melanoma, ascending doses of nivolumab have been studied as monotherapy in sequence with ipilimumab (N = 33) or in combination with ascending doses of ipilimumab (N = 53).

In the sequential cohorts, 1 mg/kg and 3 mg/kg nivolumab cohorts have been studied. Subjects were required to have prior ipilimumab and their last treatment must have occurred within 4 – 12 weeks. As such, based on ipilimumab pharmacokinetics, pharmacodynamically active ipilimumab was present at the outset of treatment in all subjects. Based on this, the monotherapy safety profile in the sequential cohorts was expected to differ from the monotherapy safety profile reported in CA209003. Therefore, the most relevant safety data for nivolumab monotherapy is from CA209003 (described above). The pooled safety data from CA209004 is only provided below for reference.

In each of the combination cohorts in this multi-arm study, ipilimumab was administered once every 3 weeks for 4 doses with nivolumab administered once every 3 weeks for 8 doses. Starting at week 24, ipilimumab and nivolumab were administered once every 12 weeks for 8 doses. The three initial dose escalation cohorts consisted of Cohort 1 (nivolumab 0.3 mg/kg plus ipilimumab 3 mg/kg; n = 14), Cohort 2 (nivolumab 1 mg/kg plus ipilimumab 3 mg/kg; n = 17) and Cohort 3 (nivolumab 3 mg/kg plus ipilimumab 3 mg/kg; n = 6). The study was subsequently amended to include Cohort 2a which evaluated nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (n = 16). Safety data were pooled to summarize the overall findings.

At least one AE, regardless of whether they were attributed to the therapy, has been reported in 81 (94.2%) of the 86 subjects (Table 11.2.1-1). A numerically higher number of subjects in the combination therapy treatment groups experienced severe AEs, regardless of causality and treatment related, than those in the monotherapy treatment group.

In the combination treatment groups, the following DLTs were observed during the dose escalation in the combination cohorts:

- Cohort 1: Grade 3 elevated AST/ALT (1 subject);
- Cohort 2: Grade 3 uveitis (1 subject) and Grade 3 elevated AST/ALT (1 subject);
- Cohort 3: Grade 4 elevated lipase (2 subjects) and Grade 3 elevated lipase (1 subject).

Based on these data, Cohort 2 was identified as the maximum tolerated dose (MTD) and Cohort 3 exceeded the MTD. The most frequent treatment related AEs were rash (54.7%), pruritus (47.2%) and fatigue (37.7%). The most frequently reported treatment related severe AEs in the combination group were lipase and AST elevation (13.2% each), ALT elevation (11.3%), followed by diarrhea (5.7%) and rash (3.8%).

Treatment related serious adverse events (SAEs) were reported in 49% of patients in the combination treated subjects (N = 53) and most frequently severe SAEs were: AST elevation (13.2%), ALT elevation (11.3%), lipase elevation (5.7%) and diarrhea, colitis and renal failure (3.8% each). Ten of the 53 (18.9%) treated subjects in the combination cohort

discontinued therapy due treatment-related adverse events, the most frequent of which were ALT, AST elevation, lipase elevation, renal failure or pneumonitis. No drug-related deaths were reported. Median time to event onset was approximately 61 days (range: 4 to 114 days).

Immunotherapy and Breast Cancer:

The interrelationship between the tumor, the tumor microenvironment and the function of infiltrating immune cells at the tumor site, is important for generating efficacious anti-tumor immune response (Naito 1998). It is also well established that the magnitude of the tumor burden leads to local suppression and apoptosis of the infiltrating T-Cells.[37] In breast cancer, the influence of tumor burden on the generation of tumor antigen-specific cytotoxic T-lymphocytes (CTLs) was investigated in a phase I/II clinical trial in the metastatic setting. The study evaluated the cytotoxic function in an adoptive immunotherapy model in 2 patient study arms, one with macroscopic disease and other with a complete remission (CR). The results revealed that the cytotoxic function was sustained in only one CR patient, suggesting that tumor burden had an inverse effect on the function of the generated CTLs.[38]

The tumor bearing host ability to mount an immune response to tumor antigens is dampened by the effect of $T_{regs,}$ a subset of CD^+ T cells. These cells have a potent ability to suppress immunity by inhibiting cytotoxic lymphocytes, and are thought to play role in tolerance to tumor and self antigens. [39] In experimental models, when the number or function of T_{regs} was reduced, a surge in the anti-tumor response was shown.[40] Furthermore, recent studies have demonstrated that immunophenotypic features of tumor infiltrating lymphocytes in breast cancer patients are associated with prognostic factors and survival rates. For example, investigators have shown increased numbers of tumor infiltrating T_{regs} and peripheral blood T_{regs} in patients with breast cancer and other tumors, compared to healthy donors.[41] In addition, significantly higher numbers of T_{regs} have been demonstrated in patients with invasive breast cancer versus ductal carcinoma *in situ* (DCIS). Particularly high numbers of T_{regs} were present in patients with higher risk tumors including high-grade tumors, tumors with lymph node involvement, and estrogen receptor (ER) negative tumors. Furthermore, T_{regs} numbers correlated with relapse rates and even overall survival. [42] Thus, tumor infiltration by immune cells appears to play a critical role in the tumorigenesis of breast cancer and therapies that can effectively modulate this immune response likely represent a promising opportunity for therapeutic innovation.

The presence of tumor infiltrating lymphocytes (TILs) has recently demonstrated prognostic (and predictive impact in breast cancer specifically.[62-65] Furthermore, PD-1/PD-L1 blockade alone has demonstrated approximately 20% response rates in the palliative breast cancer setting. [66, 67]

Rationale for Combined Therapy with Cryoablation and Ipilimumab in Cancer

Novel strategies that combine a cytoreductive modality (i.e. intra-tumoral cryoablation) with immune-modulation are promising strategies for the management of tumors. For example, combination of CTLA4 blockade with thermal ablation demonstrated synergistic anti tumor response in a B16 murine melanoma model.[16] Furthermore, our collaborators at MSKCC (Dr. James Allison and colleagues) have shown that combination therapy with cryoablation and CTLA4 blockade successfully mediated rejection of metastatic prostate cancer lesions and prevented the growth of secondary tumors in mice (Figure 1).

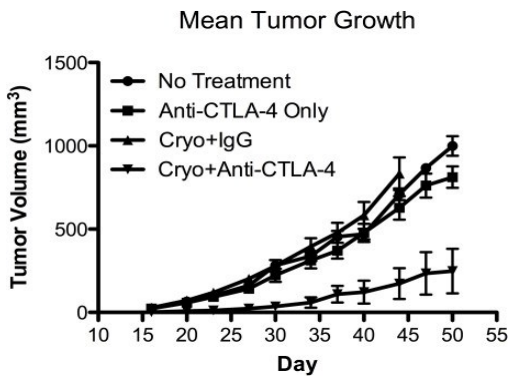


Fig 1. Cryoablation and anti-CTLA-4 combination therapy synergize to mediate rejection of a second prostate tumor challenge.

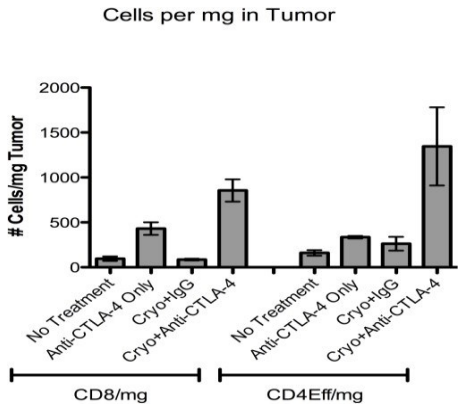


Fig 2. CD4^{+}FoxP3^{-} effector T cells and CD8^{+} T cells infiltrate the secondary tumors of combination treated mice.

Dr. Allison and colleagues have also shown that secondary tumors from combination treated mice were infiltrated with tumor specific CD8^{+} T cells (Figure 2) and demonstrated an increase in the effector T cell (T_{eff}) to regulatory T cell (T_{reg}) ratio (Figure 3). Clinical and pre-clinical studies have suggested that an elevated CD8 to Treg ratio at the tumor site is associated with better clinical outcomes (Quezada 2006).

