MCC-15-11083

T-Cell Immune Checkpoint Inhibition Plus Hypomethylation for Locally Advanced HER2-Negative Breast Cancer - A Phase 2 Neoadjuvant Window Trial of Pembrolizumab and Decitabine Followed by Standard Neoadjuvant Chemotherapy

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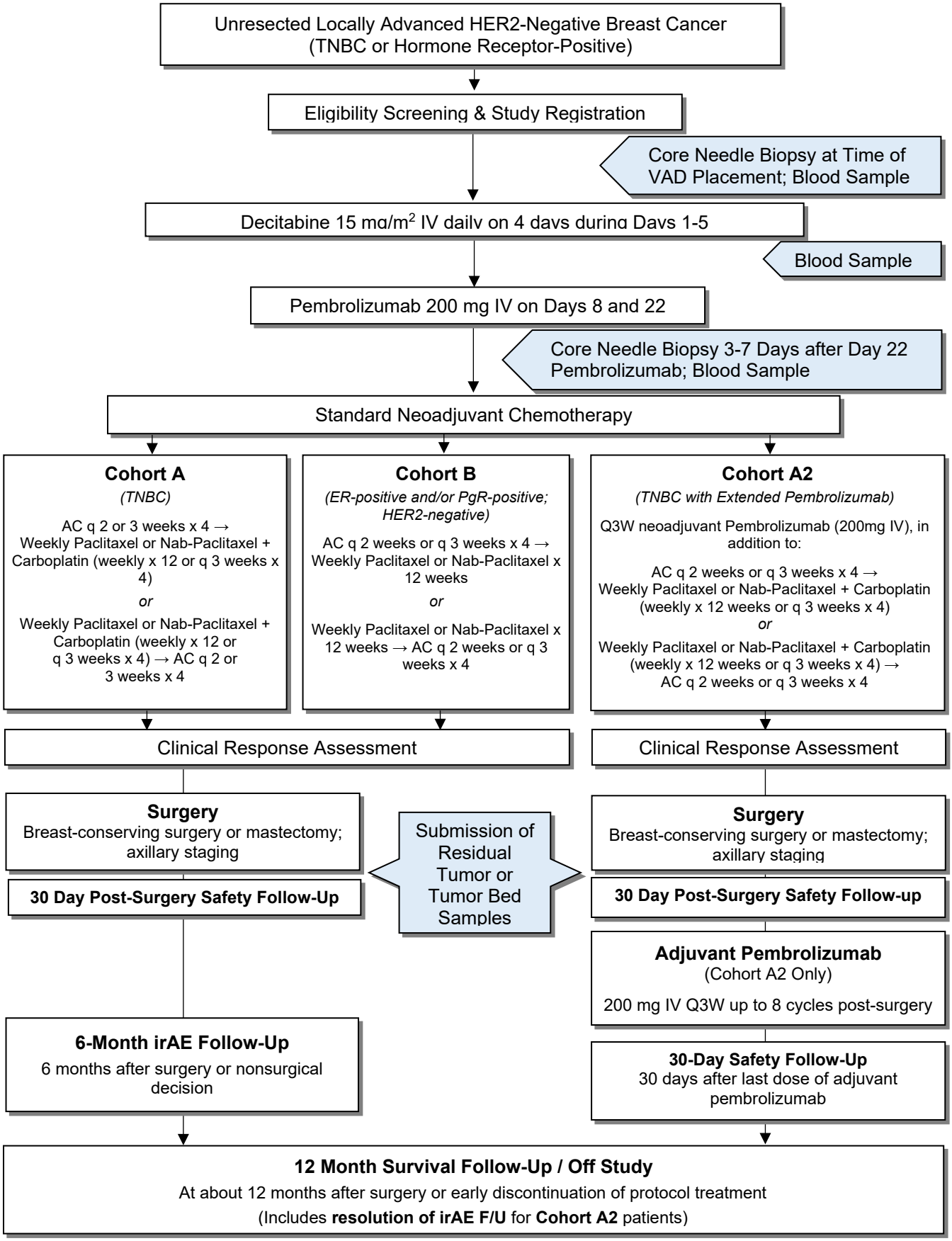
LIST OF ABBREVIATIONS

AC	doxorubicin and cyclophosphamide
AE	adverse event
AEOSI	adverse events of special interest
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
BCT	breast-conserving therapy
CBC	complete blood count
CR	complete response
CrCL	creatinine clearance
CRF	case report form
CTA	cancer testis antigens
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	cytotoxic T lymphocyte-associated protein 4
CTRL	Clinical and Translational Research Laboratory
DNAMTi	DNA methyltransferase inhibitors
DSMC	Data and Safety Monitoring Committee
ECI	event of clinical interest
EDV	early discontinuation visit
ER	estrogen receptor
FDA	Food and Drug Administration
H&E	hematoxylin and eosin
HER2	human epidermal growth factor receptor 2
HR	hormone receptor
IHC	immunohistochemistry
INR	international normalized ratio
irAE	immune-related adverse event
IRB	Institutional Review Board
LPBC	lymphocyte-predominant breast cancer
MCC	Massey Cancer Center
MDS	myelodysplastic syndrome
MDSC	myeloid-derived suppressor cell
MISP	Merck Investigator Studies Program
NCI	National Cancer Institute
NSAID	non-steroidal anti-inflammatory drug
pCR	pathologic complete response
PD-1	programmed cell death-1
PgR	progesterone receptor
PRMC	Protocol Review and Monitoring Committee
RCB	Residual Cancer Burden
SAE	serious adverse event
SAR	suspected adverse reaction
SJS	Stevens-Johnson Syndrome
SSAR	serious suspected adverse reaction
TEN	toxic epidermal necrolysis
TIL	tumor-infiltrating lymphocyte
TNBC	triple-negative breast cancer
TRegs	regulatory T cells
UP	unanticipated problem
VAD	vascular access device
VCU	Virginia Commonwealth University
WCBP	woman of child-bearing potential

STUDY SCHEMA



1 BACKGROUND

1.1 Immunotherapy of Cancer – Promise and Limitations

Over the past 4 decades, the promise of immunotherapy for treating cancer has waxed and waned, but has recently enjoyed a dramatic renaissance of enthusiasm. For many years, the successes of manipulating the immune response to induce regression of cancers were largely limited to murine models, human melanoma, and renal cell carcinoma, with little cause for optimism in the treatment of epithelial malignancies, which are the most common causes of cancer death (4-6).

Immunotherapeutic approaches can be roughly divided into 2 main categories – active and passive. Active immune therapies are aimed at stimulating and activating the host's own immune system to recognize and destroy cancer cells. This category includes vaccines, infusion of immunologically active cytokines, and altering the tumor itself to make it more immunogenic. Passive immunotherapy refers to treatments that involve infusing antigen-targeted molecules or cells into the host, which then attack the tumor directly, either causing tumor cell destruction or inhibition of tumor growth. These passive approaches include adoptive cellular therapy, such as infusion of T lymphocytes which have been removed from the patient, manipulated and expanded in vitro, and then reinfused into the host, sometimes in combination with recombinant cytokines that encourage expansion of the cells and increase their activity (7). For example, tumor-infiltrating lymphocytes grown from biopsy samples of melanoma tumors have induced dramatic regression in patients with metastatic disease. More recently, T cells genetically modified to express chimeric antigen receptors (CAR T cells) created from antibody-antigen combining regions and T-cell signaling molecules have been used successfully to treat hematologic malignancies (8-10). Monoclonal antibodies, either alone or linked to cytotoxic molecules, are another example of passive immunotherapy that has had considerable success (11). To a varying degree, monoclonal antibodies that recognize cancer cell surface targets may be both passive and active, in the sense that antibodies bound to cancer cells can directly inhibit signaling by the target molecule and can also trigger host immune cells (eg, natural killer [NK] cells) to attack and kill the cancer cells (12) (13).

The most recent wave of excitement about immunotherapy has been generated by what may be considered a "hybrid" form of therapy that is both passive and active. This approach, termed "immune checkpoint inhibition", uses monoclonal antibodies to inhibit mechanisms that suppress T-cell activation and function, and will be discussed in more detail below (14) (15).

1.2 Obstacles/Escape from Immunotherapy

The success of immunotherapy for cancer has been hampered by a variety of highly effective escape mechanisms that allow cancers to avoid immune destruction. These obstacles, in fact, have been added to the so-called "hallmarks" that characterize many cancers (16). The escape mechanisms include: lack of non-self antigens, immunoediting or loss of immunogenic tumor antigens, low expression of major histocompatibility complex (MHC) molecules required for antigenic peptide epitope presentation, secretion of immunosuppressive cytokines (eg, TGF-β, interleukin 10 (IL-10), vascular endothelial growth factor [VEGF]), induction/infiltration of suppressor cells (myeloid-derived suppressor cells [MDSCs]), T regulatory cells [Tregs]), and stimulation of immune

checkpoint molecules on T cells (PD-1, CTLA-4) (14, 15, 17-26). Overcoming these obstacles will likely be necessary for host immunity to have a significant impact on cancer.

1.3 Evidence for Role of Host Immune Response in Breast Cancer

Although immune therapies for breast cancer have had limited success, with the prominent exception of monoclonal antibodies targeting HER2 (11), evidence of the importance of host immune responses to breast cancer has been available for at least 4 decades. For example, Di Paola et al reported more than 40 years ago that lymphocyte infiltration in breast cancers and signs of immune activation in the regional lymph nodes were highly predictive of better patient outcomes (27). More recently, immunohistochemical (IHC) assessment of breast cancers has shown that CD8+ T cells infiltrating the tumor or stroma of breast cancers was predictive of a better prognosis (28-31). We have also identified a 5 immune-related gene signature based on RNA microarrays that identifies patients with a high likelihood of not experiencing a recurrence (32).

Moreover, lymphocytic infiltration of breast cancers and associated gene expression profiles, assessed using core biopsy tumor samples, have been shown to correlate strongly with responses to neoadjuvant chemotherapy, as well as the benefit of adding a platinum compound to the regimen of neoadjuvant chemotherapy for triple-negative breast cancer (TNBC) (33-36) (Figure 1 and Figure 2).

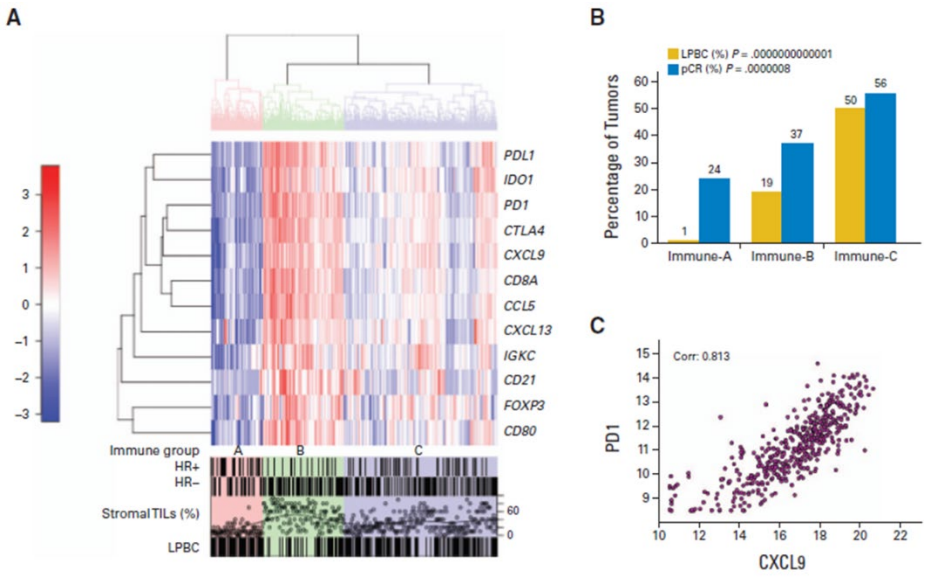


Figure 1. Expression Patterns of Immune-Related Genes Correlate with Lymphocyte Infiltration

In 481 tumors from GeparSixto: A. Hierarchic clustering of 12 immunologically relevant genes showed 3 different immune groups of tumors with different expression of immunologic genes and different amounts of tumor-infiltrating lymphocytes (TILs). Immune Group A - low expression of all immune genes; immune group B - intermediate expression levels; immune group C - high expression of immunologic genes. Corresponding levels of stromal TILs for each tumor as well as lymphocyte-predominant breast cancer (LPBC) status is shown in lower section of panel B. Evaluation of CXCL9 and PD-1 mRNA levels showed positive correlation (corr) between both markers (Pearson correlation coefficient, 0.813). C. Three immune clusters were significantly different for percentage of LPBC tumors and pathologic complete response (pCR) rate.

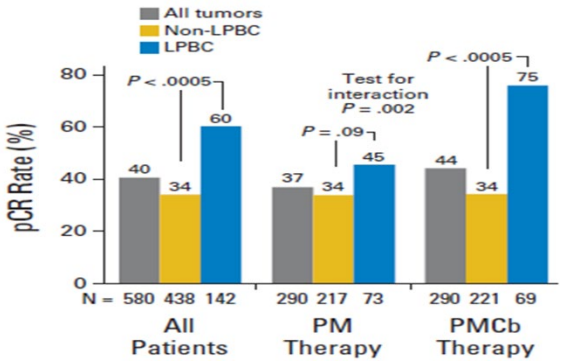


Figure 2. Lymphocyte-Predominant Breast Cancers have Higher pCR Rates

Pathologic complete response (pCR) rates increased in tumors with LPBC phenotype, compared with tumors with non-LPBC phenotype in the GeparSixto cohort. (LPBC: lymphocyte-predominant breast cancer; PM: nonpegylated liposomal doxorubicin; PMCb: PM with carboplatin)

1.4 Immunotherapeutic Approaches to Breast Cancer

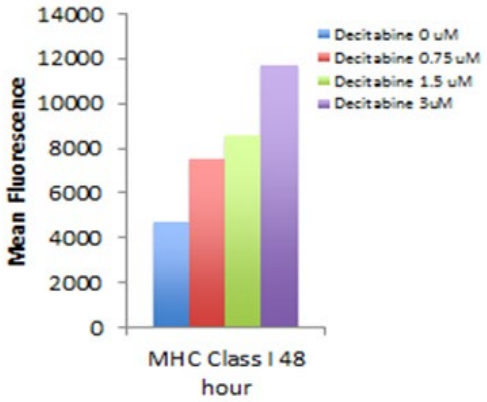
Until recently, immunological approaches to breast cancer therapy have focused on vaccines targeting HER2, mammaglobin, telomerase reverse transcriptase, MUC-1, and other potential breast cancer-specific antigens. There have certainly been "successes" in the sense of inducing immune responses, but the clinical gains have been modest, at best (37). Recently, evidence of clinical activity against breast cancer using immune checkpoint inhibiting agents has been reported (38-42). While response rates have been in the mid- to low teens, these studies, along with the immunological correlates with prognosis and response to chemotherapy, suggest the potential of these agents to have a positive impact, especially when combined with other immunologic manipulations.

1.5 DNAMTi Can Overcome Resistance to/Escape from Immunotherapy

A large number of studies, including research from our laboratories, have demonstrated that DNA methyltransferase inhibitors (DNAMTi) exert an array of effects that may increase the likelihood of responses to immune therapies (29, 43-49). Immunoediting, or the elimination through genetic or epigenetic changes of tumor antigen expression with a reduction in immunogenicity, is a normal process that cancer cells undergo in order to evade and escape the immune system (18). Epigenetic alterations include excessive deacetylation of histones as well as excessive methylation of the genome, thereby silencing or inactivating numerous genes that are involved in tumor suppression, apoptosis, antigen presentation, and those that serve as tumor antigens in dedifferentiated cells (50) (51). Indeed, loss of HER2/neu has been shown to be related to genome methylation (52). In previous studies, MHC Class I molecules have also been shown to be silenced through this mechanism, diminishing the immune response (51, 53, 54). The DNA methyltransferase inhibitors 5-aza-2'-deoxycytidine (decitabine) and azacitidine are global hypomethylation agents, and it has been suggested that, through liberation of silenced tumor antigens, these agents may increase responses to immunotherapy, including vaccines (43, 54-56).

Strategies utilizing DNA methyltransferase inhibition in combination with immunotherapy have been tested in clinical trials. Odunsi et al recently published their findings that an NY-ESO-1 CTA-targeting cancer vaccine combined with decitabine treatment resulted in enhanced immune responses in advanced platinum-resistant ovarian cancer (44). We have also used this strategy to increase host immune responses to multiple myeloma in patients (57). Also, in our center, it has been shown in a HER2 transgenic murine breast cancer model that antigen-negative variants (ANV) that have lost expression of HER2 emerge as recurrences after immune rejection of HER2-expressing tumors. Moreover, treatment of tumor cells from these ANV tumors with decitabine in vitro restored HER2 expression (52).

Tumors can also escape from immune attack by inducing immunosuppressive responses in the form of an increase in splenic and circulating MDSCs and regulatory T cells (58-60). Recruitment of MDSCs by tumors has also been shown to be altered by demethylating agents and to be highly dependent on methylation (43). In our own laboratory we have demonstrated that decitabine treatment of 4T1 mammary carcinoma cells increased expression of tumor antigens (HER2 and cancer testis antigens) and antigen-presenting molecules (MHC Class I) and increased immunogenicity, assessed by interferon-gamma secretion from tumor-sensitized T lymphocytes co-cultured with 4T1 tumor cells (Figure 3, Figure 4, and Figure 5).



MHC Class I		
	Decitabine in uM	Mean Fluorescence
48 hour	3	11666
	1.5	8582
	0.75	7537
	0	4670
24 hour	3	6620
	1.5	7154
	0.75	6255
	0	4916

Figure 3. Decitabine Resulted in Increased MHC Class I Expression in 4T1 Cells

MHC: major histocompatibility complex

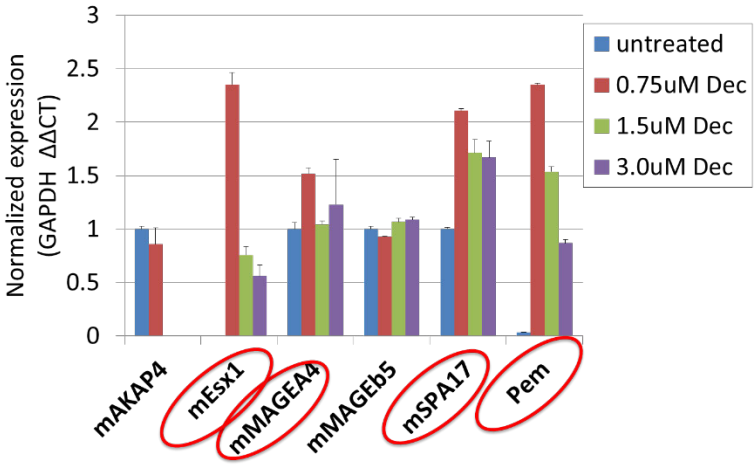


Figure 4. Decitabine Increased 4T1 Expression of Cancer Testis Antigens

Dec: decitabine

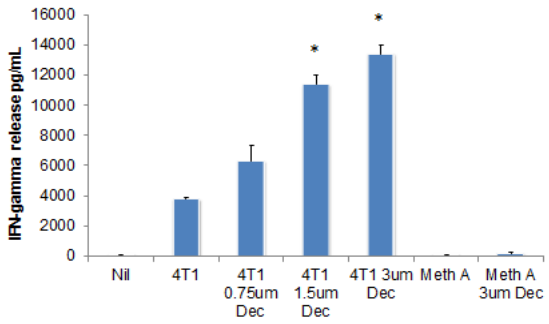


Figure 5. Decitabine Increased Interferon-Gamma Release Compared to Untreated 4T1 Cells

In vivo treatment of 4T1 tumor-bearing syngeneic mice dramatically decreased the tumor-induced MDSC burden in spleen, liver, and peripheral blood, and increased the efficacy of adoptive immunotherapy with T cells (61) (Figure 6 and Figure 7).

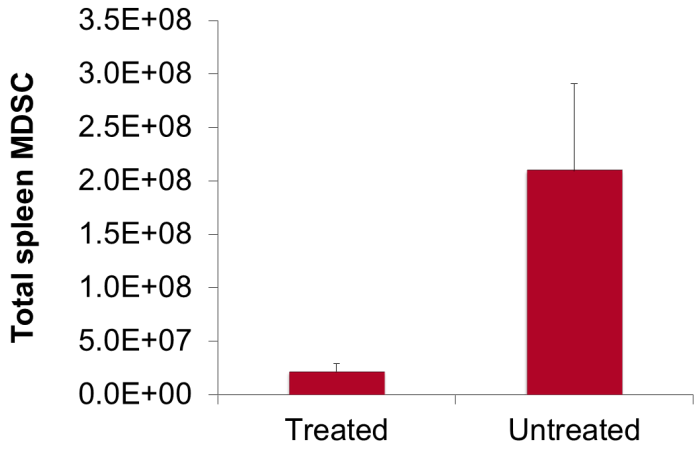


Figure 6. Decitabine Decreased Total Spleen MDSCs in 4T1 Tumor-Bearing Mice

MDSC: myeloid-derived suppressor cell

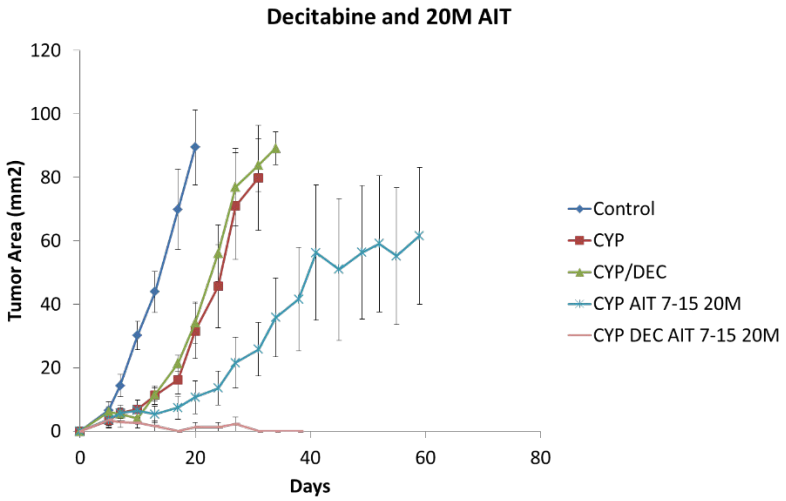


Figure 7. Decitabine Enhances Effects of AIT in Slowing Tumor Growth

Decitabine (DEC) in combination with adoptive immunotherapy (AIT) with T lymphocytes expanded in IL-7 + IL-15 resulted in a significant decrease in 4T1 tumor size compared to AIT alone (CYP: cyclophosphamide).

A group at Johns Hopkins has recently demonstrated that treatment with a combination of azacitidine, entinostat (an HDAC inhibitor), anti-CTLA-4 antibody and anti-PD-1 antibody could cure advanced 4T1 mammary carcinomas in syngeneic mice (62). They attributed an effect on MDSCs as the major mechanism accounting for this therapeutic effect. Interestingly, in contrast to our results with decitabine, they did not observe a significant effect on MDSCs with azacitidine alone in this murine model.

Luo et al showed that treatment of breast cancer cell lines with the next-generation hypomethylating agent, guadecitabine, upregulated MHC-I expression in response to interferon-γ (63).

Altered epigenetic regulation including DNA hypermethylation by DNAMT1 has been implicated as one of the causes of TNBC tumorigenesis (64). DNAMT1 expression is associated with poor breast cancer survival, and it is overexpressed in TNBC subtype. DNAMT1 inhibitors exert anti-tumorigenic effects against TNBC cells. This includes the hypomethylating agents azacitidine, decitabine, and guadecitabine that might sensitize TNBC patients to immune checkpoint blockade therapy (64).

1.6 Immune Checkpoint Inhibition

As mentioned above, immune checkpoint pathways, which function to limit autoimmunity under normal circumstances, have been shown to be a significant obstacle to immunologic destruction of cancers. Binding of B-7 on an antigen-presenting cell to CTLA-4 on a T cell recognizing an antigen on the same cell causes inhibition of T-cell activation, rather than stimulation. At the effector phase of T-cell response to a tumor cell, binding of PD-L1 to the programmed death receptor (PD-1) leads to negative regulation of the T-cell response to the tumor antigen. Monoclonal antibodies to these receptors have now been shown to induce tumor regressions in a high proportion of patients, dramatically increasing progression-free survival (PFS) compared to standard therapies. This has been demonstrated not only in melanoma, but also in bladder cancer, lung cancer, and, as noted

above, in breast cancer. Indeed, for patients with advanced lung cancer, anti-PD-1 antibody has been shown to be superior to taxane chemotherapy (14, 15, 22-26, 65-68). Toxicities are largely attributable to autoimmune or inflammatory responses, which is not surprising (69). However, the frequency of severe toxicities with anti-PD-1 antibodies has generally been lower than with the anti-CTLA-4 compounds, which were available earlier. For example, in patients with advanced melanoma, the anti-PD-1 nivolumab was superior to ipilimumab (anti-CTLA-4) in terms of PFS as well as toxicity (70). Although some trials of anti-PD-1 agents selected patients whose tumors were positive for PD-L1 for inclusion, it is not clear that baseline expression of PD-L1 is an obligate predictor of benefit from anti-PD-1 therapy (67) (66). This may be because, as has been shown for 4T1 murine mammary carcinoma, immune attack actually induces PD-L1 expression by the tumor cells, which do not express this cell surface marker in vitro (71).

Based on the information reviewed here, we hypothesize that combined treatment of breast cancer patients with a DNAMT inhibitor and anti-PD-1 antibody will:
1) increase the lymphocytic infiltration into primary breast cancers; 2) alter the gene expression pattern in primary breast cancers to a pattern more indicative of CD8+ T-cell responses; 3) increase tumor antigen expression in the tumor; 4) decrease infiltration of breast cancer with immunosuppressive cells, such as MDSCs and Tregs; and 5) increase the likelihood of pCR after the administration of standard neoadjuvant chemotherapy.

1.7 Proposed Immunotherapy Regimen

1.7.1 Decitabine

1.7.1.1 Initial Overview

Decitabine, a nucleoside metabolic inhibitor, was approved by the Food and Drug Administration (FDA) in 2006 for treatment of patients with myelodysplastic syndromes (MDS). Decitabine hypomethylates DNA by inhibiting DNA methyltransferase.

The most commonly occurring adverse reactions observed following administration of decitabine in MDS clinical trials include neutropenia, thrombocytopenia, anemia, fatigue, pyrexia, nausea, cough, petechiae, constipation, diarrhea, and hyperglycemia. However, in this study, patients do not have impaired bone marrow function prior to therapy and they will receive a short course of decitabine (only one cycle of decitabine administered once daily for a total of 5 doses). Therefore, we anticipate that decitabine will be tolerated well with very few AEs.

1.7.1.2 Rationale for Revised Decitabine Dosing

The initial MCC-15-11083 study design specified that decitabine 20 mg/m^2/day would be administered on 5 consecutive days (100 mg/m^2 total) during the first week of neoadjuvant study therapy. Grade 3 or 4 neutropenia was observed in 3 of the first 5 patients during the weeks following administration of decitabine. No infectious complications occurred during the neutropenia in these patients, but to minimize the risk of infection, maintain the study-specified treatment schedule, and avoid

potential delays in initiating chemotherapy, the decision was made in Protocol Version 6 to decrease the decitabine dosing to 15 mg/m^2 and the number of decitabine doses from 5 to 4 for a total dose of 60 mg/m^2.

1.7.2 Pembrolizumab

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4, which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail, which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ, and ZAP70, which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T cells, B cells, Tregs, and NK cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in patients with melanoma. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda (pembrolizumab) is approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor. Examples of additional indications include non-

small cell lung cancer (NSCLC), head and neck squamous cell carcinoma, classical Hodgkin lymphoma, primary mediastinal large B-cell lymphoma, urothelial carcinoma, microsatellite instability-high cancer, and gastric/gastroesophageal junction cancer.

Adverse reactions that have been reported in at least 20% of patients with melanoma receiving pembrolizumab include fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia, and diarrhea. As described in Section 9.2.13 immune-mediated reactions (eg, immune-mediated pneumonitis, colitis, hepatitis, hypophysitis, nephritis, hyperthyroidism, and hypothyroidism) can occur.

1.7.3 Safety Lead-in Phase

1.7.3.1 Original Safety Lead-in Plan

In this study, a fixed dose of 200 mg of pembrolizumab will be administered on 2 treatment days following a short course of decitabine (a total of 5 doses administered on 5 consecutive days). We anticipate that there will be very few AEs associated with the planned immunotherapy in this neoadjuvant window trial. However, a safety lead-in phase will be conducted in the initial 6 patients enrolled in the study for identification of any clinically significant immune-related adverse events (irAEs) that occur during immunotherapy and the dose-dense AC portion of neoadjuvant chemotherapy (see Section 3.2).

1.7.3.2 Extended Safety Lead-in Plan

One of the first 5 patients treated during the safety lead-in phase developed immune-related Guillain-Barré syndrome. Also, in the first 5 patients, 2 developed grade 3 neutropenia and 1 developed grade 4 neutropenia. Based on the observed neutropenia, a decision was made to reduce the total dose of decitabine from 100 mg/m^2 delivered on 5 days to 60 mg/m^2 delivered on 4 days.

In view of these toxicities, the decision was made to extend the safety lead-in by 5 additional patients to a total of 11 patients. The number of additional patients required was solved so that the 5 additional patients to the 6 already planned would yield an 80% likelihood that the posterior probability that the irAE rate was less than 30%. The posterior probability of the irAE rate was modeled as a beta distribution with parameters (1+X_{new}) and (9-X_{new}), where X_{new} corresponds to the number of irAEs in the remaining 6 patients to be enrolled. The prior distribution for the irAE rate was defined as a beta distribution with parameters 1 and 4, corresponding to the information obtained from the first 5 patients.

1.8 Rationale for the Neoadjuvant Chemotherapy Regimens

In HER2-negative breast cancer, it is standard practice to administer an anthracycline, an alkylating agent, and a taxane during neoadjuvant treatment (72). One of the most commonly used regimens is doxorubicin/cyclophosphamide (AC), followed by paclitaxel

(73). AC will be administered every 2 or 3 weeks with weekly paclitaxel or Nab-paclitaxel following completion of AC.

Bines et al reviewed 15 ongoing or retrospective clinical trials in which nearly 5000 patients with breast cancer were treated with a taxane followed by AC in at least 2 of the study arms in either the neoadjuvant or adjuvant therapy setting. No disadvantages in efficacy or toxicity of administering the taxane prior to AC were identified (74). Therefore, at the treating medical oncologist's discretion, the chemotherapy sequence may be reversed by administering AC after completion of the weekly paclitaxel or Nab-paclitaxel regimen (75).

The TNBC cohort will include the addition of carboplatin to paclitaxel or Nab-paclitaxel. In TNBC, there is emerging evidence that the administration of carboplatin may increase the pCR rate following neoadjuvant chemotherapy, which is generally associated with improved survival outcomes (76-78). Additionally, it has been shown by Denkert et al that the addition of carboplatin to neoadjuvant anthracycline/taxane-based chemotherapy significantly improved pCR rates in tumors with a positive immune score and TILs (34).

1.9 Rationale for Addition of Extended Pembrolizumab in Cohort A (Added with Version 10)

The results of the Keynote-522 trial (Funded by Merck Sharp & Dohme [a subsidiary of Merck]; ClinicalTrials.gov NCT03036488) showed that among patients with early TNBC, the percentage with a pCR was significantly higher among those who received pembrolizumab plus neoadjuvant chemotherapy than among those who received placebo plus neoadjuvant chemotherapy (1). Recently, the addition of pembrolizumab was also reported to increase event-free survival compared to placebo (79). Based on these results, the inclusion of pembrolizumab with neoadjuvant chemotherapy and continuation of pembrolizumab as postoperative adjuvant therapy was added with Protocol Version 10 as a treatment option for Cohort A. This extended pembrolizumab regimen has recently been approved by the US FDA (Pembrolizumab [Keytruda] prescribing information, dated August 2021).

Beginning with Protocol Version 11, patients receiving this extended pembrolizumab regimen will be designated as Cohort A2. This cohort was created to facilitate increased clarity in assessment schedules, analysis populations, and overall study schema. At sites active on Protocol Version 10 or later, TNBC patients initially registered to Cohort A may be reassigned to Cohort A2 per investigator discretion. Reassignment to Cohort A2 must be documented before initiating standard neoadjuvant chemotherapy on study.

1.10 Rationale for Revised Definition of Hormone Receptor Status

MD Anderson Cancer Center investigators reported clinical outcomes of primary breast carcinomas with complete information on ER status in a retrospective study of 9639 patients treated between January 1990 and December 2011. Patients were separated into 3 groups based on the percentage of tumor cell nuclei with positive staining for ER. The largest group of 7764 patients (80.5%) had ≥ 10% positive staining, while 250 (2.6%) had 1-9% positive staining and 1625 (16.9%) had < 1% staining. At a median follow-up of 5.1 years, the patient cohorts with tumors with 1-9% ER staining or < 1% ER staining had worse distant recurrence-free survival ($P < 0.0001$), recurrence-free survival ($P < 0.0001$) and overall survival ($P < 0.0001$) rates compared with patients with ≥ 10% ER staining. Patients with 1-9% ER staining had similar distant recurrence-free, recurrence-free, and

overall survival rates (P = 0.8), (P = 0.96) and (P = 0.1), respectively, as patients with < 1% ER staining (80).

A more recent study using the PAM50 gene signature to determine intrinsic subtypes in 60 ER weakly positive (Allred scores 3-5) breast cancers and 88
ER-negative (Allred scores 0-2) breast cancers were evaluated in a recently reported retrospective study. Among the breast cancers with lower proportion and intensity of staining for ER, only 6 (10%) were of luminal subtype, 24 (40%) were HER2 enriched, and 30 (50%) were basal like. The distribution of subtypes in the ER-negative patients were very similar with luminal subtypes in 5 (6%), HER2 enriched in 34 (39%), and basal like in 49 (56%). With a median follow-up of 54 months, no statistical differences in recurrence (p = 0.53) or death (p = 0.41) were identified between the Allred 3-5 and Allred 0-2 groups (81).

In summary, the underlying biology and clinical outcomes of HER2-negative tumors with lower proportion and intensity of staining for ER on IHC (1-9% or Allred scores 3-5) are very similar to those considered negative for ER staining (IHC < 1%, Allred 0-2). Therefore, patients with < 10% staining for ER and PgR by IHC will define the TNBC cohort.

1.11.2.1 Studies Using Tumor Samples

Multiplex fluorescent staining of multiple biomarkers on the same formalin-fixed, paraffin-embedded (FFPE) tumor slide will be performed with the Opal staining system and the Vectra microscope (PerkinElmer). This multiplex approach allows for 6 markers, in addition to DAPI nuclear staining, for each slide. Markers of immune cell subsets including helper T cells (CD4), cytotoxic T cells (CD8), B cells (CD20), and regulatory T cells (FOXP3) can be analyzed along with additional markers of tumoral immune evasion (PD-L1) and cytokeratin (CK) as a marker of epithelial cells to delineate stroma versus tumor. Multiple markers will be analyzed in 2-slide sets as a discovery component of this clinical trial. Biomarkers of interest at this time include the following:

- Subtypes of immune cells, including helper T cells (CD4), cytotoxic T cells (CD8), B cells (CD20), regulatory T cells (FOXP3), and MDSCs (CD33+/HLA-DR low/neg) (84)
- Immune checkpoints PD-L1, PD-1, and LAG3

Analysis of CK will be performed on all slides in order to delineate between tumor and stroma for characterization of spatial distribution of immune cells.

Additionally, tissue will be evaluated for analysis of RNA expression, which may predict for a positive immune response. Work by Drs. Ascierto and Bear identified gene expression signatures that correlated with improved outcomes in breast cancer patients in retrospective analysis (32, 85). Immunologic gene expression profiles associated with high pCR rates were also described by Denkert et al (34). Evaluation of gene expression signatures, which may be associated with improved outcomes will be analyzed, as well as the evaluation of how epigenetic modulation with decitabine affects these expression signatures.

Unstained FFPE slides from both core needle biopsies and the surgical resection specimen will be sent to QualTek Molecular Laboratories, the laboratory contracted by Merck for their proprietary PD-L1 staining.

A portion of the resected tumor obtained in this study will be sent to a third party laboratory for gene expression analysis to elucidate genes associated with function and modulation of the PD-1/PD-L1 axis. Tumor biopsy FFPE samples will be checked for quality, presence of tumor, and overall size/condition by H&E staining. Sufficient material, about 5 slides on average, will be sent to Almac for RNA isolation.

A set of more than 680 genes focused largely on genes known to be involved in T-cell biology, immune regulation, cellular markers of TILs, and tumor-associated macrophages will be evaluated on collected samples. Nanostring analysis is particularly well suited to gene expression studies from FFPE material where RNA degradation due to cross-linking results in less than optimal results from conventional gene expression platforms (eg, RTqPCR, microarrays), which require enzymatic steps to create cDNA or cRNA. The Nanostring Counter System, in contrast, provides direct binding and quantitation of mRNA species, and can be used to obtain high quality data from partially degraded (FFPE) samples, which can in turn be correlated with findings by IHC and routine histopathologic analysis.

1.11.2.2 Studies Using Blood Samples

Blood samples will be collected at 3 time points: baseline, post-decitabine, and post-pembrolizumab. These samples will be used for the evaluation of circulating MDSCs by flow cytometry $CD33^{+}$, $CD11b^{+}$, $HLA\text{-}DR^{low/neg}$, CD15, and CD14.