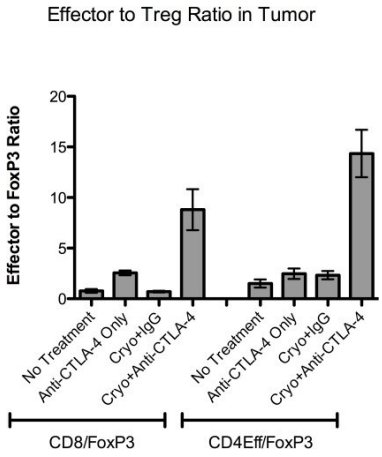


Fig 3.Combination treated tumors have an increased Teff to Treg ratio compared to controls

The safety of cryoablation combined with CTLA4 blockade has been demonstrated in the pre-operative breast cancer setting by our group.[56]

Rationale for Biomarker Research

As part of the completed pilot study of pre-operative ipilimumab and/or cryoablation, immunologic correlatives were conducted on both PBMCs and TILs. Tumors treated with combined ipilimumab plus cryoablation exhibited a higher ratio of $CD8^+Ki67^+$ proliferating T_{eff} cells to $CD4^+CD25^+FOXP3^+$ T_{reg} cells suggesting that combined therapy may favorably shift the effector/regulatory T-cell ratio. [56] This effect was not seen with Ki67-negative T_{eff} cells. One possible explanation is that cryoablation depletes both T_{eff} and T_{reg} cells, and that concurrent ipilimumab induces proliferation of residual or immigrating T_{eff} cells. We also demonstrated that cryoablation and ipilimumab enhances systemic immune activation (as measured by IFNγ and $ICOS^{hi}$ T-cells by flow cytometry) and intratumoral T-cell proliferation (as measured by TcR sequencing and Ki67+CD8+ T-cells by flow cytometry.[57]

In addition, T-cell repertoire analysis was conducted on both core biopsy and mastectomy TILs (Page 2014) In cryo-ablated specimens, T-cell counts were reduced, however the infiltrate was more polyclonal with low clonal overlap, indicating influx of new clones. Combination cryoablation plus ipilimumab resulted in an expansion of a greater number of T-cell clones intratumorally, versus cryoablation or ipilimumab alone (Table 3.1, unpublished data).

Table 3.1: T-cell Clonal expansion following ipilimumab plus cryoablation

	Median # TIL clones expanding by			Median # PBMC clones expanding by		
	$\geq 10^2$ amplicons	$\geq 10^3$ amplicons	$\geq 10^4$ amplicons	$\geq 10^2$ amplicons	$\geq 10^3$ amplicons	$\geq 10^4$ amplicons
Cryo	199	2	0	697	1	0
Ipi	750	22	1	545	5	0
Cryo+Ipi	2010	85	1	830	1	0

These data suggest that cryoablation may induce new clones secondary to antigen presentation, and that the addition of ipilimumab may induce proliferation of a subset of tumor-reactive infiltrating clones.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a pilot, single-institution study, wherein one group of 6 women ≥18 years of age with operable, ≥1.5 cm, histologically-confirmed, invasive carcinoma of the breast and no evidence of distant metastases, for whom surgery is planned, will be enrolled. The 6 participating women will be treated with ipilimumab, nivolumab and cryoablation of the dominant primary tumor prior to the planned surgery.

At the time of the initial MSKCC surgical consultation, potentially eligible candidates will be identified, informed consent forms provided, and surgery-related dates requested/set per standard-of-care. As soon as possible, potentially eligible patients will be evaluated by 1) the principal investigator (Dr. Elizabeth Comen), a designated consenting medical oncologist for a medical oncology assessment or the referring surgeon and 2) consenting professionals from radiology for assessment of amenability to cryoablation, if feasible. All available (per the standard of care) imaging (MMG +/- breast US +/- breast MRI) will be reviewed to determine eligibility for cryoablation and thus, study participation. Whenever feasible, the radiology evaluation will be arranged prior to the presurgical testing date so that baseline research bloods may be collected after the patient is confirmed eligible and together with the non-study presurgical bloods. Prior to protocol intervention, consent will take place at the first available appointment with a consenting professional.

Consenting, eligible women, with tumors amenable to MRI or US-guided cryoablation will be enrolled. All study interventions (including the cryoablation, ipilimumab/nivolumab administration, and imaging dates) will be defined by the pre-determined, non-study surgical date. **Research study appointments/interventions will not interfere with the planned, standard-of-care, surgery arrangements.**

4.2 Intervention

During study intervention planning, the standard of care for patients with early stage primary breast cancer diagnosis and treatment of as well as the timing of the definitive surgery schedule will not be altered in any way for patients participating in this study. If a patient experiences side effects from intervention resulting in a delay of their planned surgery date, this will be a failure of the primary endpoint. The planned intervention of this study is to obtain image guided biopsies, percutaneous MRI or US guided breast tumor cryoablation, and ipilimumab/nivolumab administration.

Most patients will have an outside biopsy confirming the diagnosis of breast cancer at the time of the initial surgical consultation. However, because the cryoablation procedure will cause near complete tumor destruction (that in turn will cause immunologic effect and antitumor response), pre-cryoablation breast biopsies will be performed. At least three 18G MRI or US-guided core biopsies of the primary breast tumor will be obtained (together with core biopsies of any suspicious lymph nodes as clinically or radiographically indicated) if feasible. Two core breast specimens will be provided to the MSKCC Breast and Imaging Center (BAIC) pathology department for routine (standard of care) testing. Additional core biopsy specimens will be sent to the immune monitoring facility (IMF) for research purposes as detailed in section 12. If not all three core biopsies can be performed, this will not make the patient ineligible.

The MRI or US-guided biopsies will be performed by investigators from the Breast Radiology Service with the assistance of investigators from Interventional Radiology and Anesthesiology as needed. Image guided percutaneous cryoablation can be performed through a small nick making it a minimally invasive procedure that has been performed safely in cancer patients for many years. The cryoablation will be performed 7-10 days prior to the surgery date.

Ipilimumab and nivolumab are fully human, monoclonal anti-CTLA4 antibodies that have shown anti-tumor activity and are now US FDA approved for the treatment of patients with unresectable advanced melanoma (unresectable stage III or stage IV).

Recent studies have shown that administration of ipilimumab in the pre-operative setting is safe and does not cause delay of surgery in bladder cancer.[12] In this study, ipilimumab and Nivolumab will be administered 1-5 days prior to core biopsy + cryoablation and 8-15 days prior to the planned surgery.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Cryoablation

Cryoablation has been successfully adopted for the treatment of solid tumors for many years. Initially it was an intraoperative procedure, but with advanced engineering devices for percutaneous cryoablation has been made possible. In addition the use of imaging to guide the ablation provides increased confidence of appropriate targeting of the tumor. The cryoablation will be performed percutaneously under MRI or US image guidance. Once the probes are properly placed in the tumor freeze-thaw cycles will be performed. The probes will then be removed. The patients will be observed for a short time prior to expected same day discharge.

5.2 Ipilimumab and Nivolumab:

Ipilimumab (BMS-734016), a fully human monoclonal antibody that recognizes CTLA4, is considered an immunotherapeutic drug and is currently in development as an anti-cancer agent. The proposed mechanism of action for ipilimumab is interference of the interaction of CTLA4 with CD80 (B7-1) and CD86 (B7-2) molecules expressed on antigen-presenting cells (APC), with subsequent blockade of the inhibitory modulation of T-cell activation promoted by the CTLA4:CD80/CD86 interaction.

Nivolumab (BMS-936558), a fully human monoclonal antibody that recognizes PD1, is considered an immunotherapeutic drug and is currently in development as an anti-cancer agent. The proposed mechanism of action for nivolumab is interference with the interaction between PD1 and PDL1. T cell influx into tumors results in the release of IFN-γ, which up-regulates PD-L1 expression by tumor cells. PD-L1 binds to PD-1, which is expressed by activated T cells, generating a negative signal that causes T cell exhaustion (inhibiting the ability of T cells to recognize and kill their targets). During antigen presentation by dendritic cells, PD1 can also act as a checkpoint inhibitor where a negative signal can be sent by its binding to either PD-L1 or the closely related (and dendritic cell–specific) negative regulatory ligand PD-L2.[68]

Concomitant Medications

Participants will be advised not to take steroids, immunosuppressants, or anti-inflammatory medications such as NSAIDS from the day of trial entry and until 30 days after surgery unless recommended by the study Principal Investigator (or co-investigators) for the treatment of immune related adverse events (irAEs) or adrenal insufficiency.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

Describe the characteristics of the patient/subject population.

6.1 Subject Inclusion Criteria

Subjects must meet the following criteria at screening to be eligible to participate in the study.

- Women age 18 years or older
- Confirmed histologic diagnosis of invasive adenocarcinoma of the breast, including MSKCC pathology confirmation
- ER, PR and HER2 testing in progress (i.e. on outside or MSKCC biopsy report)
    - HER2-positive pathology is permitted
- Operable tumor measuring ≥1.5 cm in maximal diameter
    - Any nodal status
    - Multifocal and multicentric disease is permitted.
    - Synchronous bilateral invasive breast cancer is permitted
    - The tumor should be more than 5 mm from the skin.
- No indication of distant metastases
- Breast surgery planned
- Tumor amenable to cryoablation as determined by radiologist
- ECOG performance status score of 0 or 1.
- Screening laboratory values must meet the following criteria:
    i. White blood cells (WBCs) ≥ 2000/μL
    ii. Absolute neutrophil count (ANC) ≥ 1500/μL
    iii. Platelets ≥ 100 x 10^3/μL
    iv. Hemoglobin ≥ 11.0 g/dL
    v. Serum creatinine ≤ 2 mg/dL (or glomerular filtration rate ≥ 40 ml/min)
    vi. AST ≤ 2.5 x upper limit of normal (ULN)
    vii. ALT ≤ 2.5 x ULN
    viii. Bilirubin within normal limits (except subjects with Gilbert's syndrome, who must have total bilirubin < 3.0 mg/dL)
    ix. Negative HIV screening test
    x. Negative screening tests for Hepatitis B and Hepatitis C.
        Patients with positive results that do not indicate true active or chronic infection may enroll after discussion and consensus agreement by the treating physician and principal investigator.
- Women of childbearing potential (WOCBP) must be using an acceptable method of contraception to avoid pregnancy throughout the study and for at least 3 months after the last dose of ipilimumab in such a manner that the risk of pregnancy is minimized. See below for the definition of WOCBP.
- WOCBP must have a negative serum pregnancy test within 14 days prior to the first dose of ipilimumab/nivolumab.
- Women must not be breastfeeding.

- Willing to adhere to the study visit schedule and the prohibitions and restrictions specified in this protocol.

Definition of WOCBP

Women of childbearing potential include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal. Post menopause is defined as:

- Amenorrhea ≥ 12 consecutive months without another cause and a documented serum follicle stimulating hormone (FSH) level >35 mIU/mL
- Women with irregular menstrual periods and a documented FSH level > 35 mIU/mL
- Women on hormone replacement therapy (HRT)

Women who are using oral contraceptives, other hormonal contraceptives (vaginal products, skin patches, or implanted or injectable products), or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy, or are practicing abstinence or where their partner is sterile (e.g., vasectomy) should be considered to be of childbearing potential.

6.2 Subject Exclusion Criteria

1) Inflammatory breast cancer
2) Medical history and concurrent diseases
   a) Subjects with active, known or suspected autoimmune disease. Subjects with vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.
   b) Any serious or uncontrolled medical disorder that, in the opinion of the investigator, may increase the risk associated with study participation or study drug administration, impair the ability of the subject to receive protocol therapy, or interfere with the interpretation of study results.

3) Prohibited Treatments and/or Therapies
   a) Chronic use of immunosuppressants and/or systemic corticosteroids (used in the management of cancer or non-cancer-related illnesses). However, use of corticosteroids is allowed for the treatment of immune related Adverse Events (irAEs), or adrenal insufficiency.
   b) Any non-oncology vaccine therapy used for prevention of infectious diseases within 4 weeks prior to first dose of ipilimumab/Nivolumab.
   c) Prior treatment with a CD137 agonist, ipilimumab or other CTLA4 inhibitor;
   d) Prior investigational agents within 2 weeks prior to first dose of ipilimumab/Nivolumab;
   e) Prior therapy with any anti-cancer agents including chemotherapy, adjuvant chemotherapy, immunosuppressive agents, surgery or radiotherapy within 2 weeks prior to first dose of ipilimumab/Nivolumab.

7.0 RECRUITMENT PLAN

The Evelyn H. Lauder Breast Center, in the Breast and Imaging Center at MSKCC provides a large referral base, with approximately 2000 new breast cancer referrals each year, with the vast majority of these representing early stage diagnoses. In 2014, the last year for which we have complete data, 719 (41%) of the 1774 women undergoing surgery at MSKCC for resectable invasive breast cancer underwent total mastectomy (with 59% undergoing breast conserving surgery). We anticipate an average accrual rate of 2-3 patients per month with complete accrual of 6 patients within 2-3 months. At the time of the initial MSKCC surgical consultation, potentially eligible candidates will be identified, informed consent forms provided, and surgery-related dates requested/set per standard-of-care. As described in detail in Section 4.1, at the time of initial presentation in the surgery clinic, potentially eligible study candidates will be identified by the surgeon and referred for an appointment with the Principal Investigator (Dr. Elizabeth Comen) or a consenting medical oncologist and an appointment with consenting professionals from radiology, if feasible, to evaluate eligibility for cryoablation. Prior to protocol intervention, consent will take place at the first available appointment with a consenting professional.

8.0 PRETREATMENT EVALUATION

After the patient is identified as a potentially appropriate study candidate at the time of the initial breast surgery consultation, an appointment will be made with the Principal Investigator (Dr. Elizabeth Comen) or another consenting medical oncologist. An appointment will also be made with consenting professionals from radiology, if feasible, to evaluate eligibility for cryoablation and to review potential complications. Eligibility for safe cryoablation will include an assessment of how close the tumor is to various structures. The tumor should be more than 5 mm from the skin. The chest wall and associated structures represent less of a complication risk. The observation of the ice ball with imaging during the procedure should mitigate these potential risks. Prior to protocol intervention, consent will take place at the first available appointment with a consenting professional. If the patient's tumor is amenable to cryoablation and informed consent has been signed, additional appointments will be arranged accordingly. Informed consent must be performed within 28 days prior to ipilimumab/nivolumab administration.

<u>Baseline blood tests to be drawn with non-study presurgical blood tests, within 14 days prior to ipilimumab/nivolumab administration:</u>

Comprehensive metabolic panel (COMP): aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), total bilirubin, calcium, creatinine, glucose, total protein, BUN, sodium, potassium, chloride, bicarbonate

Complete blood cell count with differential and platelet count (CBC)

Thyroid stimulating hormone (TSH), free T4, T3, ACTH, FSH/LH, prolactin, testosterone levels

HIV, Hepatitis C, and Hepatitis B screening tests

Research tests: Blood (4 CPT tubes) for immune response studies will be sent to the IMF (currently located on the 15th floor of the Zuckerman building) for exploratory studies. These baseline blood samples may be drawn on the day of the ipilimumab/Nivolumab injection (if applicable), but should be drawn prior to the injection.

All other screening assessments should be performed within 14 days prior to ipilimumab/nivolumab administration:

- Medical history, demographic data, adverse events, physical examination, ECOG PS, 12-Lead ECG.

No delay in surgery will be made to accommodate participation in the protocol.

9.0 TREATMENT/INTERVENTION PLAN

Patients will undergo breast biopsy with cryoablation 7-10 days prior to the planned surgery, and ipilimumab with nivolumab administration 1-5 days before cryoablation. All patients will undergo surgery at the pre-determined day defined at the initial surgical consultation as detailed in section 4.1.

9.1 Ipilimumab and Nivolumab:

Women will receive ipilimumab and nivolumab 1-5 days prior to US or MRI-guided core biopsy and cryoablation date. Intravenous ipilimumab will be administered as a single 1 mg/kg dose to be infused intravenously over 90 minutes in the chemotherapy suite of the BAIC. Intravenous nivolumab will be administered as a single 3 mg/kg dose to be infused intravenously over 60 minutes in the chemotherapy suite of the BAIC.

In a study reported by Dr. Wolchok of various doses of ipilimumab and nivolumab in combination in metastatic melanoma, there was a 98% AE rate, a 93% treatment related AE rate, a 72% grade 3/4 AE rate, a 53% treatment related grade 3/4 AE rate and a 49% serious AE rate.[61] Toxicity with both drugs administered at 3mg/kg was considered unacceptable, and nivolumab at 1mg/kg with ipilimumab at 3mg/kg was considered the maximum dose with an "acceptable" level of AEs - a threshold which is unlikely to be "acceptable" in the curative setting. Thus, combination therapy with ipilimumab administered at 1mg/kg and nivolumab administered at 3mg/kg was recommended and will be the dose we will administer for this study.

9.1.1 Dose Calculations

Calculate Total Dose as follows for ipilumumab:

Patient body weight in kg x [1 mg/kg] = total dose in mg

Calculate Total Infusion Volume as follows:

Total dose in mg ÷ 5 mg/mL = infusion volume in mL

Calculate Rate of Infusion as follows:

Infusion volume in mL ÷ 90 minutes = rate of infusion in mL/min.

For example, a patient weighing 114 kg (250 lb) would be administered 114 mg of ipilimumab (114 kg x 1 mg/kg = 114 mg) with an infusion volume of 22.8 mL (114 mg ÷ 5 mg/mL = 22.8 mL) at a rate of approximately .25mL/min (22.8 mL ÷ 90 minutes) in 90 minutes.

9.1.2 Storage, Preparation, and Administration

Identification
Ipilimumab is available in 5 mg/mL single-use vials (10 mL or 40 mL). The sterile solution in the vial is clear and colorless to pale yellow. Ipilimumab is administered via intravenous infusion only.

Nivolumab is available in 10 mg/mL single-use vials (10 mL). The sterile solution in the vial is clear and colorless to pale yellow. Nivolumab is administered via intravenous infusion only.

Packaging and Labeling
BMS will provide ipilimumab and nivolumab at no cost for this study. Ipilimumab and nivolumab will be provided in open-label containers. The labels will contain the protocol prefix, batch number, content, storage conditions, and dispensing instructions along with the Investigational New Drug (IND) caution statement. Ipilimumab will be supplied at a concentration of 5 mg/mL in vials containing 10 ml or 40 mL solution. Nivolumab will be supplied at a concentration of 10 mg/mL in vials containing 10 ml solution.

Storage, Handling, and Dispensing

Storage

Ipilimumab and nivolumab must be stored in a secure area according to local regulations. The investigator must ensure that it is stored in accordance with the environmental conditions as determined by BMS and defined in the Investigator Brochure or SmPC/reference label. Ipilimumab and nivolumab must be stored at a temperature ≥ 2°C and ≤ 8°C.