2 OBJECTIVES

2.1 Primary Objective

To determine and quantify if treatment with neoadjuvant decitabine followed by pembrolizumab increases lymphocyte infiltration into tumor and/or stroma in patients with locally advanced, HER2-negative breast cancer

2.2 Secondary Objectives

2.2.1 To evaluate the safety and toxicity of sequential decitabine plus pembrolizumab followed by AC, weekly paclitaxel (or paclitaxel plus carboplatin) or Nab-paclitaxel (or Nab-paclitaxel plus carboplatin) administered as neoadjuvant therapy

In patients with locally advanced, HER2-negative breast cancer who have received neoadjuvant decitabine and pembrolizumab:

2.2.2 To determine if the study treatment increases the proportion of tumors with ≥ 60% tumor or stromal area infiltrated with lymphocytes (ie, LPBC)

In patients with locally advanced, HER2-negative breast cancer who have received neoadjuvant decitabine and pembrolizumab followed by a standard neoadjuvant chemotherapy regimen:

2.2.3 To determine the rate of pCR in the breast and lymph nodes (pCR breast and nodes)

2.2.4 To determine the rate of Residual Cancer Burden (RCB) Index value of 0-1 (Section 10.2.3) following all neoadjuvant therapy

2.2.5 To determine the rate of clinical complete response in the breast and lymph nodes (cCR breast and nodes) following all neoadjuvant therapy

2.2.6 To characterize the alteration of T lymphocyte and other host cell infiltration and immune response gene signatures in breast cancers resulting from treatment with decitabine and pembrolizumab

2.2.7 To evaluate the correlation of pre-existing and post-immunotherapy immune response signatures with response to neoadjuvant chemotherapy

2.2.8 To correlate the Merck proprietary PD-L1 assay results with response to therapy

2.2.9 To evaluate the level of circulating MDSCs at baseline, following treatment with decitabine alone, and following treatment with pembrolizumab administered after decitabine

2.2.10 To examine the PFS rate at 12 months (±30 days) following surgery or the decision not to undergo surgery

3 STUDY DESIGN

3.1 General Description

This study is a 2-cohort, open-label, multicenter, phase 2 study of a short course of immunotherapy consisting of sequential decitabine followed by pembrolizumab administered prior to a standard neoadjuvant chemotherapy regimen for patients with locally advanced HER2-negative breast cancer. (Refer to Section **Error! Reference source not found.** for the definition of HER2-negative breast cancer.) The primary efficacy objective is to determine if the immunotherapy increases the presence and percentage of tumor and/or stromal area of infiltrating lymphocytes prior to initiation of standard

neoadjuvant chemotherapy. At enrollment, patients will be assigned to one of 2 cohorts based on hormone receptor status.

- Cohort A - patients with HER2-negative, hormone receptor-negative breast cancer (defined as both ER and PgR with < 10% positive staining on IHC)

  Note: **before beginning standard neoadjuvant chemotherapy**, patients in Cohort A may be reassigned to Cohort A2 to receive extended pembrolizumab as part of new standard neoadjuvant and postoperative adjuvant therapy. See Section 5.1.3.

- Cohort B - patients with HER2-negative, hormone receptor-positive breast cancer (defined as either ER or PgR with ≥ 10% positive staining on IHC)

Both cohorts will receive the identical doses and treatment schedules of decitabine and pembrolizumab followed by a standard neoadjuvant chemotherapy regimen. Both cohorts will receive 4 cycles of AC and 12 doses of weekly paclitaxel or Nab-paclitaxel. Paclitaxel or Nab-paclitaxel will be combined with carboplatin for Cohorts A and A2 (TNBC). The sequence of the 2 regimens will be at the discretion of the treating medical oncologist following the safety lead-in phase (refer to Table 1).

For the primary endpoint, Cohorts A and A2 will be evaluated together, separate from Cohort B. Secondary endpoints will be evaluated as described in Sections 3.7 and 13.4.2.

Note regarding cohort assignment: when patients have multifocal disease with heterogeneous hormone receptor (ER/PgR) status, enrollment cohort assignment may be determined by treating investigator based on his/her assessment of the most clinically relevant hormone receptor phenotype; rationale for this assignment will be documented.

Note regarding option of adjusting standard neoadjuvant regimen: when a treating investigator determines after enrollment that it is in the patient's best interest to omit or add carboplatin to the weekly paclitaxel or Nab-paclitaxel regimen, and that plan differs from the cohort-assigned treatment plan, the treating investigator will confer with the Sponsor-Investigator about the reason for variance from cohort-assigned standard neoadjuvant treatment, and the outcome of the discussion will be documented.

The safety objective will be to evaluate the safety and toxicity of sequential decitabine plus pembrolizumab followed by standard neoadjuvant therapy. If the breast tumor is resectable following completion of all protocol therapy, breast-conserving surgery or mastectomy and axillary surgical staging (either sentinel node biopsy and/or axillary dissection) will be performed. Patients in Cohort A2 will continue to standard adjuvant pembrolizumab treatment.

3.2 Safety Lead-In Phase

A safety lead-in phase will be conducted during immunotherapy and dose-dense AC in the first 11 patients enrolled irrespective of cohort. Clinically significant irAEs (listed in Section 8.6) will be reported in an expedited manner (Section 8.10), and accrual will be halted until the safety lead-in phase has been completed. Recommendations for clinical evaluation to confirm that an AE is immune related (ie, an irAE) are provided in Section 6.8.

Determination of adequate safety will be based on assessment of irAEs through dose-dense AC. The safety evaluation plan is summarized below:

- The initial 11 patients will be followed through dose-dense AC. Accrual will be held until the last patient enrolled during the safety lead-in phase has completed dose-dense AC.
- If ≤2 of the first 11 patients experience a clinically significant irAE (Section 8.6) before completion of dose-dense AC, study accrual may continue and the safety lead-in phase will be completed.
- If 3 or more of the initial 11 patients experience a clinically significant irAE (Section 8.6) before completion of dose-dense AC, accrual to the study will be suspended until the Massey Cancer Center (MCC) Data Safety and Monitoring Committee (DSMC) reviews the reported irAEs and determines whether study revision or study closure is required.

3.3 Site Participation

3.3.1 MCC Affiliate Network Participation

Eligibility assessment, patient informed consent, administration of the investigational components of the treatment regimen (ie, decitabine and pembrolizumab), performance of the research biopsies, collection of blood samples for correlative studies, and administration of all neoadjuvant chemotherapy to patients enrolled during the safety lead-in phase will be performed at MCC at the VCU Medical College of Virginia location. Patients referred by collaborating MCC affiliate member physicians and enrolled in the study after the safety lead-in phase has been completed will return to the referring physician for administration of the standard chemotherapy regimen and for breast surgery by their initial breast surgeon unless the patient requests transfer of care to MCC.

3.3.2 Multicenter Site Participation

MCC-15-11083 will be conducted at multicenter sites. [redacted]

3.4 Requirements for Collection of Samples for Correlative Studies

Correlative studies will be conducted using tumor samples procured by core needle biopsy performed at MCC at baseline and 3-7 days following administration of the last administered dose of pre-chemotherapy pembrolizumab. For patients who have surgery, samples from the surgical resection specimen will also be collected for the correlative studies. Correlative blood samples will be collected at 3 time points.

3.5 Study Accrual

The projected sample size for the MCC-15-11083 study is 32-50 patients. Accrual is expected to be completed in about 72 months from the time of the first patient enrollment.

3.6 Primary Endpoint

The increase in percent of tumor and stroma with infiltrating lymphocytes from baseline pre-treatment biopsy to post-immunotherapy biopsy following administration of decitabine followed by pembrolizumab

3.7 Secondary Endpoints

3.7.1 Using criteria in the National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0), all AEs captured as described in Section 8.9

3.7.2 The percentage of patients meeting criteria for LPBC following treatment with decitabine and pembrolizumab compared to the percentage before treatment (LPBC is defined as breast cancer with ≥ 60% intratumoral or stromal area with infiltrating lymphocytes.)

3.7.3 The proportion of patients with pCR in the breast and post-therapy lymph nodes defined as the absence of any invasive cancer in the resected breast specimen and absence of cancer on H&E evaluation of all resected lymph nodes following completion of neoadjuvant therapy (ypT0/is; ypN0). Note: patients in Cohort A2, who receive prolonged pembrolizumab treatment, will be evaluated separately from those who receive only pre-chemotherapy pembrolizumab

3.7.4 The proportion of patients with no or minimal residual disease in the resected breast and axillary specimen defined as RCB Index value 0 or 1 (Section 10.2.3). Note: patients in Cohort A2, who receive prolonged pembrolizumab treatment, will be evaluated separately from those who receive only pre-chemotherapy pembrolizumab

3.7.5 The proportion of patients with cCR defined as the absence of tumor based on physical examination of the breast and nodes following completion of all neoadjuvant therapy. Note: patients in Cohort A2, who receive prolonged pembrolizumab treatment, will be evaluated separately from those who receive only pre-chemotherapy pembrolizumab

3.7.6 Enumeration of T cells and immune cell subsets, including CD8+ cytotoxic T cells, CD4+ helper T cells, FOXP3+ regulatory T cells, CD20+ B cells, and MDSCs in the tumor sample procured by core needle biopsy following completion of sequential decitabine followed by pembrolizumab compared to the number of these cells in tumor samples procured at baseline

3.7.7 Evaluation of expression of PD-L1 within tumor, stroma, and infiltrating immune cells at baseline and following immunotherapy

3.7.8 Correlation of intensity of PD-L1 expression by Merck assay as it relates to pCR rates from chemotherapy

3.7.9 Evaluation of MDSCs identified in blood samples post-decitabine and post-pembrolizumab compared to MDSCs found in blood samples collected at baseline

3.7.10 Proportion of patients who are alive and have not had disease progression or relapse at 12 months following surgery (or the decision not to undergo surgery). Note: patients in Cohort A2, who receive prolonged pembrolizumab treatment, will be evaluated separately from those who receive only pre-chemotherapy pembrolizumab

4 PATIENT SELECTION

4.1 Inclusion Criteria

A patient must meet all of the following inclusion criteria to be eligible for this study.

Inclusion Criteria	Yes	No	N/A
4.1.1 Invasive adenocarcinoma of the breast diagnosed by core needle biopsy			
4.1.2 Breast cancer determined to be HER2-negative per current American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) HER2 Guidelines (If IHC was performed, IHC 0 or 1+; if fluorescence in situ hybridization [FISH] or other in situ hybridization test, dual probe HER2/CEP17 ratio < 2.0 with an average HER2 copy number < 4.0 signals/cell)			
4.1.3 Breast cancer determined to be hormone receptor-positive **or** hormone receptor-negative defined as follows: • Hormone receptor-positive: ≥ 10% staining by IHC for **either** estrogen receptor (ER) **or** progesterone receptor (PgR) • Hormone receptor-negative: < 10% staining by IHC for both ER **and** PgR Note: Refer to Section 1.10 for the rationale of the definitions provided above, to Section 3.1 for cohort assignments, and Section 13.2 regarding accrual limitations based on hormone receptor status.			
4.1.4 Locally advanced breast cancer defined as any of the following per American Joint Committee on Cancer (AJCC) Staging Criteria: Note: Imaging methods that may be used for tumor measurement to determine eligibility include breast ultrasound and breast MRI. Mammography may not be used. • T2 based on tumor measurements by physical examination or imaging and **with** clinically positive regional lymph nodes (cN1 or cN2), irrespective of hormone receptor status • Hormone receptor-negative patients with tumor size of 3-5 cm measured by physical examination or imaging with clinically negative regional lymph nodes (cN0) • Any T3 based on tumor measurements by physical examination or imaging, irrespective of hormone receptor status • Any T4 (including inflammatory breast cancer), irrespective of hormone receptor status			

Inclusion Criteria	Yes	No	N/A
4.1.5 Ipsilateral axillary lymph nodes must be evaluated by MRI or ultrasound within 12 weeks prior to study registration to determine clinical nodal status. If imaging is suspicious or abnormal, an FNA or core biopsy of the questionable node(s) on imaging is required. Nodal status should be classified according to the following criteria: • Nodal status – negative – Imaging of the axilla is negative; OR – Imaging of the axilla is suspicious or abnormal AND FNA or core biopsy is negative. • Nodal status – positive – FNA or core biopsy of node(s) is cytologically or histologically suspicious or positive			
4.1.6 Breast imaging performed prior to study registration as follows: • Ipsilateral breast – within 12 weeks • Contralateral breast – within 24 weeks			
4.1.7 Age ≥ 18 years			
4.1.8 Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (see Appendix 1)			
4.1.9 Adequate bone marrow function as defined below: • Absolute neutrophil count (ANC) ≥ 1,500/mm^3 • Platelet count ≥ 100,000/mm^3 • Hemoglobin ≥ 10.0 g/dL			
4.1.10 Adequate renal function as defined below: Serum creatinine ≤ upper limit of normal (ULN) for the lab or a calculated creatinine clearance ≥ 60 mL/min (see Appendix 2 for the Cockcroft-Gault formula for calculating creatinine clearance)			

Inclusion Criteria	Yes	No	N/A
4.1.11 Adequate hepatic function as defined below: • Total bilirubin ≤ ULN for the laboratory • Aspartate aminotransferase (AST) ≤ 1.5 x ULN for the laboratory • Alanine aminotransferase (ALT) ≤ 1.5 x ULN for the laboratory • Alkaline phosphatase (ALP) ≤ 2.5 x ULN for the laboratory Note: If ALP is > 1.5 x ULN, imaging to rule out bone and liver metastasis is required.			
4.1.12 LVEF assessment (ie, 2-D echocardiogram or MUGA scan) performed within 12 weeks prior to study registration indicates an LVEF ≥ 50% regardless of the cardiac imaging facility's lower limit of normal			
4.1.13 Women who are not postmenopausal or have not undergone hysterectomy must have a documented negative serum pregnancy test within 72 hours prior to initiating study treatment. Note: Postmenopausal is defined as any of the following: • Age ≥ 60 years • Age < 60 years and amenorrheic for at least 1 year with follicle-stimulating hormone (FSH) and plasma estradiol levels in the postmenopausal range • Bilateral oophorectomy			
4.1.14 A female patient who is a woman of child-bearing potential (WCBP) and a male patient with a partner who is a WCBP must agree to use a medically accepted method for preventing pregnancy for the duration of immunotherapy and neoadjuvant chemotherapy and until after completion of breast surgery or, for patients who do not receive neoadjuvant chemotherapy, **for a minimum of 6 months following the last dose of pembrolizumab or decitabine** (refer to Section 9.2.12 for information regarding methods of preventing pregnancy)			
4.1.15 Ability to understand and willingness to sign the consent form			

4.2 Exclusion Criteria

Exclusion Criteria	Yes	No	N/A
4.2.1 Breast cancer treatment for the currently diagnosed breast cancer including radiation therapy, chemotherapy, targeted therapy, or endocrine therapy prior to study registration			
4.2.2 Administration of a live vaccine within 30 days prior to initiating study treatment Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are permitted; however, intranasal influenza vaccines (eg, Flu-Mist) are live attenuated vaccines, and are not allowed.			
4.2.3 Administration of a monoclonal antibody within 4 weeks prior to initiating study treatment or has not recovered (ie, ≤ grade 1 or at baseline) from AEs due to a monoclonal antibody administered more than 4 weeks earlier			
4.2.4 Administration of any investigational agent within 4 weeks prior to initiating study treatment			
4.2.5 Evidence of metastatic disease that is extensive enough to preclude consideration of subsequent definitive surgery for the primary tumor			
4.2.6 History of ipsilateral invasive breast cancer or ipsilateral ductal carcinoma in situ (DCIS) Note: Patients with history of ipsilateral lobular carcinoma in situ (LCIS) are eligible.			
4.2.7 History of solid organ or allogeneic stem cell transplant			
4.2.8 Previous therapy for any malignancy with an anthracycline or taxane for Cohorts A and B and carboplatin for Cohort A			

Exclusion Criteria	Yes	No	N/A
4.2.9 Cardiac disease that would preclude administration of the drugs included in the study treatment regimen including, but not limited to: • Angina pectoris that requires the current use of anti-anginal medication • Ventricular arrhythmias except for benign premature ventricular contractions • Supraventricular and nodal arrhythmias requiring a pacemaker or not controlled with medication • Conduction abnormality requiring a pacemaker • Valvular disease with documented compromise in cardiac function; and symptomatic pericarditis			
4.2.10 Nervous system disorder (ie, paresthesia, peripheral motor neuropathy, or peripheral sensory neuropathy) ≥ grade 2, per CTCAE v5.0			
4.2.11 Administration of or condition requiring administration of systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to initiating study treatment Exception: Patients with conditions that can be managed with steroids equivalent to or less than an oral prednisone dose of 10 mg daily would not be excluded from the study.			
4.2.12 Previous therapy for this cancer with an anti-PD-1, anti-PD-L1, anti-PD-L2 agent, or any other immunomodulatory agent			
4.2.13 Known or presumed hypersensitivity to decitabine or pembrolizumab (or any of their excipients)			
4.2.14 Diagnosed immunodeficiency, eg, human immunodeficiency virus (HIV)			

Exclusion Criteria	Yes	No	N/A
4.2.15 Active autoimmune disease requiring systemic treatment within the past 2 years (ie, with use of disease-modifying agents, corticosteroids, or immunosuppressive drugs) or a documented history of clinically severe autoimmune disease or a syndrome that requires systemic steroids or immunosuppressive agents Note: Patients with the conditions or medical history listed below are **NOT** excluded from this study. • Vitiligo • Resolved childhood asthma/atopy • Requirement for intermittent use of bronchodilators or local steroid injections or topical steroids • Hypothyroidism stable on hormone replacement • Sjogren's Syndrome			
4.2.16 Known history or evidence of interstitial lung disease or active, non-infectious pneumonitis			
4.2.17 Known history of active bacillus tuberculosis (TB)			
4.2.18 Active infection requiring systemic therapy			
4.2.19 Known active Hepatitis B or C			
4.2.20 Pregnancy or breastfeeding			
4.2.21 Diagnosis or treatment for another malignancy within 5 years prior to study registration, with the following exceptions: complete resection of basal cell carcinoma or squamous cell carcinoma of the skin, any in situ malignancy, and low-risk prostate cancer after curative therapy			
4.2.22 Medical, psychological, or social condition that, in the opinion of the investigator, may increase the patient's risk or limit the patient's adherence with study requirements			

5 STUDY ENTRY AND WITHDRAWAL PROCEDURES

5.1 Study Entry Procedures

5.1.1 Required Pre-Registration Screening Tests and Procedures

Refer to the study calendars in Section 12 for the screening tests and procedures that are required prior to registration and for the timing of these events relative to the start of treatment.

5.1.2 Registration Process

[redacted]

- Completed registration cover sheet or request form as provided by the coordinating center
- Attestation of patient eligibility (either included in the request form above, or a completed, signed, and dated eligibility checklist)
- Signed and dated consent form

The Coordinating Center will complete the registration process by assigning a study identification (ID) number and returning a Confirmation of Registration to the registering study team. Study treatment may not begin until the Confirmation of Registration assigning a study ID number has been received from the registrar.

The registering site will enter the patient's initial enrollment data (eg, demographics, consent, eligibility, on-study) into the OnCore database within 24 hours following study registration (before the baseline core needle biopsy for sample procurement is performed).

5.1.3 Reassignment to Cohort A2 with Extended Pembrolizumab

Before beginning standard neoadjuvant chemotherapy, patients initially enrolled to Cohort A may be reassigned to Cohort A2 to receive extended pembrolizumab as part of standard neoadjuvant and postoperative adjuvant therapy as described in Sections 6.7 and 6.13, at the investigator's discretion. Patients may not initiate neoadjuvant chemotherapy with extended pembrolizumab (ie, Cohort A2 treatment) until the decision to do so has been documented in source documents and the OnCore database **and** been confirmed by the Coordinating Center.

5.2 Study Withdrawal

A patient may decide to withdraw from study participation at any time. Patients must be withdrawn from the study when any of the following occurs:

- The patient has withdrawn consent for study treatment and study procedures.
- If, in the investigator's opinion, continuation of the study requirements would be harmful to the patient's well-being.
- The patient is lost to follow-up.

The reason for and date associated with study withdrawal or removal from the study must be documented in the source documents and OnCore database.

6 STUDY TREATMENT

6.1 Baseline Tests and Procedures

Refer to the study calendar in Section 12 for requirements prior to initiation of therapy. The baseline core needle biopsy and initiation of study treatment should begin as soon as possible following study registration.

6.2 Baseline and Post-Immunotherapy Core Needle Research Biopsies

Both of the required core needle biopsy procedures for tumor sample procurement will be performed at participating sites. Patients from MCC Affiliate Network sites will have these biopsy procedures performed at the VCU MCC downtown location. For instructions, refer to Section 3.3.1.

- Prior to initiation of decitabine, tumor samples must be procured by core needle biopsy. When possible, this procedure should be performed concurrently with placement of a vascular access device (VAD) required for administration of standard chemotherapy.
- A second core needle biopsy must be performed 3-7 days after the last dose of pre-chemotherapy pembrolizumab (ie, after the second dose of pembrolizumab). The biopsy must be performed before initiation of standard neoadjuvant chemotherapy.

  Note: If a decision is made that the second pembrolizumab dose will not be administered, the biopsy should still be performed, within 7 days of the decision (prior to standard neoadjuvant chemotherapy).

- Ultrasound guidance may be used for either the pre-or post-immunotherapy biopsies if the tumor is not easily palpable.
- Four (4) 10-14 gauge cores should be obtained at each of the 2 biopsy time points.

6.3 Marking the Primary Tumor Site Prior to Initiation of Therapy

Patients who are considered potential candidates for breast-conserving therapy (BCT) should have the primary tumor site marked in some way prior to initiating chemotherapy or, at least, prior to disappearance of the tumor clinically. This can be achieved with such methods as insertion of a radiopaque marker or clip, tattoos of the tumor boundary on the skin (especially for smaller breasts), or by making a transparent template with the tumor site marked on it. Other techniques are acceptable as long as they provide assurance that the primary tumor site can be located and excised. If a clip is used, a specimen radiograph should be performed to confirm its removal.

6.4 Summary of Study Treatment and Neoadjuvant Chemotherapy Regimens

The study treatment regimen and neoadjuvant chemotherapy regimens are outlined on Table 1.

Table 1. Summary of Study Treatment Regimen and Neoadjuvant Chemotherapy Regimens				
Patients	**Drug**	**Dose**	**Route of Administration**	**Dosing Interval Cycles/Doses**
Immunotherapy				
All Patients	Decitabine[A]	15 mg/m^2	IV infusion over 60 minutes	Days 1 through 4[B] *(total of 4 doses)*
	Pembrolizumab[C]	200 mg	IV infusion over 30 minutes	Day 8 and Day 22 *(total of 2 doses)*
Neoadjuvant Chemotherapy Regimen Initiated Up to 27 Days After the Last Dose of Pre-Chemotherapy Pembrolizumab (After the Post-Pembrolizumab Biopsy)[D]				
Cohort A	Doxorubicin	60 mg/m^2	IV push over 15 minutes	Every 2 or 3 weeks for a total of 4 cycles[E]
	Cyclophosphamide	600 mg/m^2	IV infusion over 30-60 minutes	
	Followed By[F]			
	Paclitaxel **Or** Nab-Paclitaxel	80 mg/m^2 125 mg/m^2	IV infusion over 60 minutes IV infusion over 30 minutes	Weekly for a total of 12 doses
	Carboplatin[G]	AUC 1.5 **Or** AUC 5	IV infusion over 30-60 minutes	Weekly x 12 (AUC 1.5) **Or** q3w x 4 (AUC 5)
Cohort A2	Doxorubicin	60 mg/m^2	IV push over 15 minutes	Every 3 weeks for a total of 4 cycles[E]
	Cyclophosphamide	600 mg/m^2	IV infusion over 30-60 minutes	
	Followed By[F]			
	Paclitaxel **Or** Nab-Paclitaxel	80 mg/m^2 125 mg/m^2	IV infusion over 60 minutes IV infusion over 30 minutes	Weekly for a total of 12 doses
	Carboplatin[G]	AUC 1.5 **Or** AUC 5	IV infusion over 30-60 minutes	Weekly x 12 (AUC 1.5) **Or** q3w x 4 (AUC 5)
	Throughout Neoadjuvant Chemotherapy			
	Pembrolizumab[C]	200 mg	IV infusion over 30 minutes	Every 3 weeks throughout neoadjuvant chemotherapy

Table 1. Summary of Study Treatment Regimen and Neoadjuvant Chemotherapy Regimens

Patients	Drug	Dose	Route of Administration	Dosing Interval Cycles/Doses
Cohort B	Doxorubicin	60 mg/m^2	IV push over 15 minutes	Every 2 or 3 weeks for a total of 4 cycles[E]
	Cyclophosphamide	600 mg/m^2	IV infusion over 30-60 minutes	
	Followed By[F]			
	Paclitaxel **Or** Nab-Paclitaxel	80 mg/m^2 125 mg/m^2	IV infusion over 60 minutes IV infusion over 30 minutes	Weekly for a total of 12 doses
Breast Surgery				
Patients Who Are Surgical Candidates	Breast-conserving surgery or mastectomy (with or without reconstruction) and axillary staging following recovery from the last cycle of neoadjuvant chemotherapy			
	Adjuvant pembrolizumab for Cohort A2 only			
	Pembrolizumab[C]	200 mg	IV infusion over 30 minutes	Every 3 weeks for up to 8 additional cycles post surgery

A. Refer to Section 6.5 and 9.1 for dose preparation and additional instructions.
B. If a dose is missed during Days 1-4, decitabine may be administered on Day 5.
C. Refer to Section 6.6 and 9.2 for dose preparation and additional instructions. For Cohort A2, omit pembrolizumab with the first dose of neoadjuvant chemotherapy if less than 3 weeks have passed since the previous dose of pembrolizumab
D. **Begin standard neoadjuvant chemotherapy as soon as possible after the second core needle research biopsy** (which should be performed 3-7 days after the last dose of pre-chemotherapy pembrolizumab) and should start by **day 49**. Refer to Section 6.7 for additional instructions.
E. **Primary prophylaxis with G-CSF is required during dose-dense AC** (see Section 6.7.4 for instructions).
F. At the treating medical oncologist's discretion following the safety lead-in, the paclitaxel regimen may be administered first followed by AC.
G. Carboplatin may be administered at AUC 5 every 3 weeks at investigator discretion (Section 6.7.2).

6.5 Administration of Decitabine

6.5.1 Decitabine Regimen for All Patients

Beginning within 2 weeks following study registration and after the core needle biopsy to procure tumor samples and placement of a VAD, administer

decitabine 15 mg/m^2 IV once daily for a total of 4 doses.

Note: When possible, doses should be given consecutively on days 1 through 4. However, if dosing on 4 consecutive days is not possible for any reason, the

schedule may be adjusted for dosing on 4 non-consecutive days, or a dose may be omitted. However, patients must receive at least 3 doses of decitabine to continue with study treatment, and decitabine dosing must be completed on or before day 5.

6.5.2 Premedications for Decitabine

Patients should be premedicated for nausea and vomiting, but should NOT receive dexamethasone or other steroids. Otherwise, the choice of antiemetic is at the investigator's discretion.

6.5.3 Administration of Decitabine

Refer to Section 9.1.5 for instructions regarding reconstitution and preparation.

- Administer reconstituted decitabine in 0.9% Sodium Chloride Injection, 5% Dextrose Injection, or Lactated Ringer's Injection.
- Administer the total dose over a period of 60 minutes (+/- 10 minutes).

6.5.4 Supportive Care

Supportive care to manage AEs related to decitabine is at the investigator's discretion with the exception that administration of steroids is NOT permitted unless required to manage a severe allergic reaction. In that case, the patient's participation in the study would be discontinued following the 30-day period for evaluation of AEs.

6.6 Administration of Pembrolizumab

6.6.1 Pembrolizumab Regimen for All Patients

- Pembrolizumab 200 mg IV will be administered on day 8 and day 22 **(total of 2 doses).**
- The first dose of pembrolizumab should be administered 1 week following the first dose of decitabine.
- Decitabine toxicities must be as follows:
  - Anemia, neutropenia, and thrombocytopenia ≤ grade 2
  - Other clinically significant toxicities (in the opinion of the investigator) ≤ grade 1
- If the patient cannot receive pembrolizumab on day 8 due to decitabine-related toxicity or for any other reason, the first dose of pembrolizumab may be delayed up to 1 week. If pembrolizumab cannot be administered by day 15, pembrolizumab will not be administered, the second research biopsy will not be performed, and standard neoadjuvant chemotherapy should begin as soon as possible.
- The second dose of pembrolizumab should be administered 2 weeks following the first dose. If the second dose of pembrolizumab cannot be given on day 22, the second dose may be delayed for up to 1 week. If the second dose cannot be

given by day 29, the second dose of pembrolizumab will be omitted and the patient should undergo breast biopsy as soon as possible followed by initiation of the standard neoadjuvant chemotherapy regimen.

- If fewer than 3 doses of decitabine have been administered, pembrolizumab will not be given, and the second research biopsy will not be performed.

6.6.2 Extended Pembrolizumab Administration in Cohort A2

See Section 6.7.2 for administration of pembrolizumab during standard neoadjuvant chemotherapy.

Patients in Cohort A2 who complete breast surgery may receive adjuvant pembrolizumab 200mg IV infusion over 30 minutes every 3 weeks for up to 8 cycles as outlined in Table 1 beginning 30 to 60 days post-surgery.

Administration of the extended pembrolizumab regimen should be according to the current prescribing information and standard practices followed by the treating medical oncologist. This includes use of premedications and supportive care before, during, and after each pembrolizumab treatment.

Note: *Patients in Cohort A2 will receive a total of 18 pembrolizumab doses (2 doses in the window segment prior to new-adjuvant chemotherapy, 8 doses during neo-adjuvant chemotherapy treatment, and up to 8 doses post-surgery)*

6.6.3 Premedications for Pembrolizumab

- Patients may be premedicated for nausea and vomiting. Choice of antiemetic is at the investigator's discretion with the exception that steroids are NOT permitted.
- Refer to Table 2 for management of infusion reactions.

6.6.4 Administration of Pembrolizumab

Refer to Section 9.2.6 for preparation instructions.

- Administer reconstituted pembrolizumab in intravenous infusion bag of either 0.9% Sodium Chloride Injection or 5% Dextrose Injection.
- Administer infusion solution over 30 minutes through an IV line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter. (Do not co-administer other drugs through the same infusion line.)
- Every effort should be made to plan for administration as close to 30 minutes as possible. However, a window of -5 minutes and +10 minutes is permitted.

6.6.5 Pembrolizumab Infusion Reaction Treatment Guidelines

Severe infusion reactions have been reported in 2 (0.1%) of 1562 patients receiving pembrolizumab in clinical trials. Management of a pembrolizumab infusion reaction is outlined on Table 2.

Table 2. Management of Pembrolizumab Infusion-related Reaction

Grade (NCI CTCAE v5.0)	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild transient reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the investigator.	None
Grade 2 Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (eg, antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hours	**Stop infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDS • Acetaminophen • Narcotics Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (eg, from 100 mL/hour to 50 mL/hour). Otherwise dosing will be held until symptoms resolve; the patient should be premedicated for the next scheduled dose. **Pembrolizumab should be permanently discontinued for patients who develop grade 2 toxicity despite adequate premedication.**	Patient may be premedicated 1.5 hour (± 30 minutes) prior to infusion of pembrolizumab with: • Diphenhydramine 50 mg po (or equivalent dose of antihistamine). • Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grade 3 Prolonged (ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (eg, renal impairment, pulmonary infiltrates) **Grade 4** Life-threatening consequences; urgent intervention indicated; pressor or ventilatory support indicated	**Stop infusion.** Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDS • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids • Epinephrine Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Permanently discontinue pembrolizumab.**	No subsequent dosing
Note: Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

6.6.6 Supportive Care

- Patients should receive appropriate supportive care measures as deemed necessary by the treating investigator. Supportive care measures for the

management of AEs with potential immunologic etiology, ie, irAEs, are outlined in Appendix 3.

- Supportive care for management of symptoms that are not thought to have potential immunologic etiology may be managed at the investigator's discretion with the exception that corticosteroids should not be administered until after the last research biopsy is performed. Any toxicity that is at least possibly related to pembrolizumab and has been determined to be an irAE will have corticosteroids administered as part of the routine management of irAEs (see Appendix 3).
- Use of G-CSF
  - G-CSF should be avoided prior to the second research biopsy, but may be administered per investigator discretion to manage neutropenia-related AEs should they occur following decitabine.
  - G-CSF subsequent to the second research biopsy is at the treating medical oncologist's discretion, as are the G-CSF dose and schedule.
  - Use of G-CSF will be captured in the database.

6.7 Standard Neoadjuvant Chemotherapy

During the safety lead-in phase of the study, all neoadjuvant chemotherapy will be administered at MCC. After completion of the safety lead-in phase, standard neoadjuvant chemotherapy may be administered at MCC Affiliate Network sites or participating sites.

6.7.1 Timing

- Standard neoadjuvant chemotherapy should begin 26-49 days after initiation of decitabine and as soon as possible following the second research biopsy.
- The second core needle biopsy for procurement of tumor samples must be performed before initiation of chemotherapy.

Cohort A2 Treatment Assignment: TNBC patients initially registered to Cohort A may be assigned to Cohort A2 in order to receive extended pembrolizumab concurrent with standard neoadjuvant chemotherapy.

Note: decision to extend pembrolizumab and Cohort A assignment must be documented prior to initiation of any neoadjuvant chemotherapy on trial as described in Section 5.1.3.

6.7.2 Chemotherapy Regimens

As outlined on Table 1, the neoadjuvant chemotherapy regimen to be administered is cohort-specific.

Note: Following the safety lead-in phase, the paclitaxel regimen may be administered prior to administration of the AC regimen at the treating medical oncologist's discretion.

- **Cohort A**

  Doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 (AC) once every 2 weeks or 3 weeks for 4 cycles

  Followed by

  Paclitaxel 80 mg/m^2 IV **or** Nab-Paclitaxel 125 mg/m^2 IV once weekly for 12 weeks + carboplatin AUC 1.5 once weekly or AUC 5 every 3 weeks for 12 weeks

- **Cohort A2**

  Doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 (AC) once every 2 weeks or 3 weeks for 4 cycles

  Followed by

  Paclitaxel 80 mg/m^2 IV **or** Nab-Paclitaxel 125 mg/m^2 IV once weekly for 12 weeks + carboplatin AUC 1.5 once weekly or AUC 5 every 3 weeks for 12 weeks

  Throughout Neoadjuvant Chemotherapy

  Pembrolizumab 200mg IV infusion over 30 minutes every 3 weeks throughout neoadjuvant chemotherapy

  Note: omit pembrolizumab with the first dose of neoadjuvant chemotherapy if less than 3 weeks have passed since the previous dose of pembrolizumab

- **Cohort B**

  Doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 (AC) once every 2 weeks or 3 weeks for 4 cycles

  Followed by

  Paclitaxel 80 mg/m^2 IV **or** Nab-Paclitaxel 125 mg/m^2 IV once weekly for 12 weeks

6.7.3 Preparation, Administration, and Supportive Care Instructions

Preparation and administration of chemotherapy should be according to the current prescribing information for each agent and standard practices followed by the treating medical oncologist. This includes use of premedications and supportive care before, during, and after each chemotherapy treatment.

6.7.4 Use of G-CSF During Standard Neoadjuvant Chemotherapy

- Primary prophylaxis with pegfilgrastim or filgrastim is **required during dose-dense AC**. Follow current prescribing information for the agent used.
- If G-CSF support is required during weekly paclitaxel or Nab-paclitaxel therapy, filgrastim **must** be used.

- Do not administer G-CSF within 24 hours of chemotherapy administration.

6.7.5 Alternative Chemotherapy Regimens

For patients who experience toxicity related to the chemotherapy regimen described in Section 6.7.2 and are not able to complete the chemotherapy as specified or who have disease progression, the treating medical oncologist may choose to:

- Discontinue chemotherapy before completion of all cycles and proceed to surgery, *or*
- Discontinue protocol-specified chemotherapy and administer an alternative neoadjuvant chemotherapy regimen

6.8 Recommended Evaluation of Potential Immune-related Adverse Events

When the patient experiences signs and symptoms of potential irAEs, the patient should be evaluated to determine if the AE is an irAE. Recommendations for evaluation of symptoms are provided on Table 3.

Refer to Appendix 3 for clinical management of irAEs.