Handling and Disposal
As with all injectable drugs, care should be taken when handling and preparing ipilimumab and nivolumab. Whenever possible, ipilimumab and nivolumab should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents applying aseptic technique. Latex gloves are required. If ipilimumab or nivolumab concentrate or solution comes in contact with skin or mucosa, immediately and thoroughly wash with soap and water. After final drug reconciliation, unused ipilimumab solution should be disposed at the site following procedures for the disposal of anticancer drugs.

Dispensing

It is the responsibility of the investigator to ensure that ipilimumab and nivolumab is only dispensed to study subjects. The ipilimumab and nivolumab must be dispensed only from official study sites by authorized personnel according to local regulations.

Drug product administration

Ipilimumab is to be administered as an IV infusion with a 0.2µm or 1.2µm in-line filter using a volumetric pump, at the 1 mg/kg dose, to complete the infusion in 90 minutes with at least 10 cc normal saline flush at the end. The total dose needed should be prepared and administered per institutional guidelines. The total dose must be calculated using the most recent subject weight (obtained on the same day of, and prior to, the infusion).

Nivolumab is to be administered as an IV infusion with a 0.2µm or 1.2µm in-line filter using a volumetric pump, at the 3 mg/kg dose, to complete the infusion in 60 minutes with at least 10 cc normal saline flush at the end. The total dose needed should be prepared and administered per institutional guidelines. The total dose must be calculated using the most recent subject weight (obtained on the same day of, and prior to, the infusion).

Separate infusion bags and filters must be used for each infusion. Nivolumab is to be administered first. The nivolumab infusion must be promptly followed by a saline flush to clear the line of nivolumab before starting the ipilimumab infusion.

9.2 Pre-cryoablation core biopsies:

At least three 18G MRI or US-guided core breast biopsies will be obtained at the core biopsy appointment for all participants, if feasible. If not all three core biopsies can be performed, this will not make the patient ineligible. Core biopsies of clinically or radiographically suspicious lymph nodes will also be obtained as indicated. Two core breast specimens will be provided to the MSKCC BAIC pathology department for routine (standard of care) testing. Additional core biopsy specimens will be sent to the IMF for research purposes as detailed in section 12. The MRI or US-guided biopsies will be performed by investigators from the Breast Radiology Service with the assistance of investigators from Interventional Radiology and Anesthesiology as needed.

9.3 Cryoablation:

Percutaneous, MRI or US-guided cryoablation will be performed with a multiple freeze-thaw cycle. Freeze-thaw cycle strategies will be individualized to the patient and their tumor. Generally two cycles will be performed with each cycle being approximately 10 minutes of freeze time. Intermittent imaging will guide the procedure to ensure the ice ball does not encroach on critical structures, including skin. The number of probes used will depend on the lesion size and location.

Prior to the procedure the operator will rate the level of technical difficulty. Immediately after the procedure the operator will rate the expectation of completeness of therapy. This information will be

used for data collection purposes for this study. Cryoablation will be performed under the care of a radiologist with support from anesthesiology as needed.

9.4 Blood samples:

All participants will undergo 4 research blood draws as indicated in the protocol schema in Section 1 (at ipilimumab/nivolumab administration, cryoablation/biopsy, surgery, and 30 (+/-10) days after surgery). Detailed collection/processing instructions are included in Section 12.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

The focus of the evaluation during the treatment will be directed to assess the safety of the procedures described above.

All patients will be observed for 30 minutes after drug injection to monitor for acute allergic reactions. All participants will be observed in the PACU after cryoablation and/or image guided core biopsies for a couple of hours prior to expected same day discharge if sedation or anesthesia is required.

No additional testing, treatment, or intervention will be performed on patient accrued to the protocol.

Efforts should be made to synchronize post procedure pain management, and to try to avoid NSAIDs (specifically COX-2 inhibitors) given their potential for immune modulation.

11.0 TOXICITIES/SIDE EFFECTS

In routine care for breast cancer, multiple cores are taken to insure adequate material for diagnosis. The potential complication associated with these procedures, such as bleeding and infection are the same as those associated with these procedures when done as part of routine care. Refer to Appendix 2 for management of immune related adverse events.

11.1 Cryoablation

Patients will be monitored for adverse events related to ablation. These events may include bleeding, infection, injury to the breast, skin injury, and pain at the ablation site. Other potential complications include paresthesias.

11.2 Infusion Reactions

Ipilimumab and/or nivolumab may induce infusion or hypersensitivity reactions. If such a reaction were to occur, it may manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypo or hypertension, bronchospasm, or other symptoms. Infusion reactions should be graded according to Common Terminology Criteria for Adverse Events (CTCAE, Version 4.0) guidelines. Treatment recommendations are provided below and may be

modified based on clinical judgment, local treatment standards and guidelines, and/or specific symptoms, as appropriate:

For Grade 1 symptoms: Mild reaction [e.g., localized cutaneous reactions including mild pruritus, flushing, rash] requires infusion rate to be decreased; intervention may be indicated.

- Decrease the rate of the study drug infusion until recovery from symptoms.
- Remain at bedside and monitor subject until resolution of symptoms. Diphenhydramine 50 mg may be administered at the discretion of the treating physician.
- When symptoms resolve, restart the infusion at the original infusion rate.

For Grade 2 symptoms: Moderate reaction [i.e., any symptom not listed above (mild symptoms) or below (severe symptoms) such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic blood pressure > 80 mm Hg], requires infusion interruption but responds promptly to symptomatic treatment [e.g., antihistamines, nonsteroidal anti inflammatory drugs, narcotics, corticosteroids, i.v. fluids]; prophylactic pre-infusion medications indicated for ≤ 24 hours.

- Discontinue the study drug infusion.
- Begin an i.v. infusion of normal saline, and treat the subject with diphenhydramine 50 g i.v. (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen).
- Remain at bedside and monitor subject until resolution of symptoms. Corticosteroid therapy may be administered at the discretion of the treating physician.
- When symptoms resolve, restart the infusion at 50% of the original infusion rate; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate.
- Monitor subject closely. If symptoms recur, immediately discontinue the infusion; no further study drug will be administered at that visit. Administer diphenhydramine 50 mg i.v., and remain at bedside and monitor the subject until resolution of symptoms.
- The amount of ipilimumab infused must be recorded.

For Grade 3 or Grade 4 symptoms: Severe reaction [e.g. bronchospasm, generalized urticaria, systolic blood pressure < 80 mm Hg, or angioedema], Grade 3: prolonged [i.e. requiring 6 or more hours to respond to symptomatic medication and/or discontinuation of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g., renal impairment, pulmonary infiltrates].

Grade 4: life-threatening; pressor or ventilatory support indicated.

- Immediately discontinue the study drug infusion. No further study drug will be administered. The amount of study drug infused must be recorded on the case report form (CRF).
- Begin an i.v. infusion of normal saline, and treat the subject as follows: Recommend bronchodilators, epinephrine 0.2 to 1.0 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for i.v.

administration, and/or diphenhydramine 50 mg i.v. with methylprednisolone 100 mg i.v. (or equivalent), as needed.

- Remain at bedside and monitor subject until recovery from symptoms.
- Subject should be monitored until the treating physician is comfortable that the symptoms will not recur.
- Treating physician should follow their institutional guidelines for the treatment of anaphylaxis

In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine or corticosteroids).

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

12.1 Safety:
Safety/tolerability is the primary end point for this project.

Safety will be evaluated for all treated subjects using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events v4.0 (CTCAE). Safety assessments will be based on medical review of adverse event reports and the results of vital sign measurements, physical examinations, and clinical laboratory tests. The incidence of observed adverse events will be tabulated and reviewed for potential significance and clinical importance. The reporting period for safety data will be from the date of first on-study dose to 12 weeks after ipilimumab/nivolumab administration.

If there are no clinical signs or symptoms of toxicity, safety bloods will be done every 2-3 weeks and will include the following:

- Comprehensive metabolic panel (COMP): aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), total bilirubin, calcium, creatinine, glucose, total protein, BUN, sodium, potassium, chloride, bicarbonate
- Complete blood cell count with differential and platelet count (CBC)
- Thyroid stimulating hormone (TSH)

If 5 of the 6 study patients are without an AE necessitating delay in surgery, the regimen will be considered safe/tolerable for further study.

12.2 Efficacy:

Although not a primary endpoint, exploratory correlative studies will be performed to evaluate anti-tumor and immunological response pre- and post-treatment in individuals. Comparisons with other study participants will also be conducted. Breast cancer tissue specimens, axillary lymph nodes specimens where available and peripheral blood samples will be studied. The planned exploratory correlative studies currently include:

Immune response:
The phenotypic and functional impact of the treatment will be explored by examining specimens from tumor tissue; peripheral blood (post treatment only). These tests will be done at the IMF (currently located in the Zuckerman building).