Table 3. Recommended Evaluation of Potential Immune-Related Adverse Events

Sign/Symptom	Recommended Evaluations/Referrals	Potential irAE
Constitutional (not attributable to cytotoxic chemotherapy)		
Malaise Dizziness Headache	• Consider following lab tests: BMP TSH and Free T4 Cortisol (7-9 am) ACTH Prolactin *Also for females:* LH and FSH *Also for males:* Testosterone • Consider endocrinology referral	Endocrinopathy/ Hypophysitis
Persistent headaches or headaches with vision changes	Consider pituitary MRI (in addition to lab tests above)	Hypophysitis
New onset ≥ grade 3 hyperglycemia in absence of pre-existing diabetes	Consider endocrinology consult	New onset Type 1 diabetes
Gastrointestinal (not attributable to cytotoxic chemotherapy)		
Abdominal pain Blood or mucous in the stool Fever Vomiting (≥ grade 2) Diarrhea • ≥ grade 2 lasting more than 48 hours (before AC); • ≥ grade 3 (after AC) Peritoneal signs ≥ Grade 2 AST, ALT or bilirubin	• Consider testing for Clostridium difficile (infectious diarrhea) • Consider evaluation for ileus or perforation • Consider colonoscopy or flexible sigmoidoscopy for lower GI symptoms. If lower endoscopy is performed, include random biopsies of colonic mucosa, even if tissue appears grossly normal during examination. Lymphocytic infiltrate in biopsy specimens are diagnostic of immune enterocolitis. • Consider upper endoscopy with random biopsies for refractory vomiting undergoing evaluation for immune enterocolitis • Consider GI consult	Enterocolitis Hepatitis
Cardiopulmonary (not attributable to cytotoxic chemotherapy)		
Cough Dyspnea Hypoxemia Chest pain	*Consider following:* • Chest x-ray or chest CT (contrast is not required to evaluate parenchyma) or bronchoscopy • If during chemotherapy or fever is present, check CBC and treat empirically for infectious pneumonia as indicated while evaluating for pneumonitis. • Cardiac evaluation	Pneumonitis Myocarditis
Neurologic (not attributable to cytotoxic chemotherapy)		
Weakness, numbness, or tingling unrelated to paclitaxel; paresis	*Consider following depending on presentation:* • Brain MRI • Neurology consultation • CSF evaluation	Immune-mediated Neurologic Event (eg, Guillain-Barre syndrome; myasthenic syndrome; encephalitis)

6.9 Breast Surgery

As soon as possible following recovery from neoadjuvant chemotherapy and after the clinical tumor assessment, the patient should undergo a lumpectomy or mastectomy. BCT should be selected according to the patient's preference and the surgeon's evaluation. The surgery may be performed by the patient's breast surgeon.

- Patients who are not considered candidates for BCT or who do not desire BCT will undergo a total mastectomy; breast reconstruction is permitted.
- Patients who are deemed to be good candidates for BCT will undergo segmental excision of the primary tumor bed. If the residual tumor is non-palpable, methods to ensure adequate excision of the primary tumor site should be used to guide the excision (Section 6.3). If the tumor location was marked with clips, a specimen radiograph should be obtained intraoperatively to document that the lesion has been removed including the clips.
- The margins of the resected specimen of patients who had BCT should be histologically free of invasive tumor and DCIS. In patients for whom pathologic examination demonstrates tumor at the margin, additional operative procedures should be performed to obtain clear margins.

If breast surgery is not performed, the reason for that decision will be documented. An EDV will be conducted within 10 days of the decision, and a safety follow-up visit will be conducted 30 days (±10 days) from the time of the decision not to undergo surgery. Documentation of irAEs will continue as described in Section 8.8.

6.10 Axillary Staging

- Staging procedures performed prior to initiation of study treatment:
  - Sentinel node (SN) biopsy prior to initiation of study treatment is discouraged.
  - Even if FNA or core biopsy of an axillary node(s) was performed before initiation of neoadjuvant therapy, surgical evaluation of the axilla (either SN biopsy and/or axillary dissection as described below) must be performed following completion of all neoadjuvant therapy, **regardless of whether the pre-treatment FNA or core biopsy was positive or negative**.
- Post-neoadjuvant therapy axillary staging is required. Use of a SN biopsy procedure following completion of neoadjuvant therapy is at the discretion of the patient's surgeon. If SN biopsy is not performed, surgical evaluation of the axilla is required.
  - If the post-neoadjuvant therapy SN biopsy is positive, additional surgical evaluation of the axilla is required.
  - If the post-neoadjuvant therapy SN biopsy is negative, further surgical nodal staging procedure is not required. If, however, the only SN identified by isotope scan is in the internal mammary nodal chain, the axilla should be explored for blue or suspicious nodes.

6.11 Prohibited Medications and Treatments

6.11.1 Cancer Treatment

Cancer treatment (eg, chemotherapy, biological therapy, immunotherapy, radiation therapy) other than the treatment specified in the protocol for this study is not permitted during neoadjuvant therapy. Adjuvant therapy is at the discretion of the primary oncologist.

6.11.2 Other Medications

- Live vaccines (examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine) are prohibited.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of suspected immunologic etiology are prohibited. Exception: Steroids equivalent to or less than an oral prednisone dose of 10 mg daily are permitted and steroids are allowed without limitation (eg, to manage standard neoadjuvant chemotherapy-induced side effects) AFTER the last research biopsy has been completed.
- Any investigational agent not specified in the protocol for this study is not permitted.

6.12 Duration of Therapy

Study treatment will be administered as outlined on Table 1 unless one of the following occurs (also see study withdrawal criteria in Section 5.2):

- AE that requires discontinuation of study treatment (see Section 7)
- Pregnancy
- Determination by the investigator that discontinuation is in the patient's best medical interest
- Patient decision to discontinue study treatment
- Withdrawal of study sponsor support

The reason for discontinuation of study treatment must be documented in the source documents and in the OnCore database.

6.13 Post-Treatment Visits

Patients who do not initiate pembrolizumab are off-study after the 30-day safety follow-up period as outlined in Section 8.8. Patients who do initiate pembrolizumab are off-study after the last PFS assessment as outlined in Section 6.13.4.

6.13.1 Early Discontinuation Visit

Patients who discontinue (ie, do not complete) study treatment at any point after initiating standard neoadjuvant chemotherapy will undergo an Early Discontinuation Visit (EDV) within 10 days of the decision to discontinue study treatment. The

reason for discontinuation of study treatment will be documented in the source documents and in the OnCore database.

6.13.2 Safety Follow-up

Safety follow-up visits will be conducted 30 days (±10 days) following surgery or the decision not to undergo surgery (all cohorts) and completion of adjuvant pembrolizumab (Cohort A2). If a patient discontinues study treatment, then every attempt should be made to perform a safety follow-up visit 30 days (±10 days) after the EDV.

6.13.3 Evaluation of AEs

The plan for evaluating and reporting AEs during treatment and follow-up is outlined in Section 8.8.

6.13.4 Immune-related AEs

The follow-up assessments noted below are required for patients who have received one or more pembrolizumab doses.

- At the safety follow-up visit conducted 30 days (±10 days) following surgery or the decision not to undergo surgery, patients will be evaluated for occurrence of irAEs. An ongoing requirement for steroid management of any irAE will be noted.
- For Cohorts A and B, a follow-up visit will be conducted 6 months (± 30 days) following surgery or the decision not to undergo surgery. The following will be assessed:
  - the status of irAEs noted at the 30-day safety follow-up visit as having a steroid management requirement. An ongoing requirement for steroid management of previously known irAEs will be noted.
  - the occurrence, in the interim since the 30-day safety follow-up visit , of any new (late-occurring) irAE(s) requiring steroid management. An ongoing requirement for steroid management of new (late-occurring) irAEs will be noted.
- For Cohort A2, irAEs will be assessed at 12 months (± 30 days) after the end of neoadjuvant treatment or surgery.

6.13.5 Postoperative Evaluation of Treatment Response

- All patients who have breast surgery will have a 30-day (±10 day) follow-up evaluation of surgical outcome (see Section 10.2) following surgery.
- Patients in Cohort A2 who receive adjuvant therapy will have a 30-day (±10 days) safety follow-up visit after completion of adjuvant therapy.
- Patients who discontinue or complete treatment with ongoing SD, PR, pCR, or cCR (see Section 10) remain in follow-up status for PFS by reported clinical status for up to 12 months (± 30 days) after surgery (or discontinuation of

treatment if surgery is not performed) until one of the following occurs: disease progression, relapse, or death.

7 DOSING DELAYS/DOSING MODIFICATIONS

7.1 Recording Dose Delays and Omissions

All dosing delays and omissions will be recorded in the source documents and captured in the OnCore database.

7.2 Toxicity Grading

AEs will be characterized and graded according to NCI CTCAE v5.0.

7.3 Decitabine Treatment Modification

- Because the decitabine treatment plan is to only administer a total of 4 doses on 4 out of 5 consecutive days, there are no planned dose reductions for decitabine.
- If 2 or more decitabine doses must be omitted, study treatment will be discontinued.
- The only AE requiring discontinuation of decitabine is ≥ grade 3 allergic reaction.

7.4 Pembrolizumab Treatment Modification

7.4.1 First Pembrolizumab Dose

See Section 6.6.1 for instructions regarding the timing of pembrolizumab if completion of decitabine was delayed.

7.4.2 Second Pembrolizumab Dose

There are no planned reductions in the pembrolizumab dose. AEs requiring omission of the second pembrolizumab dose are the following:

- ≥ grade 3 infusion reaction (see Table 2)
- grade 3 maculopapular rash
- ≥ grade 2 bullous dermatitis
- ≥ grade 3 Stevens-Johnson Syndrome
- ≥ grade 4 toxic epidermal necrolysis
- ≥ grade 2 myocarditis
- ≥ grade 2 pneumonitis
- ≥ grade 2 diarrhea persisting for at least 2 days despite medical intervention
- ≥ grade 3 diarrhea
- ≥ grade 2 enterocolitis
- ≥ grade 2 pancreatitis

- ≥ grade 2 creatinine increase
- ≥ grade 2 increase in AST and/or ALT
- ≥ grade 2 increase in total bilirubin
- ≥ grade 3 hyperthyroidism
- ≥ grade 2 hypophysitis
- new onset of type 1 diabetes mellitus
- ≥ grade 2 Guillain-Barre syndrome
- ≥ grade 3 hyperglycemia
- ≥ grade 2 vasculitis
- sclerosing cholangitis
- other severe or ≥ grade 3 irAEs
- persistent grade 2 irAEs that do not recover to ≤ grade 1 by 3 weeks following administration of the first dose of pembrolizumab

7.4.3 Extended Pembrolizumab Doses (Cohort A2)

Dose modifications for extended pembrolizumab doses (Cohort A2) will be at the investigator's discretion based on current prescribing information.

7.5 Neoadjuvant Chemotherapy Dose Modifications

Dose modifications during neoadjuvant chemotherapy will be at the treating medical oncologist's discretion based on current prescribing information for each agent and standard practices followed by the treating medical oncologist.

If toxicity occurs during AC that requires discontinuation of AC prior to completion of 4 cycles, weekly paclitaxel with carboplatin (Cohorts A and A2) or weekly paclitaxel (Cohort B) may be initiated when the patient has recovered from the AC-related toxicity.

If the paclitaxel regimen is given first and toxicity occurs requiring discontinuation prior to completion of the regimen, AC may be initiated when the patient has recovered from the paclitaxel-related and/or carboplatin-related toxicity. Refer to Section 6.7.5 for additional information.

Note: AEs occurring during standard neoadjuvant chemotherapy may be related to immunotherapy (ie, irAEs). Refer to Section 6.8 for recommended evaluation of potential irAEs, Section 8.6 for a list of clinically significant irAEs, and Appendix 3 for recommended supportive care for irAEs.

8 ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

8.1 Definitions

8.1.1 Adverse Event (AE)

AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

8.1.2 Suspected Adverse Reaction (SAR)

Any AE for which there is a reasonable possibility that the drug caused the AE. “Reasonable possibility” means that there is evidence to suggest a causal relationship between the drug and the AE.

An AE with an attribution of possible, probable, or definite (see Section 8.1.8) is a SAR.

8.1.3 Serious AE (SAE) or Serious SAR (SSAR)

An AE or SAR is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death,
- a life-threatening AE,

  An AE or SAR is considered to be “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
- inpatient hospitalization or prolongation of existing hospitalization,

  Planned inpatient hospitalizations, eg, for planned surgery, or those that occur for logistical reasons, eg, to complete a therapy that cannot be completed due to outpatient clinic business hours, are exempt from SAE reporting. Events that prolong such hospitalizations and otherwise meet reporting criteria are, however, still subject to SAE reporting requirements.
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgement, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.4 Unexpected SAR

A SAR is considered “unexpected” if:

- it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed;
- or, if an investigator brochure is not required, is not consistent with the risk information described in the prescribing information.

"Unexpected" as used in this definition, also refers to SARs that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the drug under investigation.

8.1.5 Unanticipated Problem

UPs include any incident, experience, or outcome that meets all of the following criteria:

- unexpected (in terms of nature, severity, frequency) given (a) the research procedures that are described in the protocol-related documents, such as the research protocol and informed consent document approved by the institutional review board (IRB); and (b) the characteristics of the patient population being studied;
- related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places patients or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.1.6 AE Description and Grade

The descriptions and grading scales found in CTCAE v5.0 will be utilized for AE reporting.

8.1.7 AE Expectedness

AEs can be 'Unexpected' or 'Expected'.

- Expected AEs are those AEs, the specificity and severity of which, that are consistent with the listings for decitabine found in Section 9.1 and the FDA-approved prescribing information for Dacogen, and for pembrolizumab found in protocol Section 9.2 or in the most current version of the Keytruda Investigator's Brochure. Refer to the prescribing information for each of the drugs included in the standard neoadjuvant chemotherapy regimen.
- Unexpected AEs are those AEs occurring in one or more patients participating in the study, the nature, severity, or frequency of which is not consistent with either:
  - The known or foreseeable risk of AEs associated with the procedures involved in the research that are described in (a) the protocol-related document, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent

document, and (b) other relevant sources of information, such as product labeling and package inserts; or

- The expected natural progression of any underlying disease, disorder, or condition of the patient(s) experiencing the AE and the patient's predisposing risk factor profile for the AE.

8.1.8 AE Attribution

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

8.2 Known AEs

The known AEs for decitabine can be found in protocol Section 9.1 and in the decitabine (Dacogen) prescribing information. The expected AEs for pembrolizumab can be found in protocol Section 9.2 and in the pembrolizumab (Keytruda) Investigator's Brochure. Refer to the prescribing information for the known AEs for each of the drugs included in the neoadjuvant chemotherapy regimen.

8.3 Pregnancy and Lactation

If a patient inadvertently becomes pregnant while on treatment with either decitabine or pembrolizumab, study treatment will immediately be discontinued. The patient will be contacted at least monthly to determine the patient's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor-Investigator within 24 hours if the outcome is a serious adverse experience (eg, death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Coordinating Center. If a male patient impregnates his female partner, the Sponsor-Investigator must be informed immediately; the same reporting requirements will apply.

Pregnancy or lactation occurring through the 30-day follow-up period will be reported as a UP. Also, refer to Section 8.9 for instructions regarding reporting pregnancy and lactation.

8.4 Secondary Malignancy

A secondary malignancy is a new cancer caused by previous treatment for a malignancy, eg, chemotherapy or radiation therapy. Metastatic disease is not a secondary malignancy. Any secondary malignancy should be reported via expedited reporting mechanisms. (Refer to Section 8.9 regarding reporting of secondary malignancy.)

8.5 Event of Clinical Interest

Events of clinical interest (ECIs) for this trial include:

- An overdose of pembrolizumab, defined as any pembrolizumab dose of 1,000 mg or greater (≥ 5 times the study-specified dose), that is not associated with clinical symptoms or abnormal laboratory results will be reported as non-serious ECI using the terminology “accidental or intentional overdose without adverse effect”

  An AE that is associated with (“results from”) the overdose of pembrolizumab, the AE is reported as a SAE, even if no other seriousness criteria are met. Such an event will also be noted as an ECI in the study database.

- From initiation of study treatment through the 30 day follow-up period, occurrence of all of the following at the same time will be reported as an ECI:
  - An elevated AST or ALT lab value that is ≥ 3 x ULN; and
  - an elevated total bilirubin lab value that is ≥ 2 x ULN and, at the same time,
  - an alkaline phosphatase lab value that is < 2 x ULN, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.

  Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

8.6 Immune-related Adverse Events

For this trial, an irAE is an immune-mediated AE that is possibly, probably, or definitely related to pembrolizumab.

Clinically significant irAEs will be reported in an expedited manner (See Section 8.10 and Table 4 and Table 5). Refer to Section 8.7 for a list of clinically significant irAEs.

Serious irAEs will be reported as SAEs regardless of whether clinically significant or not.

Recommendations for evaluation of AEs to determine if the AE is an irAE are provided in Section 6.8, requirements for expedited reporting of irAEs are specified in Section 8.10, and supportive care recommendations for irAEs are outlined in Appendix 3.

8.7 Clinically Significant Immune-related Adverse Events

Clinically significant irAEs identified for consideration are listed below. When a severity grade is specified, the grading is based on CTCAE v5.0 terminology and criteria. When a severity grade is not specified, the development of that toxicity at any grade warrants review to determine whether it qualifies as a clinically significant irAE.

- Pneumonitis (≥ grade 2)
- Myocarditis (≥ grade 2)
- Diarrhea (≥ grade 3 persisting for a minimum of 2 days despite usual medical intervention, responded to steroids, and has no other apparent cause)

- Enterocolitis (≥ grade 2)
- Pancreatitis (≥ grade 2)
- Severe skin reactions
  - Bullous dermatitis (≥ grade 2)
  - Stevens-Johnson Syndrome (≥ grade 3)
  - Toxic epidermal necrolysis (≥ grade 4)
- Creatinine increased (≥ grade 2)
- Hepatic toxicity
  - Elevation in AST or ALT (≥ grade 3)
  - Total bilirubin ≥ 2 x ULN
- Hypophysitis (≥ grade 2)
- Adrenal insufficiency (≥ grade 2)
- Hyperthyroidism (≥ grade 3)
- Hyperglycemia (≥ grade 3)
- Guillain-Barre syndrome (≥ grade 2)
- Myasthenia gravis (≥ grade 2)
- Other severe or ≥ grade 3 treatment-related AEs determined to be irAEs

8.8 Time Period and Grade of AE Capture

All AEs, regardless of grade or attribution, will be captured from the time of study registration until initiation of standard neoadjuvant chemotherapy. This includes any AEs occurring during biopsy (eg, hemorrhage).

For patients who do not initiate pembrolizumab, all AEs will be captured until 30 days following the last dose of decitabine or until another cancer treatment is initiated, whichever occurs first.

For patients who initiate pembrolizumab, irAEs (clinically significant and non-clinically significant) will be captured as described in Section 6.13.4.

Clinically significant irAEs will be reported in an expedited manner through the end 30-day follow-up period (Section 8.6).

SAEs will be captured from the start of study treatment until 30 days (± 10 days) following surgery or until a decision is made to forego surgery. All ECIs and other reportable events (eg, pregnancy, pregnancy outcomes, lactation, second or secondary malignancies) will be captured from the start of study treatment through end of the 30-day follow-up period.

All UPs will be captured from the start of study treatment through the end of the 30-day follow-up period.

8.9 Procedures for Recording Events

- AEs, irAEs, SAEs, UPs, ECIs, and other reportable events will be recorded in MCC's OnCore Clinical Trials Management System.
- In most cases, it is acceptable to record in OnCore only the highest grade of a toxicity occurring during a particular study segment when an event has serial fluctuations in grade over time.
- SAEs will be entered into the OnCore SAE domain. UPs will be entered into the OnCore Deviations domain. An SAE that is both an SAE and a UP will be entered in both domains.
- AEs requiring expedited reporting must also be recorded by routine AE capture (ie, in AE eCRFs).