The exploratory objectives of this study are:
1. To evaluate the effect of cryoablation and ipilimumab/nivolumab administration on: lymphocyte phenotype and serum cytokines, disease related biomarkers, antibody responses to selected antigens, and humoral and cellular responses to tumor antigens and recall non-tumor antigens.
2. To examine tumor samples for pathologic correlates of clinical activity before and after treatment, including (but not limited to) the abundance and characteristics of inflammatory infiltrates (e.g., CD8 and CD4 cells and expression of some molecules on lymphocytes and tumors, respectively), and,
3. To evaluate serological and cellular immune correlates of toxicity and/or clinical activity

To this end, the specimens to be collected for IMF evaluation include:
- At least 1 core biopsy sample (collected on the core biopsy date)
- Surgery specimen
- Four research blood samples:
  1. Baseline blood on the day of pre-surgical testing or on the day of ipilimumab/nivolumab injection prior to drug administration
  2. Blood on day of core biopsy/cryoablation
  3. Blood on the day prior to or day of surgery
  4. Blood 30 (+/-10) days after surgery.

For the each blood draw, 4 CPT tubes will be collected.
- **Flow cytometric analysis**:
  1) T-cells: activation and memory markers
  2) Dendritic cells: activation and maturation markers and markers of inhibitory/tolerogenic DCs
- **ELISA:** to identify relevant peripheral blood cytokines
- **CBC:** to quantify Absolute lymphocyte count (ALC).

Pathology*:* Pre (core biopsies) and post (surgical tissue) treatment evaluation of the tumor will be performed for each study participant. The planned exploratory studies to evaluate treatment response include:

- Evaluation of T cell proliferation as measured by Ki-67.
- Expression of the B7-H1 inhibitory molecule (Ghebeh 2007)
- Lymphocyte infiltration including perivascular infiltration.
- Specific immunohistochemistry (IHC) staining to further define particular lymphocytic populations such as T-regulatory cells (Tregs) ($CD4^+CD25^+$*$FOXP3^+$*), effector T cells (Teff) ($CD4^+/CD8^+$) and the ratio between Tregs and Teff.
- Using immunohistochemistry (IHC) staining to identify subsets of dendritic cell (DC) populations (i.e. tolerogenic versus immunogenic)Sentinel or non-sentinel lymph

node tissue, where available, will also be evaluated by immune profiling as described above.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Subjects participating may withdraw from the study at any time and for any reason.

Subjects may be removed from the study at any time due to severe unacceptable side effects, life-threatening events, (as described in Section 11), patient's non-compliance with the defined treatment plan, the patient becomes pregnant or the patient's request to withdraw consent.

For all participants, failure to have the planned surgery at MSKCC will result in removal from the study.

Patients will be replaced if they withdraw from the study after signing consent but prior to completing all study assessments.

14.0 BIOSTATISTICS

Definitive surgery confers cure for many women with early stage breast cancer and it is therefore imperative that any experimental pre-operative therapeutic innovation not undermine the timing of curative-intent surgery. Consequently, the primary endpoint for this study is safety/tolerability, as defined by the number of patients who do not develop grade 3 or 4 adverse events that necessitates a delay in surgery. Therefore, if 5 or 6 of the 6 patients are without an AE necessitating delay in surgery, the regimen will be considered safe/tolerable for further study. The probability of observing 5 or 6 patients (out of 6) who for whom therapy is safe/tolerable is 0.53 if the true safety/tolerability rate is 0.75, 0.65 if the true safety/tolerability rate is 0.80, and 0.89 if the true safety/tolerability rate is 0.90. This rule is based on the time period from start of the investigation regimen to the date of surgery, which is estimated to be approximately 15 days. Safety data up to 12 weeks following surgery will also be evaluated and reported.

Study design and analysis of the data will be performed with the assistance of Sujata Patil, PhD, Division of Epidemiology and Biostatistics.

This is a pilot study using innovative methods and if cryoablation and single-dose ipilimumab/nivolumab is deemed safe, will result in the design of a larger trial. As such, there is no formal statistical analysis or sample size calculation for this study. All analysis will be descriptive in nature and the sample size is dictated by drug availability and budget constraints.

We have several exploratory secondary analyses. As noted above, all analyses will be evaluated descriptively and graphically. Serum and tumor biomarker levels (including lymphocytes and DC's) and serum cytokines will be plotted over the study timepoints. The ratio of specific intra-tumoral and serum t cell populations (i.e. CD4+FOXP3+ T-regulatory cells (Tregs) to CD8+Effector cells) will also be plotted over study timepoints. Other endpoints that will be summarized descriptively include ALC, and absolute T cell count.

Estimated accrual will be approximately 2-3 patients per month, making targeted completion of accrual within approximately 2 to 3 months of the study initiation.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 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.

15.2 Randomization

The study will not include any randomization.

16.0 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 coordination of activities of the protocol study team.

The data collected for this study will be entered into a secure database at MKSCC. Data from this trial will be entered in the Clinical Research Data Base (CRDB). Source documentation will be available to support the computerized patient record.

16.1 Quality Assurance

Weekly 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.

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.

16.2 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.htm* 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.

17.0 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 toxicities and side effects. Every effort will be made to keep study records private. Neither the patient's name nor anything else that could identify the patient will be used in any reports or publications that result from this study. Trained staff at Memorial Hospital, the Food and Drug Administration will be able to review the medical records if necessary. The patient may terminate her participation in the study at any time during the trial.

Patients will not be charged for the following costs associated with this study:

- The cost of ipilimumab and nivolumab (administration will be charged).
- Cryoablation procedure
- Research blood tests
- Research biopsy for all patients

17.1 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).

17.2 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 be submitted electronically to the SAE Office as follows:

For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

For all other trials: 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.

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office

17.2.1 Additional Information on Serious Adverse Event (SAE) Reporting

Collection of Safety Information

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

Although overdose and cancer are not always serious by regulatory definition, these events should be reported on an SAE form and sent to BMS in an expedited manner. An overdose is defined as the accidental or intentional ingestion or infusion of any dose of a product that is considered both excessive and medically important.

Note that all pregnancies, regardless of outcome, must be reported to the sponsor on a Pregnancy Surveillance Form, not an SAE form. All pregnancies must be reported and followed to outcome, including pregnancies that occur in the female partner of a male study subject. See Section 8.4 for instructions on reporting pregnancies.

Nonserious Adverse Events
All adverse events that are not classified as serious.

Assignment of Adverse Event Intensity and Relationship to Investigational Product
All adverse events, including those that are serious, will be graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE), Version 4.0.

The following categories and definitions of causal relationship to investigational product as determined by a physician should be used for adverse events:

- Definite: The AE is clearly related to the intervention.
- Probable: The AE is likely related to the intervention.
- Possible: The AE may be related to the intervention.
- Unlikely: The AE is doubtfully related to the intervention.
- Unrelated: The AE is clearly NOT related to the intervention.

Collection and Reporting
Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: onset, duration, intensity, seriousness, relationship to investigational product, action taken, and treatment required. If treatment for the AE was administered, it should be recorded in the medical record.

The investigator shall supply the sponsor and Ethics Committee with any additional requested information, notably for reported deaths of subjects.

Collection and Reporting for Serious Adverse Events
The investigator should notify BMS of any SAE occurring after the previously specified time period that is believed to be certainly, probably, or possibly related to the investigational product or protocol-specified procedure.

All SAEs whether related or unrelated to the ipilimumab, must be immediately reported to BMS Worldwide Safety (by the investigator or designee) within 24 hours of becoming aware of the event. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

All SAEs should be faxed or emailed to BMS at:

Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

For studies conducted under an Investigator IND, any event that is both serious and unexpected must be reported to the FDA as soon as possible and, in no event, later than 7 days (death or life-threatening event) or 15 days (all other SAEs) after the investigator's or institution's initial receipt of the information. BMS will be provided with a simultaneous copy of all adverse events filed with the FDA. SAEs should be reported on the MedWatch Form 3500A, which can be accessed at:

http://www.accessdata.fda.gov/scripts/medwatch/.

MedWatch SAE forms should be sent to the FDA at:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

All SAEs should simultaneously be faxed or e-mailed to BMS at:

Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

Serious adverse events, whether related or unrelated to investigational product, must be recorded on the SAE page and reported expeditiously to BMS (or designee) to comply with regulatory requirements. An SAE report should be completed for any event where doubt exists regarding its status of seriousness.

All SAEs must be immediately reported by confirmed facsimile transmission (fax) and mailing of the completed SAE page. In some instances where a facsimile machine is not available, overnight express mail may be used. If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.) In selected circumstances, the protocol may specify conditions that require additional telephone reporting.

If the investigator believes that an SAE is not related to the investigational product, but is potentially related to the conditions of the study (such as withdrawal of previous therapy, or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE page.

If an ongoing SAE changes in its intensity or relationship to the investigational product, a follow-up SAE report should be sent immediately to the sponsor. As follow-up information becomes available it should be sent immediately using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization.

Handling of Expedited Safety Reports

In accordance with local regulations, BMS will notify investigators of all SAEs that are suspected (certainly, probably, or possibly related to the investigational product) and unexpected (i.e., not previously described in the Investigator Brochure). In the European Union (EU), an event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). investigator notification of these events will be in the form of an expedited safety report (ESR).

Other important findings which may be reported by the sponsor as an ESR include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (eg, animal) study, important safety recommendations from a study data monitoring committee, or sponsor decision to end or temporarily halt a clinical study for safety reasons.

Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the Investigator Brochure. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.

In addition, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).

Nonserious Adverse Events

The collection of nonserious AE information should begin at initiation of investigational product. Nonserious AE information should also be collected from the start of a placebo lead-in period or other observational period intended to establish a baseline status for the subjects.

If an ongoing nonserious AE worsens in its intensity, or if its relationship to the investigational product changes, a new nonserious AE entry for the event should be completed. Nonserious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for nonserious AEs that cause interruption or discontinuation of investigational product, or those that are present at the end of study participation. Subjects with nonserious AEs at study completion should receive post-treatment follow-up as appropriate.

All identified nonserious AEs must be recorded and described in the medical record.

Pregnancy

Sexually active WOCBP must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized. Before enrolling WOCBP in this clinical study, the investigator must review the guideline about study participation for WOCBP which can be found in the GCP Manual for Investigators. The topics include the following:

- General Information
- Informed Consent Form
- Pregnancy Prevention Information Sheet
- Drug Interactions with Hormonal Contraceptives
- Contraceptives in Current Use
- Guidelines for the Follow-up of a Reported Pregnancy.

Before study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during study participation and the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form documenting this discussion.

All WOCBP MUST have a negative pregnancy test within 72 hours before receiving ipilimumab. The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not receive ipilimumab and must not be enrolled in the study.

In addition, all WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for subject safety). The investigator must immediately notify BMS of this event and record the pregnancy on the Pregnancy Surveillance Form (not an SAE form). Initial information on a pregnancy must be reported immediately to BMS, and the outcome information provided once the outcome is known. Completed Pregnancy Surveillance Forms must be forwarded to BMS according to SAE reporting procedures.

Any pregnancy that occurs in a female partner of a male study participant should be reported to the sponsor. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy

follow-up procedures should be considered if indicated. In addition, the investigator must report to BMS, and follow-up on information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants should be followed for a minimum of 8 weeks.

Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded in the medical record.

18.0 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.

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20.0 APPENDICES

Appendix 1: Study Timetable

Visit	**Visit #1:** 1st Surgical Appointment	**Visits #2 and #3:** MD and IR evaluation appointments (to be coordinated whenever feasible)	**Visit #4:** Ipilimumab/ nivolumab date	**Visit #5:** Core biopsy/ cryoablation date	**Visit #6:** Safety assessment (1 day prior or day of surgery)	**Visit #7:** Surgery date	**Visit #8 +:** Safety assessment 2-3 weeks post-surgery and every 2-3 weeks thereafter until 12 weeks post ipi/nivo
Potential candidates identified	X						
Surgery date set	X						
Presurgical testing date set	X						
Cryoablation amenability determined by Radiology		X					
Consent signed		X					
Ipilimumab/nivolumab date set/appt with MD		X					
Ipi/nivo infusion			X				
Core biopsy x≥3				X			
Research Blood samples*			X	X	X		X[1]
Safety blood work (CBC, Comp, TSH)							X
Pathology review	X						
History & Physical exam	X	X	X		X		X

***** Baseline study bloods (CBC, COMP, TSH, Free T4, T3, ACTH, FSH, LH, Prolactin, Testosterone levels, HIV, Hep B, Hep C), EKG, vital signs, and research bloods may be tested with routine pre-surgical testing or on the day of ipilimumab/nivolumab administration. HIV, Hep B, and Hep C can be drawn within 60 days of study registration.

§this appointment will be coordinated with off-study surgery follow-up appt if feasible, visits 9 and 10 (if applicable) will be done every 2-3 for safety assessments up to 12 weeks after ipilimumab/nivolumab administration

1. Post surgery research bloods will be collected 30 days (+/-10 days) after surgery completion.

Appendix 2: General Recommendation for Management of Suspected Immune Related Adverse Events (irAE)

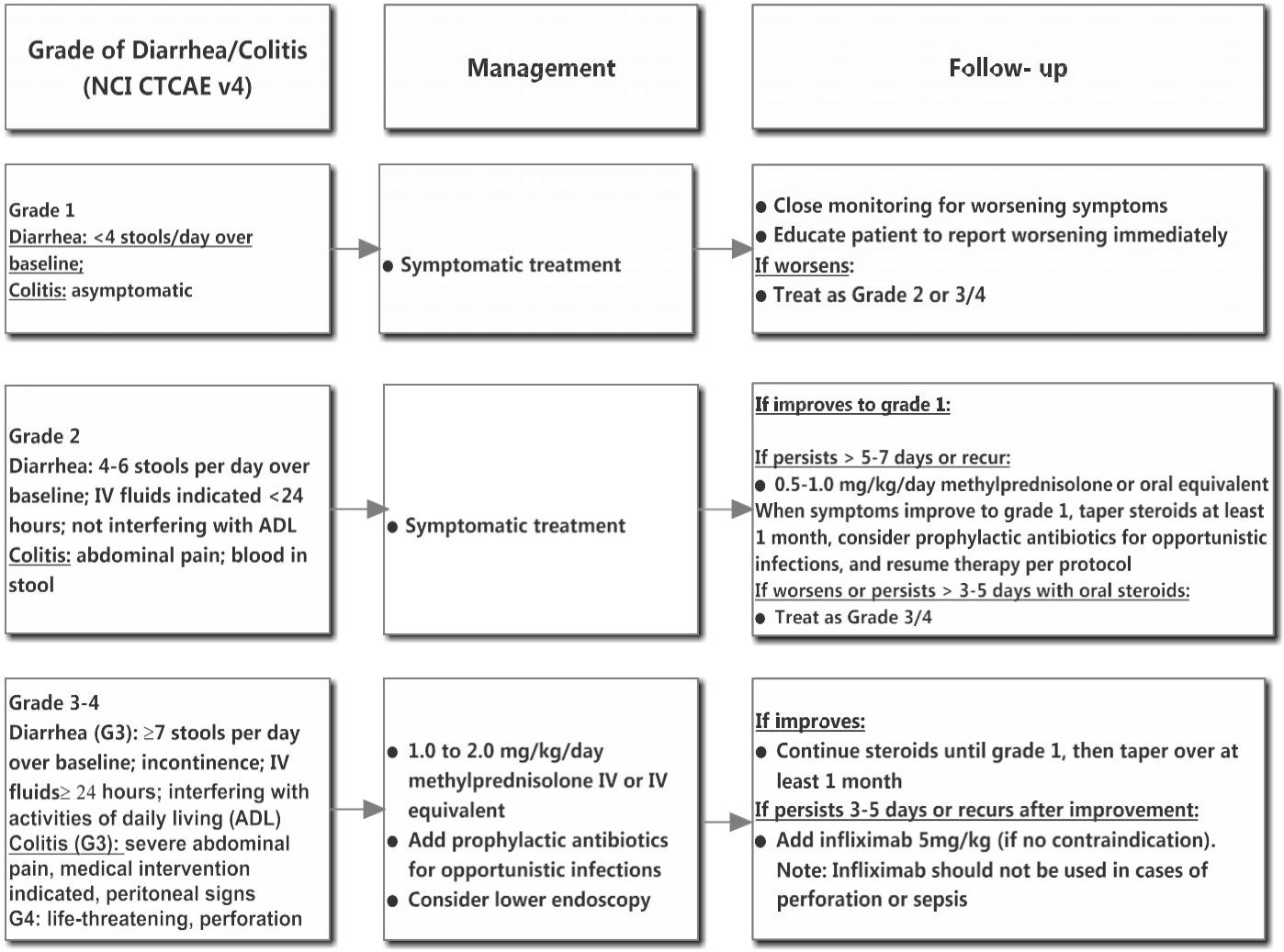
A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated.

Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is recommended.