8.10 Expedited Reporting Procedures

Participating sites will refer to Table 4 for expedited reporting requirements and instructions. The Sponsor-Investigator/Coordinating Center will refer to Table 5 for expedited reporting requirements and instructions. Also, see Section 8.6 for irAEs requiring expedited reporting.

Table 4. Expedited Reporting Requirements for Participating Sites

SAEs[A]	UPs[A]	irAEs (clinically significant)[A, B]	ECIs and other reportable events[A, C, D, E]
Sponsor-Investigator			
Merck and Company, Inc[F]	**IRB[G]**		
IRB (if applicable)[G]			

A. Report event within 1 business day of becoming aware of the occurrence, with the exception of pregnancy and lactation, which must be reported within 24 hours. A PDF of a de-identified OnCore SAE or Deviation record may be used for expedited event reporting purposes.
B. Refer to Section 6.8 for recommended evaluation of potential irAEs, and Section 8.6 for a list of clinically significant irAEs.
C. Refer to Sections 8.3, 8.4, and 8.11 for requirements and instructions for expedited reporting of pregnancy, lactation, and new primary malignancy.
D. Serious irAEs should be reported as SAEs regardless of whether clinically significant or not clinically significant.
E. Refer to Section 8.5 for a list of ECIs.
F. Refer to Section 8.11.1 for instructions for expedited reporting of SAEs to Merck Global Safety
G. Report SAEs and UPs to the IRB of record according to local institutional guidelines.

Table 5. Expedited Reporting Requirements for Sponsor-Investigator/Coordinating Center

SSARs	UPs	ECIs and other Merck-reportable events[A]
FDA[B]	**DSMC[C]** Email: [redacted]	**Merck and Company, Inc[D]**
Merck and Company, Inc[B, D]	**IRB[E]**	

A. Refer to Section 8.5 for a list of ECIs. Refer to Sections 8.3, 8.4, and 8.11 for requirements and instructions for expedited reporting of pregnancy, pregnancy outcome, lactation, and new primary malignancy.
B. Using a MedWatch Form 3500A, the Sponsor-Investigator will report to the FDA and Merck (within the timeframes shown below):
   - Any AE that meets all of the following criteria:
     i. possibly, probably, or definitely related to study drug (ie, the event is a SAR); and
     ii. serious (ie, a SSAR); and
     iii. unexpected.

     Events meeting these criteria will be reported to the FDA **within 15 days of determining that the event is reportable.**
   - A clinically important increase in the rate of SSARs **within 15 days** of determining that the event is reportable.
   - Any unexpected fatal or life-threatening SSARs **within 7 days of receipt of the information** regarding the event. Refer to Section 8.11 for requirements and instructions for expedited reporting to Merck Global Safety.
C. Report to DSMC within 1 business day of becoming aware of UP. A PDF of a de-identified Oncore Deviation record may be used for expedited event reporting purposes.
D. Refer to Section 8.11 for requirements and instructions for expedited reporting to Merck Global Safety.
E. Report each UP to the VCU IRB within 5 business days of becoming aware of the occurrence.

8.11 Requirements for Expedited Reporting to Merck and Company, Inc

8.11.1 Reports from Participating Sites and/or the Coordinating Center to Merck Global Safety

All expedited reports to Merck Global Safety (as outlined in Table 4 and/or Table 5) are to be faxed with related supporting information to the fax number listed below.

Attn: [redacted]

8.11.2 Reports from the Coordinating Center to Merck Global Safety

All expedited reports to Merck Global Safety (as outlined in Table 5) must be reported by the Coordinating Center to Merck Global Safety within 2 business days.

- AEs that are both an SAE and an ECI should be reported one time as an SAE only; however, the event must be appropriately identified as an ECI in the database.

- Although pregnancy and lactation are not considered AEs, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a patient (spontaneously reported to them) that occurs during the trial. Pregnancies and lactations that occur from the time of treatment through 120 days following the last administered dose of pembrolizumab, or 30 days following the last administered dose of pembrolizumab if the patient initiates new anticancer therapy, whichever is earlier, must be reported by the Sponsor-Investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy.

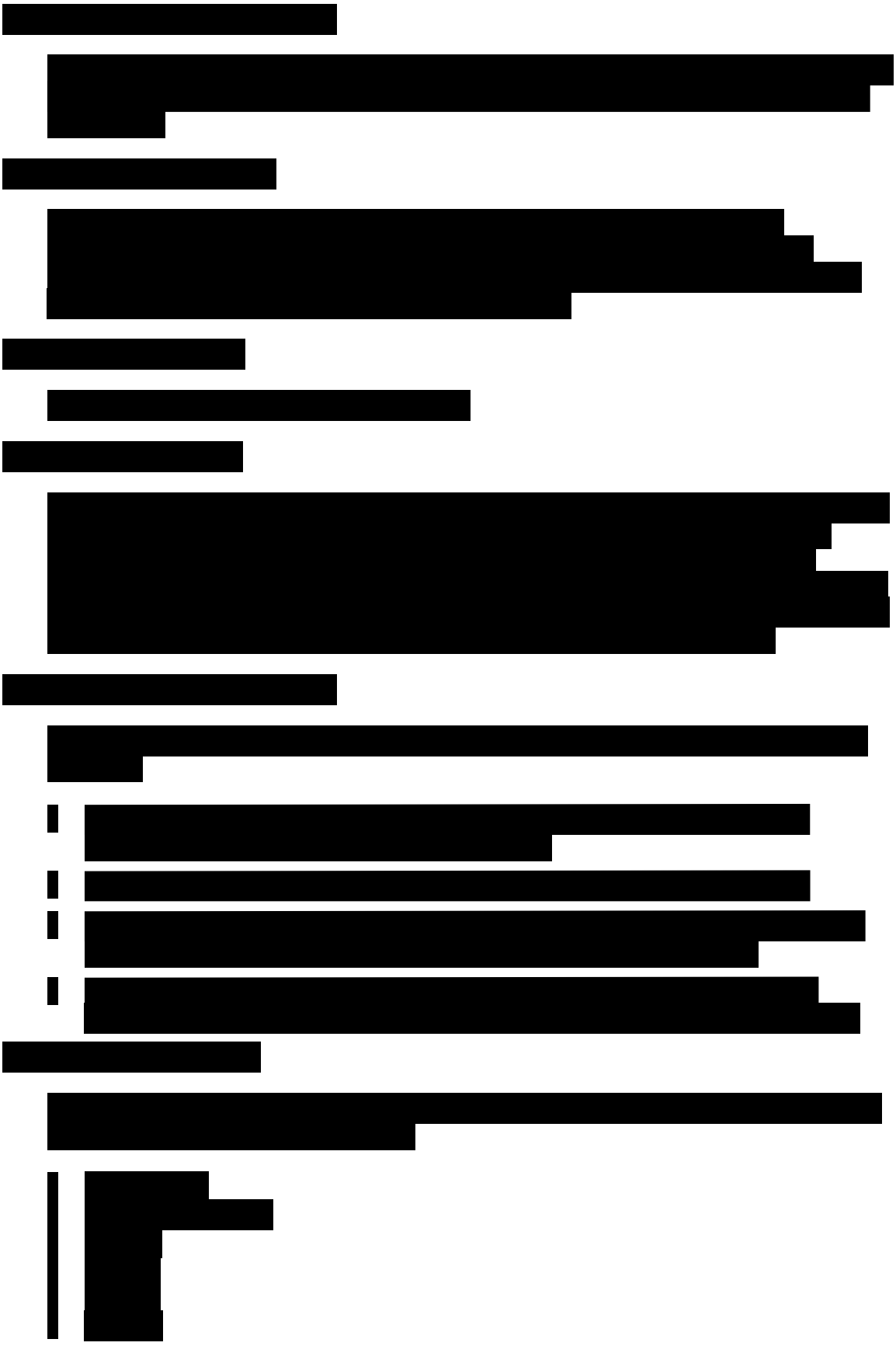
  Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage, and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (ie, health of infant) must also be reported.

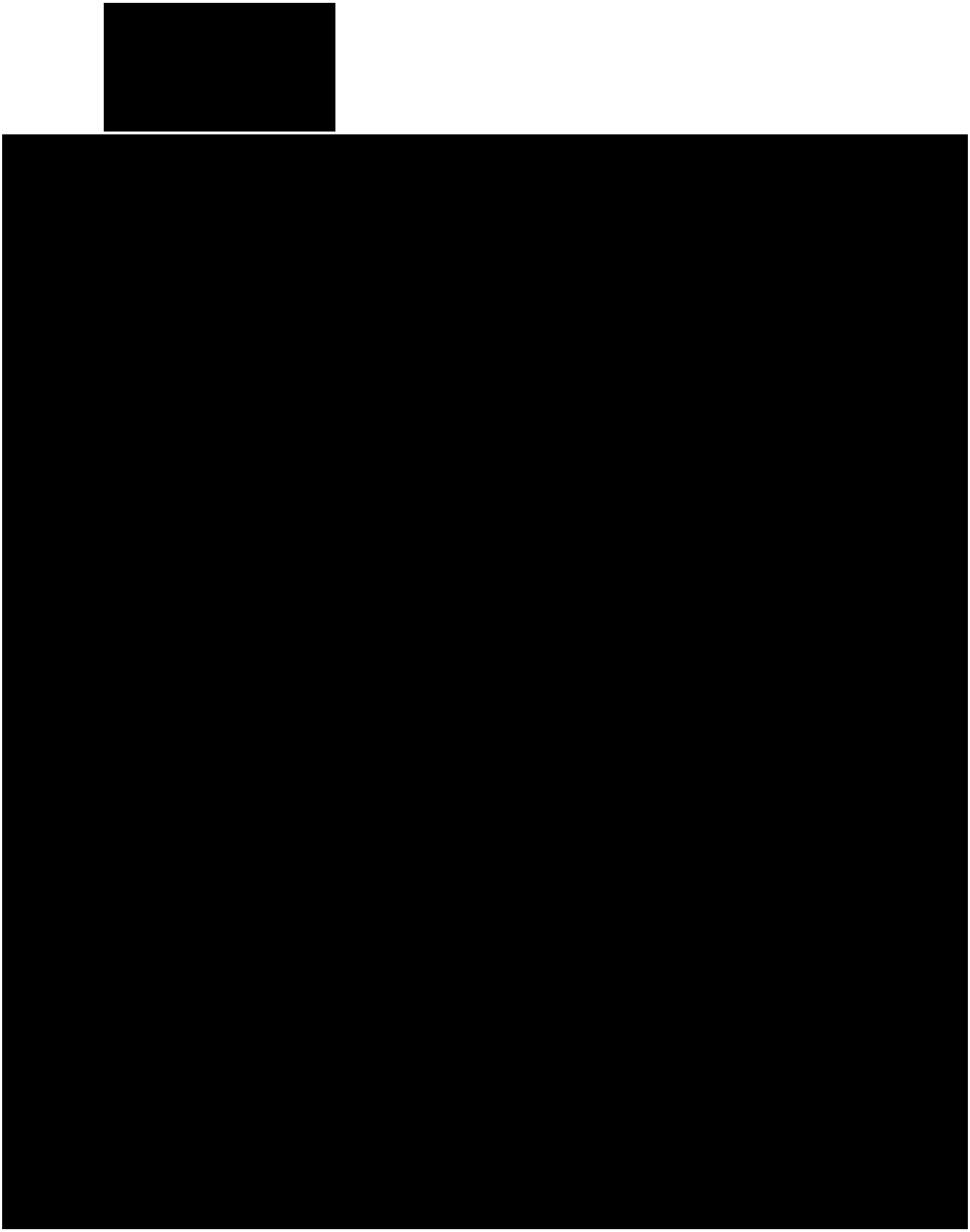
[redacted]

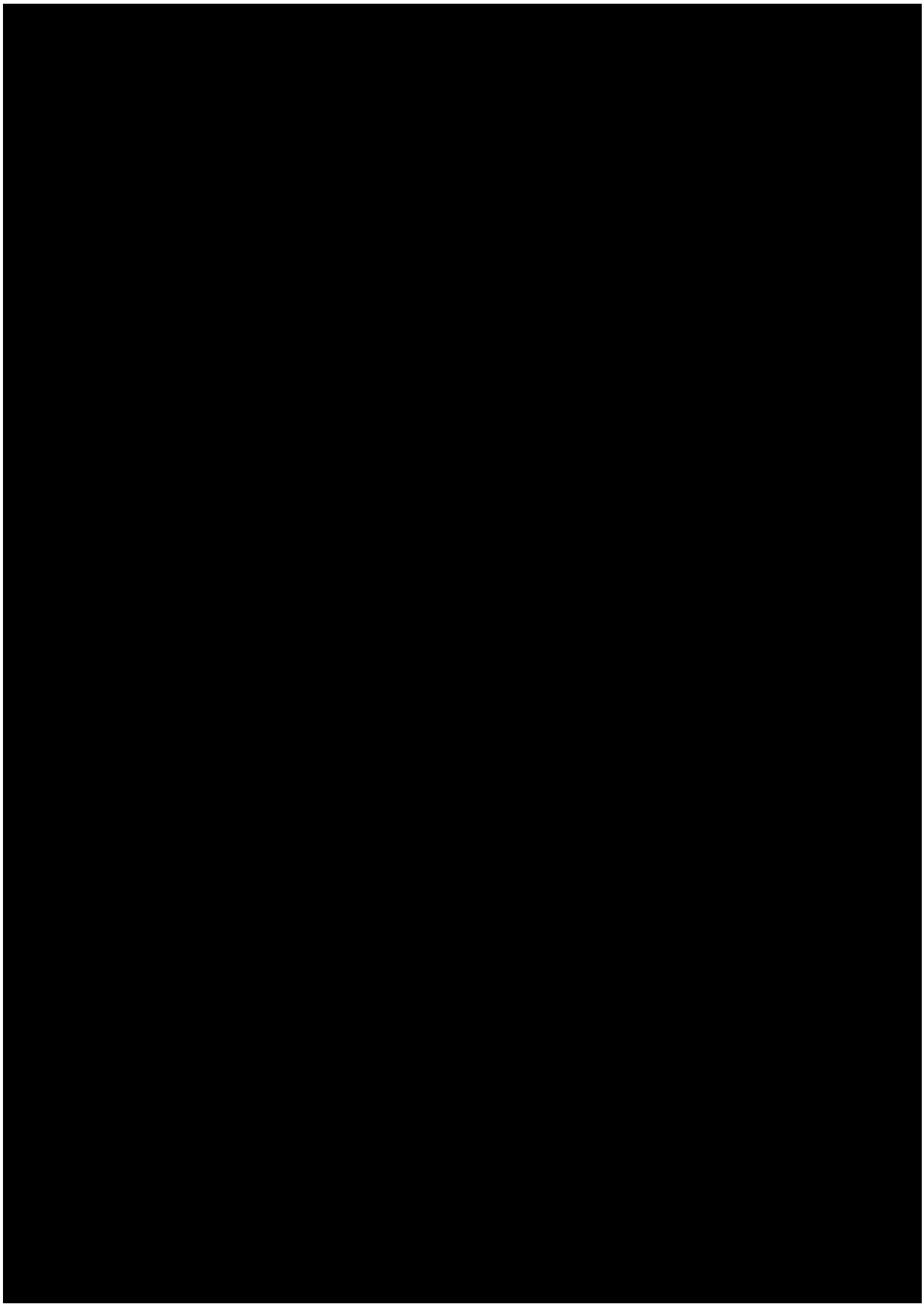
9.1 [redacted]

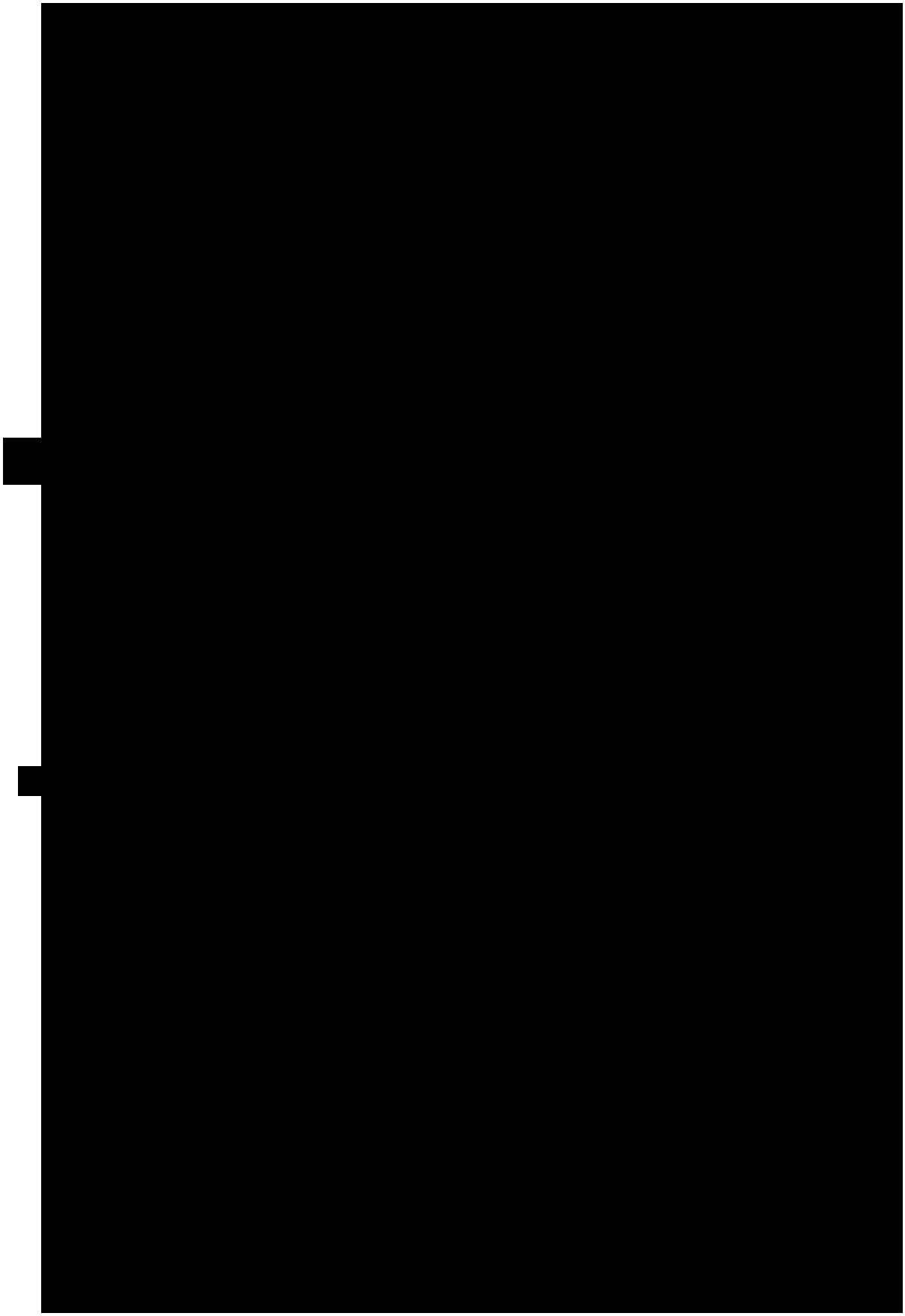
[redacted]