GI ADVERSE EVENT MANAGEMENT ALGORITHM

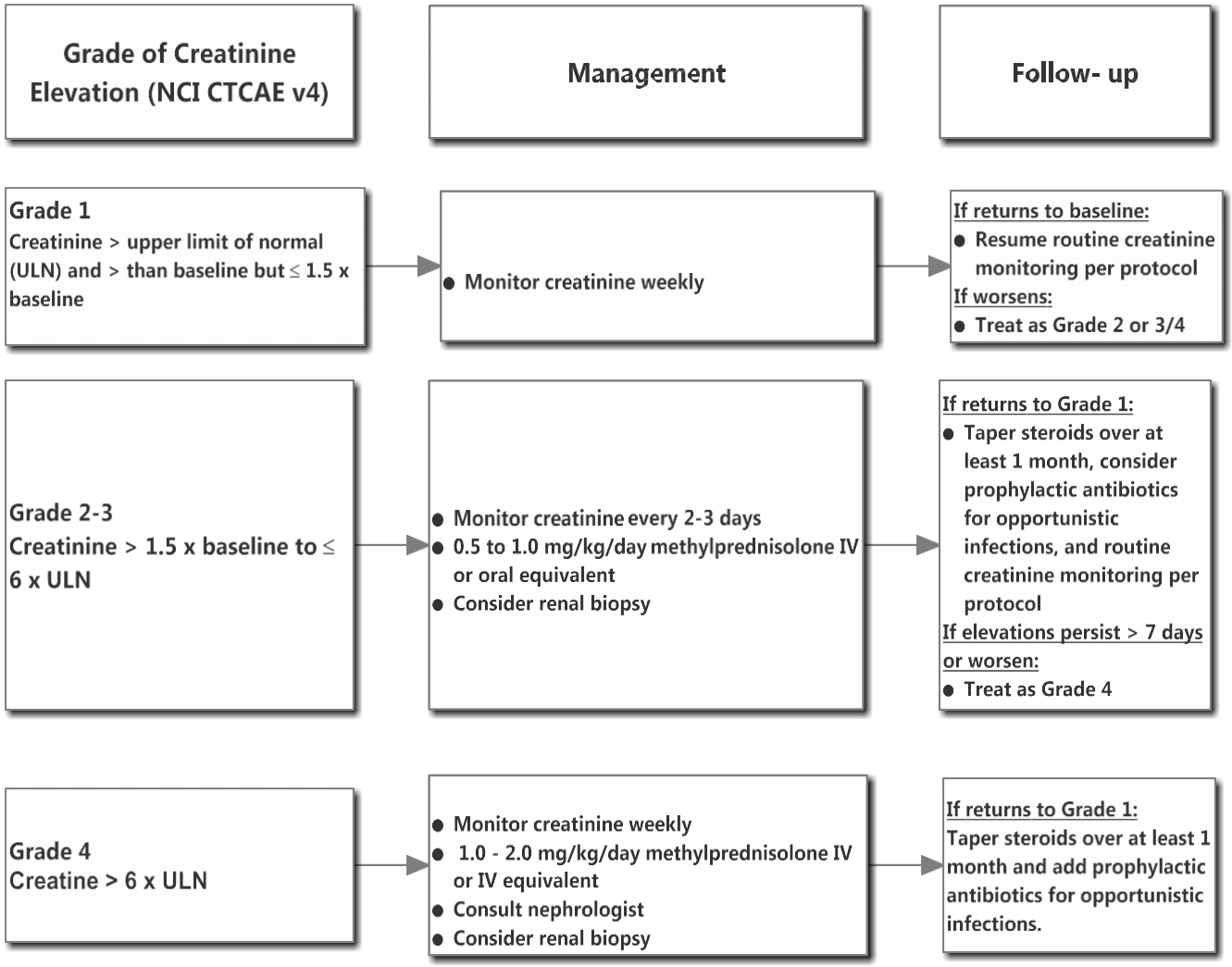
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

RENAL ADVERSE EVENT MANAGEMENT ALGORITHM

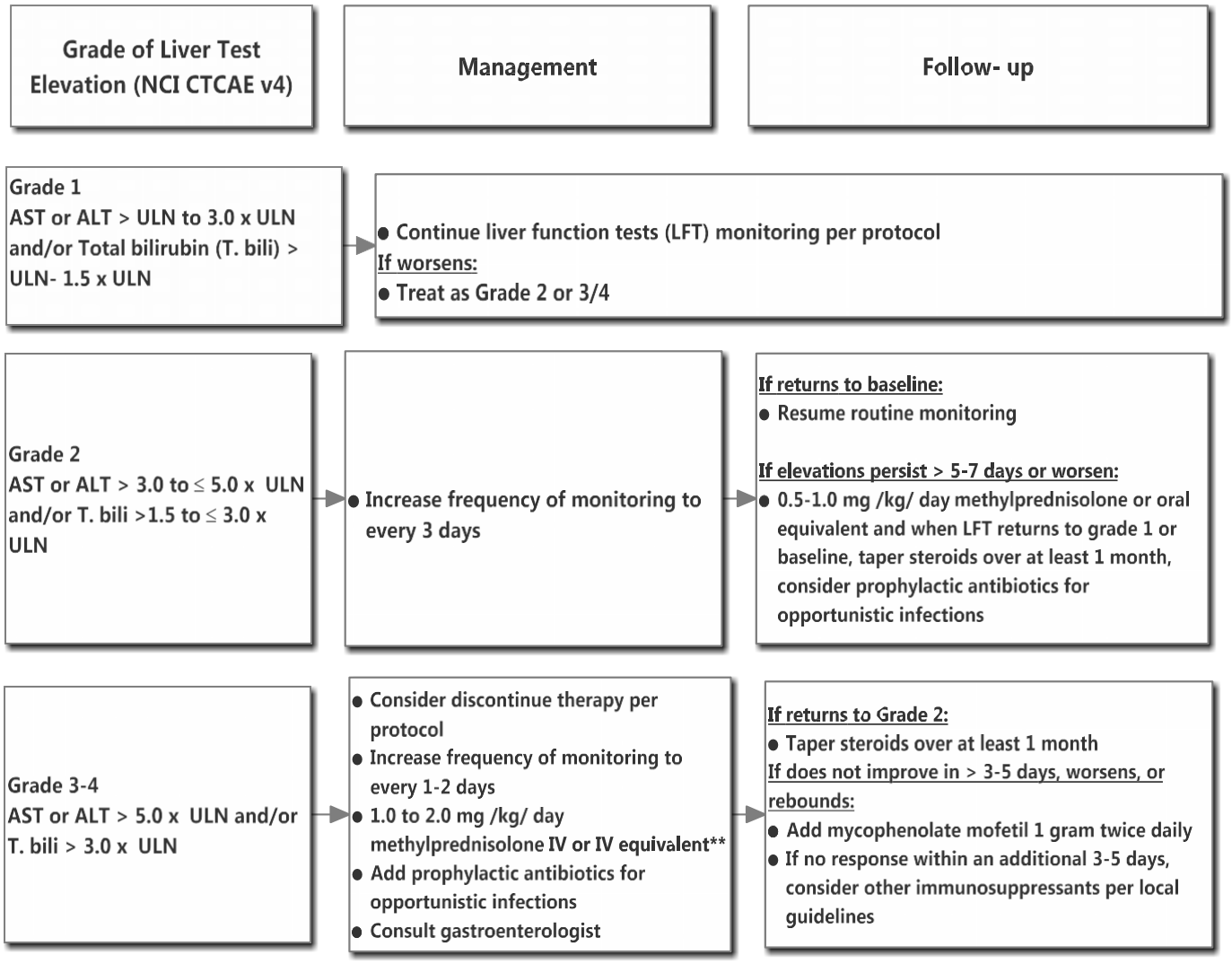
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue therapy



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

HEPATIC ADVERSE EVENT MANAGEMENT ALGORITHM

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue therapy. Consider imaging for obstruction.