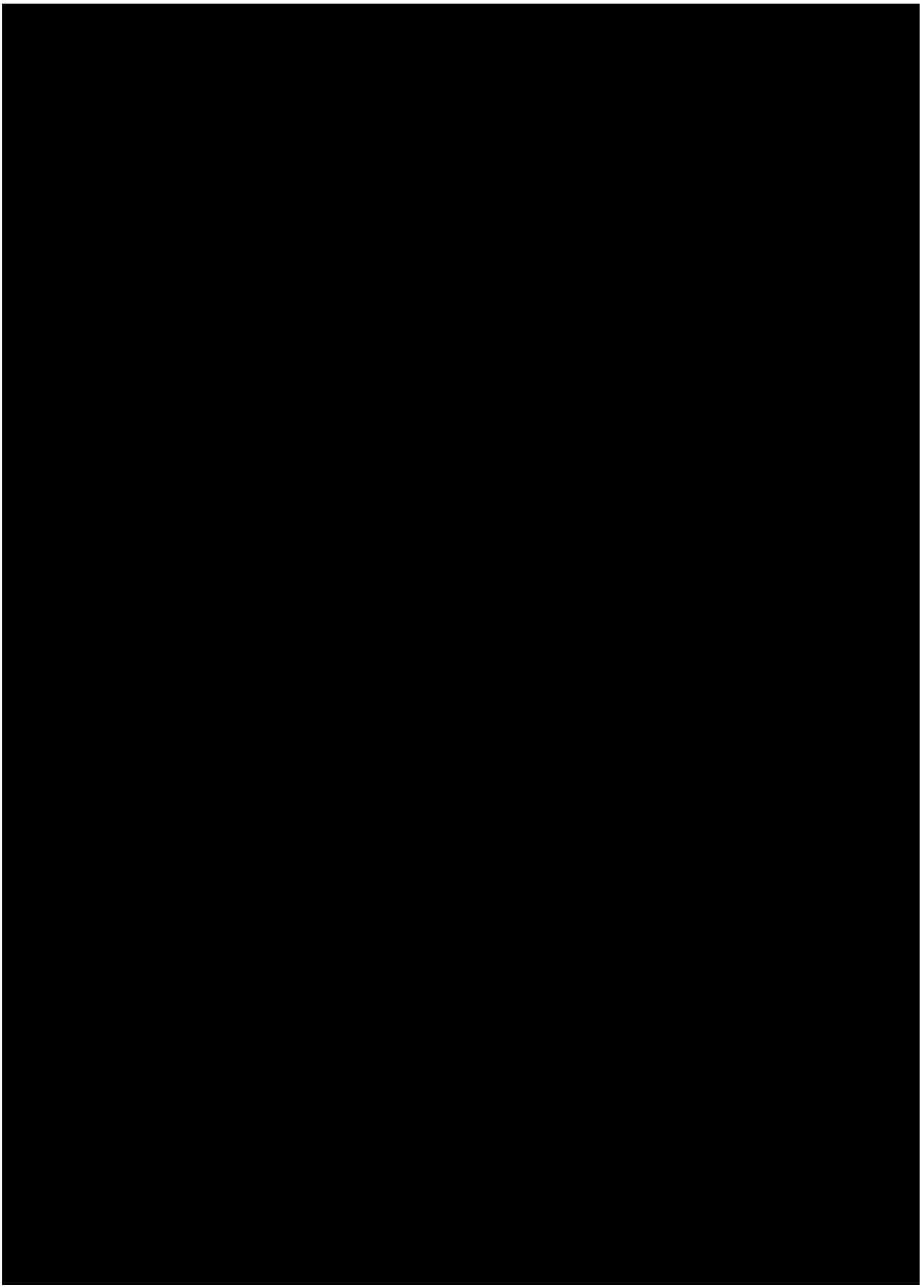
[redacted]

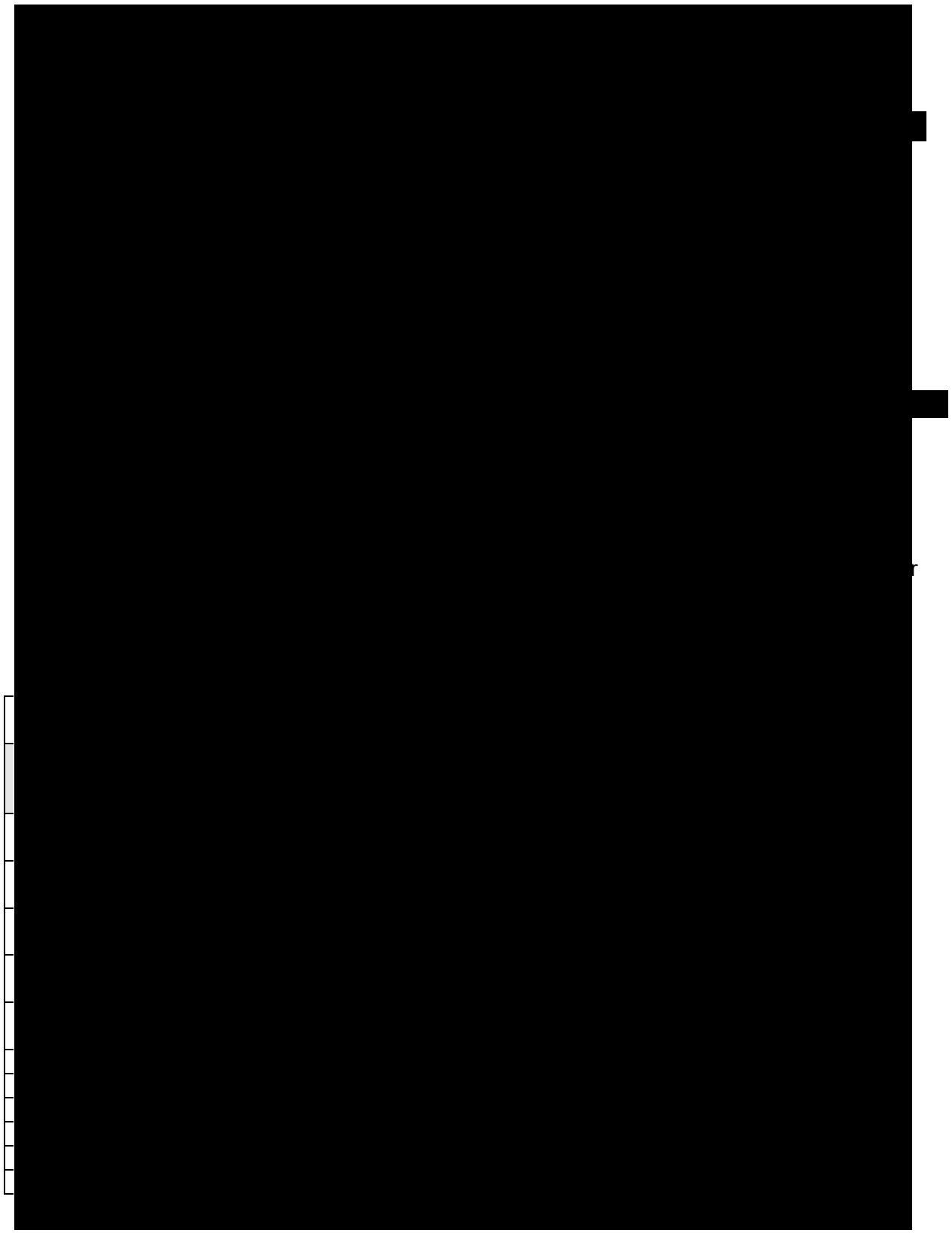


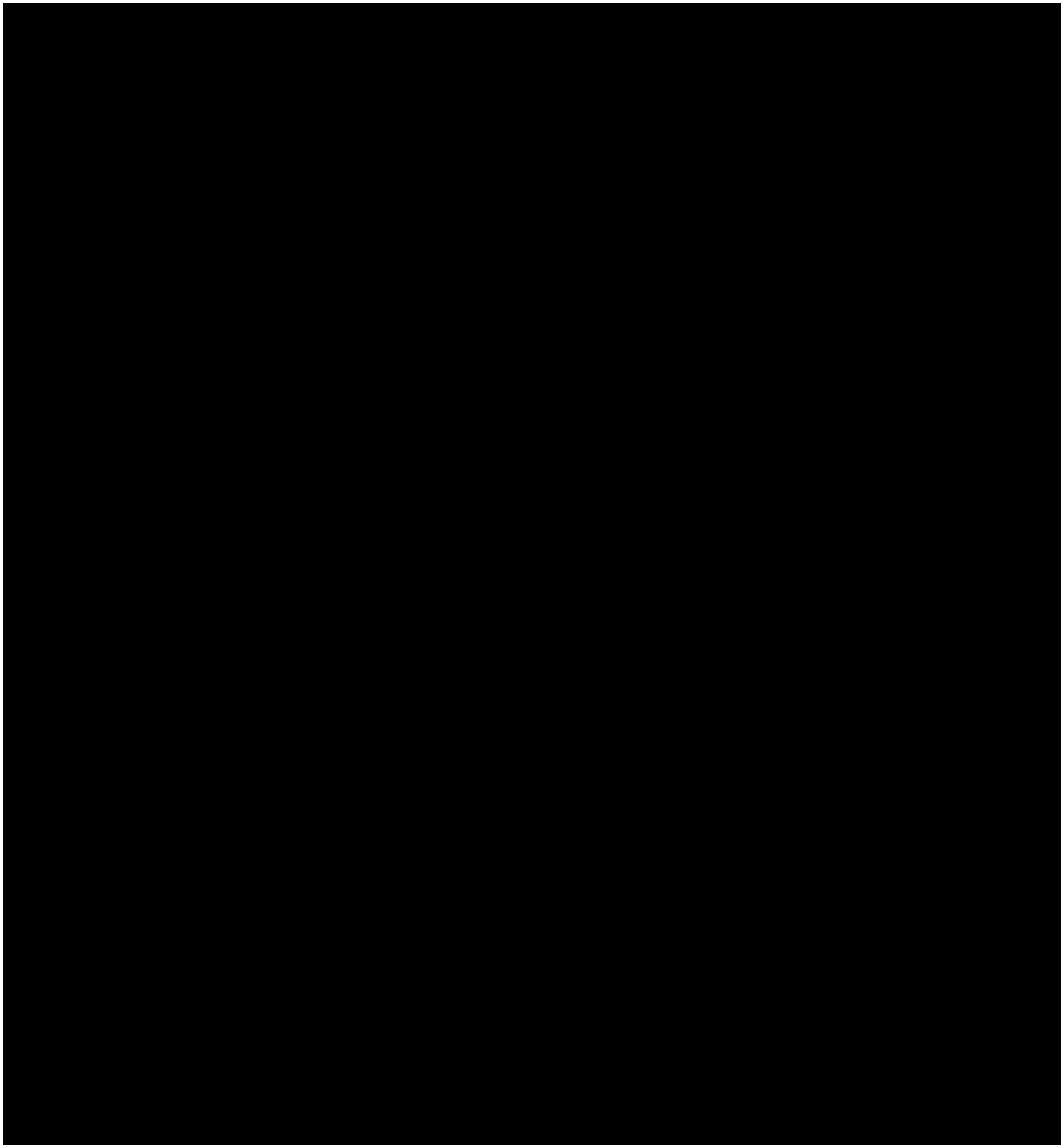












10 MEASUREMENT OF EFFECT

10.1 Assessment of LPBC

Tumor samples will be obtained by core needle biopsy at baseline (prior to initiation of any treatment) and after completion of pembrolizumab (prior to initiation of neoadjuvant chemotherapy). Lymphocyte infiltration in the tumor and stroma will be assessed as described in Section 11.3.

10.2 Pathologic Assessment of Effect

10.2.1 Timing of Evaluation

The determination of pCR will be performed by the local pathologist following examination of tissue (breast and nodes) removed at the time of surgery. See Appendix 4 for suggested procedures to evaluate the surgical specimens for determination of pCR.

10.2.2 Criteria for Evaluation of Pathologic Response

pCR in breast and axillary lymph nodes as well as non-axillary SN (pCR breast and nodes) will be determined based on the following definition:

No histologic evidence of invasive tumor cells in the surgical breast specimen, axillary nodes, or SNs identified after neoadjuvant therapy

10.2.3 Residual Cancer Burden

The RCB Index will be calculated locally and entered into the OnCore database for all patients who have residual disease. This can be done by the site pathologist or by entering data at http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3

Data collected is based on instructions provided by Symmans et al (86):

- Size of the tumor bed
- Cellularity of residual primary tumor
- Percentage of DCIS component
- Number of positive nodes
- Size of macrometastasis

10.3 Clinical Exam Assessment of Effect

To document the presence or absence of cCR, protocol-required tumor assessment by physical exam must be performed following the last cycle of neoadjuvant chemotherapy (prior to surgery).

Note: It is recommended that patients also have a breast examination for tumor assessment before each cycle of preoperative therapy to ensure that there is no disease progression.

10.3.1 Determination of cCR (Breast and Nodes)

The following criteria will be employed to determine if there has been a cCR (breast and nodes) following the last cycle of neoadjuvant chemotherapy (before surgery):

- Resolution of all palpable disease identified at baseline, *and*
- No new lesions or other signs of disease progression.

Resolution of all imaging abnormalities is not required for this endpoint.

10.3.2 Progressive Disease

Criteria to be used for determination of progressive disease are at the investigator's discretion. In the event of progressive disease, see Section 6.7.5 for further treatment instructions.

11 CORRELATIVE STUDIES

11.1 Participation in Correlative Studies

Section 1.11 outlines the plans and rationale for the correlative studies. Core needle biopsies to procure tumor samples and submission of residual tumor or, in the case of pCR, submission of tissue from the tumor bed, and collection of blood samples are study requirements.

11.2 Collection, Processing, and Distribution of Samples

- Refer to the MCC-15-11083 Biospecimen Manual
- The study team at the participating site will coordinate collection and de-identification of all correlative samples.
- MCC Clinical and Translational Research Lab (CTRL) will receive, process, store, and/or distribute the correlative blood and tumor samples as described in Sections 11.3, 11.4, and 11.5.
- The pathology department where the patient has surgery will perform the postoperative pathologic evaluation.
- Questions regarding study requirements should be directed to the Coordinating Center.

11.3 Tumor Samples from Core Needle Biopsy

11.3.1 Collection Time Points

Core needle biopsies to procure research tumor samples from the primary tumor in the breast will be performed at 2 time points:

- After study registration (before initiation of study treatment); preferably at the time of VAD placement
- Within 3-7 days after administration of the last dose of pembrolizumab (before initiation of neoadjuvant chemotherapy)

  Note: If, for any reason, no pembrolizumab is given (eg, if fewer than 3 doses of decitabine are administered or decitabine-related toxicity prevents pembrolizumab administration), the second research biopsy will not be performed.

11.3.2 Sample Requirements

Refer to the MCC-15-11083 Biospecimen Manual

At the time of each core needle biopsy procedure using a 10-14 gauge needle, 4 cores of tumor will be obtained and placed in formalin. Samples procured at the time of the 2 core needle biopsy procedures will be used for all of the following:

- Evaluation of TILs

  A tumor sample (1 slide with 4 micron thick specimen) will be used for evaluating TILs, which is the primary objective of the MCC-15-11083 study. Assessment and evaluation of TILs will be performed by VCU Anatomic Pathology and will be based on the procedures described by Salgado et al (83).

- Multiplex immunofluorescence assays

  Tumor samples (2 unstained slides, each with 4 micron thick specimen) will be analyzed using the Vectra microscope with validated antibodies according to standard operating procedures for the Opal staining system and Vectra microscope.

- Evaluation of PD-L1

  Tumor tissue from the 2 research core biopsies and, if available, from the surgical resection specimen (see Section 11.4), should be submitted on 5 unstained slides, per the QualTek Sample Handling Manual.

  Tumor samples will be shipped from the Coordinating Center to QualTek Molecular Laboratories for proprietary PD-L1 staining. (Refer to the *QualTek MISP Sample Handling Manual* for instructions regarding slide preparation and shipping.)

- Nanostring gene analysis

  FFPE tumor samples, about 5 slides on average, will be sent to Merck for provision to Almac for genomic analysis by next-generation sequencing panel (≥ 680 genes) to determine if there are gene expression patterns associated with response to decitabine and pembrolizumab and/or that predict response to neoadjuvant chemotherapy.

11.4 Tumor Samples from Surgical Specimens

Refer to the MCC-15-11083 Biospecimen Manual

11.4.1 Sample Requirements

An FFPE block of tumor or, if there was no remaining tumor (ie, pCR) an FFPE block from the tumor bed will be requested following surgical resection.

11.4.2 Testing and Analysis

Unstained slides will be cut from the block and provided to the following laboratories:

- Evaluation of PD-L1
- QualTek Molecular Laboratories – 5 unstained slides, each with 5 micron thick specimen (Refer to the *QualTek MISP Sample Handling Manual* for instructions regarding the preparation and shipping of tumor samples.)
- Multiplex immunofluorescence assays – 2 unstained slides
- Samples (2 unstained slides for each patient) will be analyzed using the Vectra microscope with validated antibodies according to standard operating procedures.

11.5 Correlative Blood Samples

11.5.1 Blood Sample Collection Time Points

Blood samples will be collected at the following time points:

- Prior to initiation of study treatment (after study registration)
- After the last dose of decitabine (before the first pembrolizumab dose)
- After the second dose of pembrolizumab (before initiation of standard neoadjuvant chemotherapy)

11.5.2 Blood Sample Collection, Labeling, and Analysis

Refer to MCC-15-11083 Biospecimen Manual

11.6 Tracking Tumor and Blood Samples

Collection and distribution of all samples will be logged by the study team at the participating site in OnCore.

12 STUDY CALENDAR

The schedule of tests, exams, assessments, collection of samples for correlative studies, and administration of study drugs are listed on 2 tables: requirements during screening and after registration (before treatment begins) on Table 9; requirements during neoadjuvant decitabine and pembrolizumab on Table 10; and cohort-specific requirements during standard neoadjuvant chemotherapy through end of study on Table 11 and Table 12.