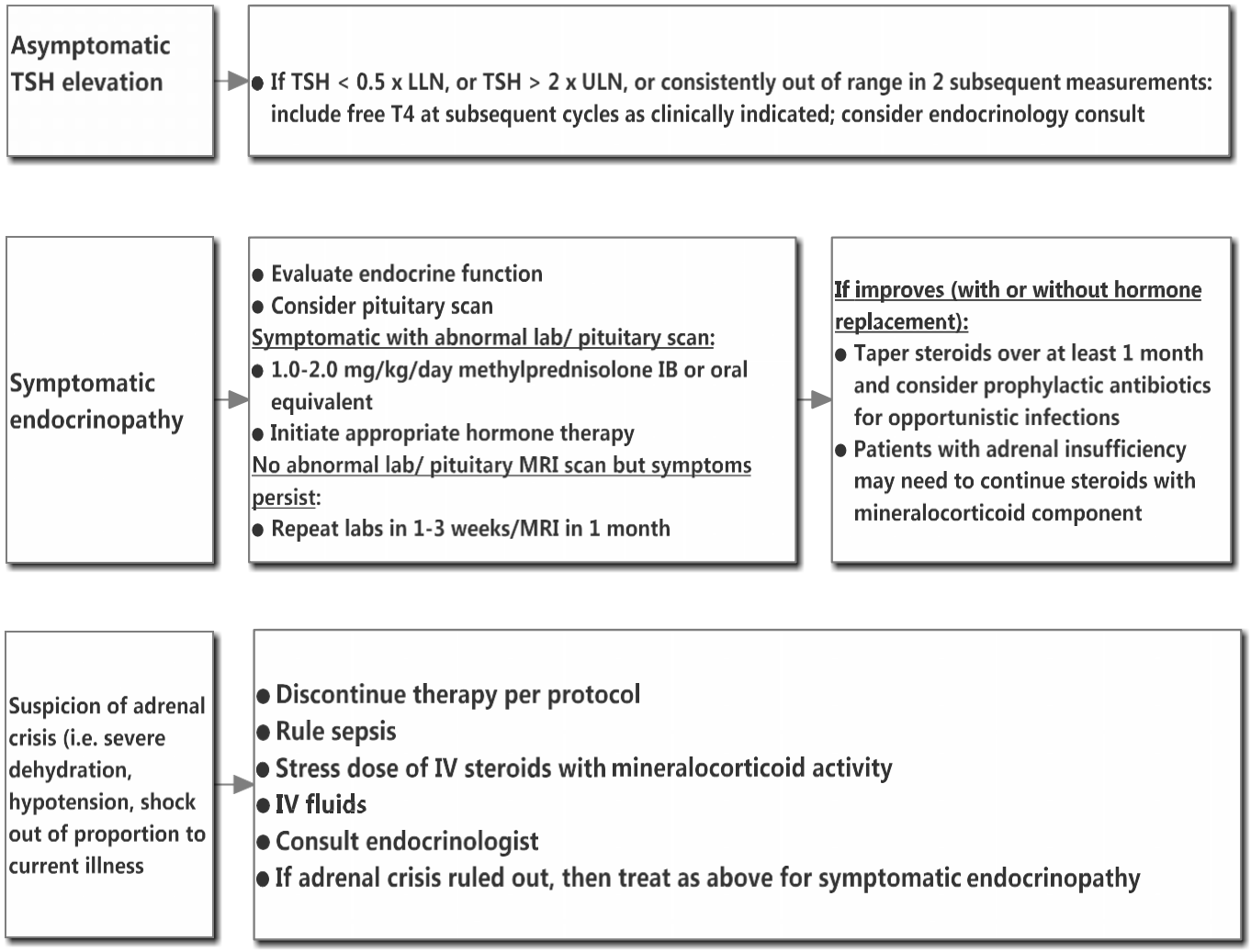


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

** The recommended starting dose for grade 4 hepatitis is 2mg/kg/day methylprednisolone IV.

ENDOCRINOPATHY MANAGEMENT ALGORITHM

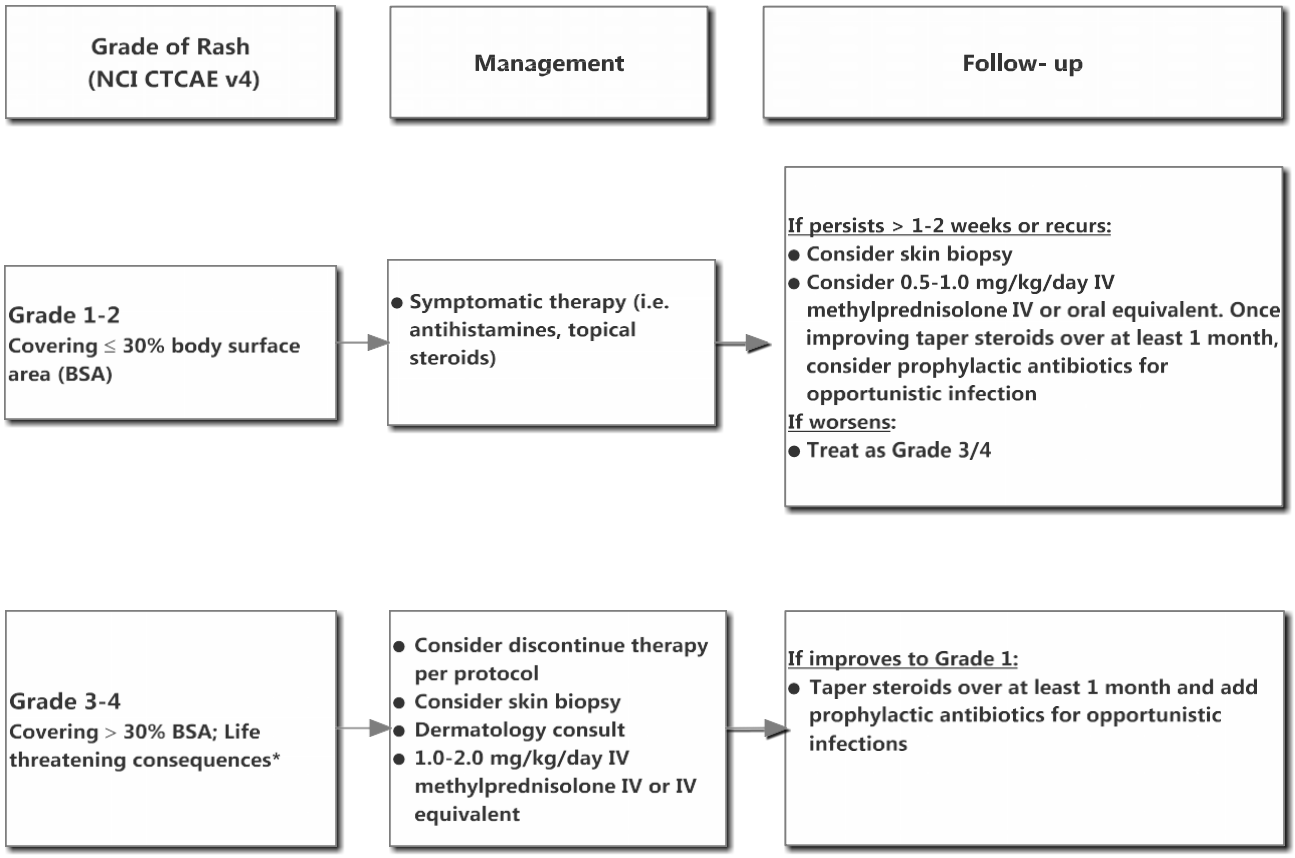
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue therapy. Consider visual field testing, endocrinology consultation, and imaging.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

SKIN ADVERSE EVENT MANAGEMENT ALGORITHM

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue therapy.

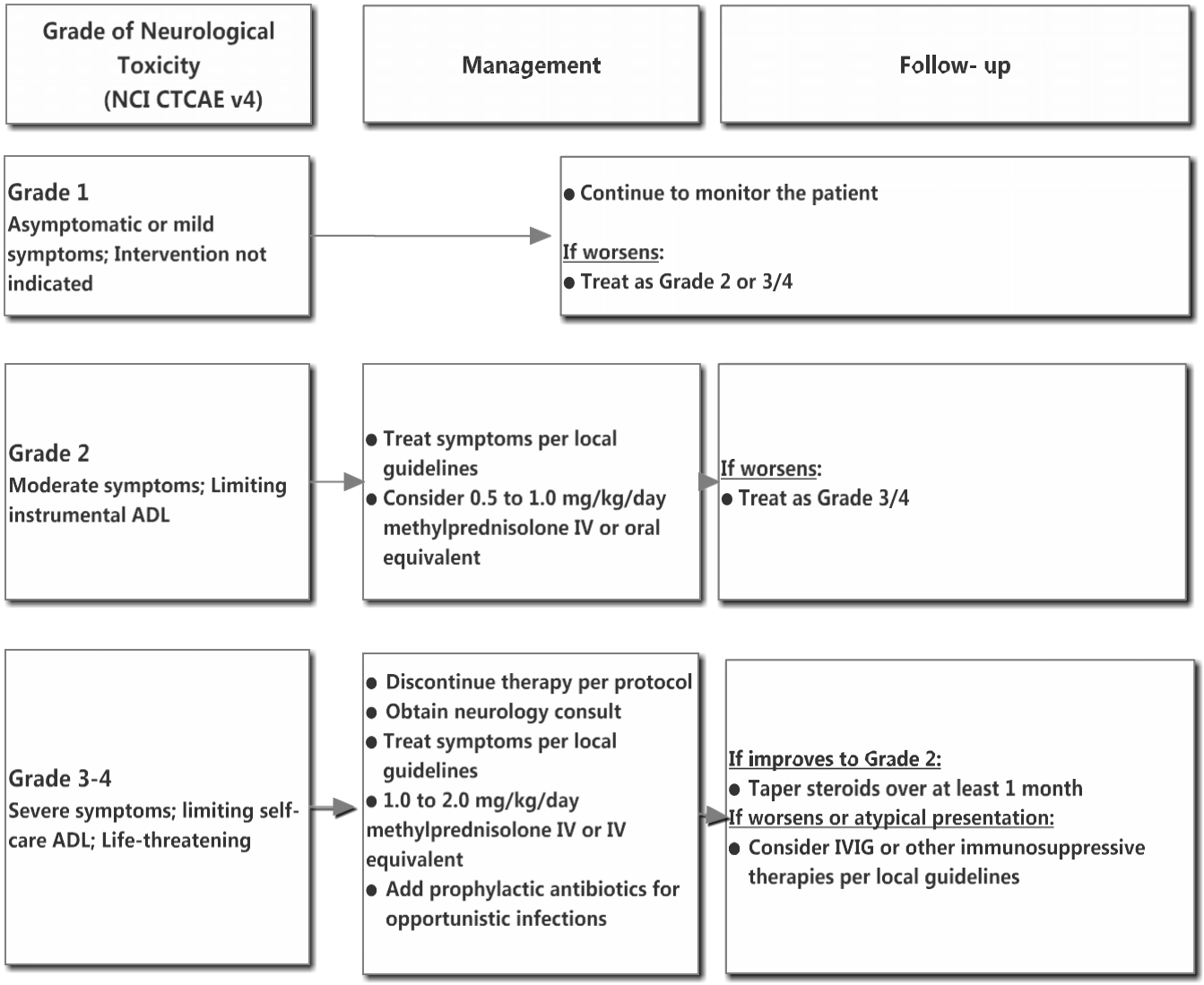


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*Refer to NCI CTCAE v4 for term specific grading criteria.

NEUROLOGICAL ADVERSE EVENT MANAGEMENT ALGORITHM

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.