Table 9. Study Requirements During Screening and Prior to Initiating Study Treatment (All Cohorts)

Assessments and Other Requirements	Screening *(Prior to Study Registration)*				After Study Registration
	Within 24 Weeks	**Within 12 Weeks**	**Within 4 Weeks**	**Within 72 Hours** *(Prior to Initiation of Treatment)*	**Before Treatment Begins**
Informed Consent			X[Error! Reference source not found.]		
Demographics			X		
Height			X		
Weight			X		
Vital Signs			X		
Performance Status[Error! Reference source not found.]			X		
Medical/Surgical History[C]			X		
Baseline Conditions			X		
Concurrent Medications[D]			X		
Physical Examination			X		
Tumor Assessment and Measurement[E]			X		
CBC, Differential, Platelet Ct			X		
Serum Chemistry[F]			X		
TSH, Cortisol		X			
Serum Pregnancy Test[G]				X	
Ipsilateral Breast Imaging[H]		X			
Contralateral Breast Imaging	X				
ECG		X			
2-D echocardiogram (or MUGA)		X			
Correlative Blood Sample					X[I]
Core Needle Biopsy for Correlative Tumor Samples					X[J]

Table 9 Footnotes:

A. If a patient has been consented but not registered within 4 weeks, reconsenting is not required unless a new IRB-approved version of the consent form is available.
B. Refer to Appendix 1 for ECOG criteria.
C. Assessment of Medical History will include, if known, tumor genomic profile (ie, MammaPrint, Oncotype, other).
D. Include over-the-counter medications.
E. **Physical exam** will be used to assess and measure the primary breast tumor and regional lymph nodes to determine clinical response after all neoadjuvant therapy as described in Section 10.3. Refer to Section **Error! Reference source not found.** regarding eligibility.
F. Chemistry includes the following: basic metabolic panel (sodium, potassium, carbonate, chloride, glucose, calcium, BUN, creatinine) and hepatic panel (ALT, AST, ALP, total bilirubin, direct bilirubin, albumin, total protein).
G. Only required for WCBP (see Section **Error! Reference source not found.** for definitions and related requirements).

H. Mammogram required at baseline but may not be used for tumor measurements. MRI (or ultrasound) also required at baseline and may be used for tumor measurements for eligibility purposes
I. Baseline blood sample can be collected on treatment day 1 but must be prior to administration of decitabine (see Section 11.5).
J. For convenience, the first research biopsy could be performed at the time of VAD placement; refer to Section 11.3 and provided MCC-15-11083 Biospecimen Manual for additional instructions.

Table 10. Study Requirements During Neoadjuvant Decitabine and Pembrolizumab Treatment Through Initiation of Standard Neoadjuvant Chemotherapy (All Cohorts)

Assessments and Other Requirements	Neoadjuvant Window Treatment Study Day (± 3 days)						Early Discontinuation (within 10 days) (*Section 6.13.1*)
	1[A]	2, 3, 4	8[B]	22[C]	25-29[D]	26-49[D]	
Weight	X		X	X			X
Vital Signs	X		X	X			X
Performance Status[E]	X		X	X			X
Physical Examination			X	X			X
Concurrent Medications[F]			X	X	X (performed prior to initiation of standard chemotherapy)		X
AE Assessment[G]			X	X			X
CBC, Differential, Platelet Count	X		X	X			X
Blood Chemistry[H]	X		X	X			X
Decitabine Administration[I]	X[J]	X[J]					
Correlative Blood Sample[K]			X		X		
Pembrolizumab Administration[I]			X[L]	X[L]			
Core Needle Biopsy for Correlative Tumor Samples					X[M]		
TSH, Cortisol						X[N]	X
Initiation of Standard Neoadjuvant Chemotherapy						X[O]	

Table 10 Footnotes

A. Except for the pregnancy test, which must be performed within 72 hours prior to initiation of treatment, Day 1 assessments do not need to be repeated if done within 14 days prior to initiation of study treatment.
B. Prior to administration of the first pembrolizumab dose.
C. Assessments, exams, and lab tests may be performed within 3 days prior to day 22 or on day 22 (prior to administration of pembrolizumab). Dose may be delayed up to day 29 (Section 6.6.1). If the 2nd dose of pembrolizumab must be delayed, day 22 requirements may be performed within 3 days before or on the day the 2nd dose of pembrolizumab is to be administered.
D. Procedures timed relative to the last dose of pre-chemotherapy pembrolizumab may shift accordingly.
E. See Appendix 1 for ECOG criteria.
F. During the follow-up visits, only capture medication required for managing (late-occurring) irAEs.
G. Assessment and reporting based on the NCI CTCAE v5.0. Refer to Sections 8.5, 8.6, 8.8, 8.10, and 8.11 regarding expedited reporting.
H. Chemistry includes the following panels: basic metabolic panel (sodium, potassium, carbonate, chloride, glucose, calcium, BUN, and creatinine) and the hepatic panel (ALT, AST, ALP, total bilirubin, direct bilirubin, albumin, and total protein).
I. Refer to Table 1 in Section 6.4 for a summary of the treatment regimen; for decitabine instructions refer to Sections 6.5 and 9.1; for pembrolizumab instructions refer to Sections 6.6 and 9.2.
J. Decitabine will be given daily for a total of 4 doses (see Section 6.5 for additional instructions).
K. See Section 11.5.
L. The first dose of pembrolizumab should be administered on day 8 but may be delayed until day 15; the second dose of pembrolizumab should be administered 2 weeks after the first dose (see Section 6.6 for additional instructions).

M. Performed 3-7 days after administration of the 2nd dose of pembrolizumab (before neoadjuvant chemotherapy begins); refer to Section 11.3 for instructions regarding the collection and initial processing of correlative tumor sample.

N. Performed prior to initiation of standard neoadjuvant chemotherapy

O. As soon as possible after the biopsy (if blood counts and chemistries are acceptable for administration of chemotherapy).

Table 11. Study Requirements From Standard Neoadjuvant Chemotherapy through End of Study (Cohorts A and B)

Assessments and Other Requirements	During Standard Neoadjuvant Chemotherapy[A] *(Section 6.7)*	Prior to Surgery (if applicable)	Breast-Conserving Surgery or Mastectomy with Axillary Staging (if applicable)	30-Day Follow-Up[B] (± 10 days)	6-Month irAE Follow-Up[B,C] (±30 days)	12-Month Follow-up[B] (± 30 days)	Early Discontinuation (within 10 days) *(Section 6.13.1)*
TSH, Cortisol	X[D]			X	X		X
Initiation of Standard Neoadjuvant Chemotherapy	X[E]						
AE Assessment[F]	X	X		X[G]	X		X
Weight							X
Vital Signs							X
Performance Status[H]							X
Physical Examination		X		X			X
Ipsilateral Breast Imaging		X		X[I]			
Tumor Measurement		X[J]		X[I,J]			
Concurrent Medications[K]					X		X
CBC, Differential, Platelet Count							X
Blood Chemistry[L]							X
Submission of Samples from Surgical Resection[M]				X			
Determination of pCR and RCB[N]				X			
Survival Status					X[O]	X[O]	

Table 11 Footnotes

A. Except for TSH and cortisol, tests and exams during neoadjuvant chemotherapy are at the discretion of the treating medical oncologist.

B. Refer to Section **Error! Reference source not found.**.

C. For all patients who received one or both pembrolizumab doses, assess at 6 months (± 30 days) after surgery or the decision not to undergo surgery by in-person visit and/or medical record review: (1) the status of irAEs noted at the 30-day time point as having a steroid management requirement (an ongoing requirement for steroid management of previously known irAEs will be noted), (2) the occurrence, in the interim since the 30-day time point, of any new (late-occurring) irAE(s) requiring steroid management (an ongoing requirement for steroid management of new [late-occurring] irAEs will be noted). Note: 6-month irAE assessment is NOT required for patients who received postoperative chemotherapy and/or immunotherapy.

D. TSH and cortisol tests are to be done prior to initiation of standard neoadjuvant chemotherapy and between the standard neoadjuvant chemotherapy regimens.

E. As soon as possible after the second research biopsy (if blood counts and chemistries are acceptable for administration of chemotherapy).

F. Assessment and reporting based on the NCI CTCAE v5.0. Refer to Sections 8.5, 8.6, 8.8, 8.10, and 8.11 regarding expedited reporting.

G. Refer to Sections 8.4 and 8.11 for reporting requirements that may extend beyond 30 days.

H. See Appendix 1 for ECOG criteria.

I. For those patients who did not proceed to surgery.

J. **Physical exam** will be used to assess and measure the primary breast tumor and regional lymph nodes following completion of all neoadjuvant therapy (prior to surgery).

K. During the follow-up visits, only capture medication required for managing (late-occurring) irAEs.

L. Chemistry includes the following panels: basic metabolic panel (sodium, potassium, carbonate, chloride, glucose, calcium, BUN, and creatinine) and the hepatic panel (ALT, AST, ALP, total bilirubin, direct bilirubin, albumin, and total protein).

M. If there was no residual tumor (ie, pCR), submission of samples of the tumor bed is required (see Section 11.4).

N. In addition to determining pCR (breast and nodes), the RCB Index will be calculated locally and entered into the OnCore database for all patients who have residual disease (see Section 10.2.3).

O. Capture disease status per Section 6.13.5.

Table 12. Study Requirements From Standard Neoadjuvant Chemotherapy through End of Study (Cohort A2)

Assessments and Other Requirements	During Standard Neoadjuvant Chemotherapy with Pembrolizumab[A] *(Section 6.7)*	Prior to Surgery (if applicable)	Breast-Conserving Surgery or Mastectomy with Axillary Staging (if applicable)	30-Day Follow-Up[B] (± 10 days)	Adjuvant Treatment Phase[C] (± 3 days) C1-≤C8 D1	30 Day Safety Follow-Up[B] (± 10 days)	12-Month Follow-up[B,D] (± 60 days)	Early Discontinuation (within 10 days) *(Section 6.13.1)*
TSH, Cortisol	X[E]			X		X		X
Initiation of Standard Neoadjuvant Chemotherapy	X[F]							
Pembrolizumab Administration	X				X			
AE Assessment[G]	X	X		X[H]	X	X[H]	X	X
Weight					X			X
Vital Signs					X			X
Performance Status[I]					X			X
Physical Examination		X		X		X		X
Ipsilateral Breast Imaging		X		X[J]		X[J]		
Tumor Measurement		X[K]		X[J,K]		X[J,K]		
Concurrent Medications[L]				X	X	X	X	X
CBC, Differential, Platelet Count					X	X		X
Blood Chemistry[M]					X	X		X
Submission of Samples from Surgical Resection[N]				X				
Determination of pCR and RCB[O]				X				
Survival Status							X[P]	

Table 12 Footnotes

A. Except for TSH and cortisol, tests and exams during neoadjuvant chemotherapy are at the discretion of the treating medical oncologist.

B. Refer to Section **Error! Reference source not found.**.

C. In general, assessments/procedures are performed on Day 1 of each cycle prior to dosing of any study treatment unless otherwise specified. Each treatment cycle is 3 weeks (21 days) for up to 8 cycles. Initiation of adjuvant therapy should begin 30-60 days post-surgery.

D. Assess at 12 months (± 30 days) after surgery or the decision not to undergo surgery by in-person visit and/or medical record review: (1) the status of irAEs noted at the 30-day time point as having a steroid management requirement (an ongoing requirement for steroid management of previously known irAEs will be noted), (2) the occurrence, in the interim since the 30-day time point, of any new (late-occurring) irAE(s) requiring steroid management (an ongoing requirement for steroid management of new [late-occurring] irAEs will be noted). Note: 12-month irAE assessment is NOT required for patients who received postoperative chemotherapy and/or immunotherapy off study.

E. TSH and cortisol tests are to be done prior to initiation of standard neoadjuvant chemotherapy and between the standard neoadjuvant chemotherapy regimens.

F. As soon as possible after the biopsy (if blood counts and chemistries are acceptable for administration of chemotherapy).

G. Assessment and reporting based on the NCI CTCAE v5.0. Refer to Sections 8.5, 8.6, 8.10, and 8.11 regarding expedited reporting.

H. Refer to Sections 8.4 and 8.11 for reporting requirements that may extend beyond 30 days.

I. See Appendix 1 for ECOG criteria.

J. For those patients who did not proceed to surgery.

K. **Physical exam** will be used to assess and measure the primary breast tumor and regional lymph nodes following completion of all neoadjuvant therapy (prior to surgery).

L. Include over-the-counter medications. During the follow-up visits, only capture medication required for managing (late-occurring) irAEs.

M. Chemistry includes the following panels: basic metabolic panel (sodium, potassium, carbonate, chloride, glucose, calcium, BUN, and creatinine) and the hepatic panel (ALT, AST, ALP, total bilirubin, direct bilirubin, albumin, and total protein).

N. If there was no residual tumor (ie, pCR), submission of samples of the tumor bed is required, except for those who do not proceed to surgery (see Section 11.4).

O. In addition to determining pCR (breast and nodes), the RCB Index will be calculated and reported by the pathology department. If RCB has not been calculated by the pathology department, information required for calculation of RCB is to be submitted to the Coordinating Center for calculation of RCB (see Section 10.2.3).

P. Capture disease status per Section 6.13.5.

13 STATISTICAL CONSIDERATIONS

13.1 Study Design

This is a single-arm phase 2 study of decitabine followed by pembrolizumab in the treatment of patients with locally advanced TNBC or ER-positive and/or PgR-positive, HER2-negative breast cancer. The primary objective is to determine if treatment with neoadjuvant decitabine followed by pembrolizumab increases lymphocyte infiltration into tumor and/or stroma in patients with locally advanced, HER2-negative breast cancer in each of the cohorts.

13.2 Sample Size and Accrual Rate

According to Loi et al, each 10% increase in TILs was associated with a 13% reduction in distant recurrence in TNBC (29), and according to Denkert et al and Issa-Nummer et al, each 10% increase in tumor or stromal TIL infiltration was associated with a significant increase (hazard ratio of 1.2) in pCR to neoadjuvant chemotherapy (34, 36).Therefore, this study is powered to detect a 10% increase in TILs from baseline to the post-treatment period. Among the TNBC patients (Cohort A), the range of the percentage of stromal lymphocyte infiltration was 3 to 85 (29), and using the range rule that SD=(min-max)/4, the approximate standard deviation (SD) is 20.5%. A sample size of 28 achieves 80% power to detect a mean of paired differences of 0.10 with an estimated SD of difference of 0.205 with a significance level of 0.05 using a one-sided paired t-test. To allow for the possibility that 10% of patients enrolled may be inevaluable, we anticipate enrolling 32 patients in order to ensure that 28 in Cohort A will be evaluable.

Among the hormone receptor-positive patients (Cohort B), the range of stromal lymphocyte infiltration was 0.5 to 72.5 (29), and using the range rule that SD=(min-max)/4, the approximate SD is 18%. A sample size of 18 achieves 80% power to detect a mean of paired differences of 0.11 with an estimated SD of difference of 0.18 with a significance level of 0.05 using a one-sided paired t-test. As Cohort A is the primary target group in this trial given that TILs are significantly more likely to be observed in TNBC, Cohort B will not over-enroll past N=18 patients to account for inevaluability.

Based on previous recruiting history, we anticipate that 2-3 patients will be enrolled per month over a period of about 24 months. With the addition of 3 months during which accrual will be held while the last patient enrolled in the safety lead-in phase completes dose-dense AC, the enrollment period was expected to be about 27 months. However, based on the initial recruitment experience and the addition of the extended pembrolizumab option, the expected enrollment period has been extended to 72 months from the time of the first patient enrollment.

13.3 Analysis Populations

13.3.1 Safety Lead-In Population

The first 11 patients who have received at least 3 doses of decitabine and at least 1 dose of pembrolizumab will be evaluable for safety during the safety lead-in phase.

13.3.2 Safety-Evaluable Population

Patients who have received at least 3 doses of decitabine and at least 1 dose of pembrolizumab will be evaluable for safety and toxicity analyses.

13.3.3 Efficacy-Evaluable Population

- For tumor immune infiltrate analysis:

  Patients who have received at least 3 doses of decitabine and at least 1 pembrolizumab dose and have had procurement of a tumor sample following the second core needle biopsy, ie, the biopsy performed after the last administered dose of pre-chemotherapy pembrolizumab

- For clinical response:

  Patients who have received at least 3 doses of decitabine, at least 1 dose of pembrolizumab, and any chemotherapy, and have had a physical examination to measure the tumor following completion of all neoadjuvant therapy

- For pathologic assessment:

  Patients who have received at least 3 doses of decitabine, at least 1 dose of pembrolizumab, and any chemotherapy, and have had breast surgery

13.4 Statistical Analysis Plan

13.4.1 Analysis of the Primary Endpoint

A one-sided paired t-test will be used to compare baseline and post-treatment percentage of tumor and stromal areas with infiltrating lymphocytes. The paired t-test will be applied separately to Cohorts A and B. Cohort A analysis of the primary endpoint will include patients in Cohort A2.

13.4.2 Analysis of Secondary Endpoints

SAEs and UPs will be reported using frequencies and percentages separately for Cohorts A and B. The total number and percentage of patients with a SAE or UP will be provided as well as the average number of SAEs and UPs for each patient.

McNemar's test will be used to compare patients with LPBC before and after treatment with decitabine and pembrolizumab. The frequency and percent of patients that achieve pCR will be tabulated and a one-sample binomial test will be used to compare the observed proportion to 0.28, the expected pCR rate in the population. All inferential tests will be considered significant at the 0.05 level.

Frequencies and proportions/percents will be reported for pCR in breast and post-therapy lymph nodes, and cCR rate. Descriptive statistics such as the mean, median, SD, and range will be reported for the number of T cells, T-cell subsets, B cells, and MDSCs in tumor samples. The distribution of cell counts and IHC positive cells will be examined to determine whether they can be modeled using a Gaussian, Poisson, or negative binomial model, for which the latter 2 are recommended for count or rate data, particularly when the distribution is skewed. Exploratory regression analyses will be performed to determine whether any baseline demographic or clinical characteristics are significantly associated with cell counts.

13.4.3 Subset Analyses

Subset analyses will be conducted to account for any variability in actual therapy given during the standard neoadjuvant phase.

14 DATA AND SAFETY MONITORING

14.1 Coordinating Center

While patients are on treatment, the Sponsor-Investigator, study team members (eg, site investigators, research nurses, clinical research associates) and Coordinating Center staff will meet at least monthly to review study status. The biostatistician will review data with the Sponsor-Investigator and Coordinating Center staff at least quarterly. This review will include, but not be limited to, reportable events and an update of the ongoing study summary that describes study progress in terms of the study schema. All meetings, including attendance, are documented.

14.2 Monitoring and Auditing

14.2.1 MCC Compliance Office

Compliance specialists in the MCC Compliance Office will provide monitoring and auditing for this study.

14.2.2 Data Safety and Monitoring Committee

The study will be reviewed by the MCC DSMC initially according to the risk level specified by the MCC Protocol Review and Monitoring Committee (PRMC) and then according to a schedule based on study status and quality indicators. The DSMC will review reports provided by the Sponsor-Investigator/study team and the MCC Compliance Office focusing on data integrity and patient safety.

15 REGULATORY COMPLIANCE AND ETHICS

15.1 Ethical Standard

This study will be conducted in conformance with the principles set forth in *The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Patients of Research* (US National Commission for the Protection of Human Patients of Biomedical and Behavioral Research, April 18, 1979).

15.2 Regulatory Compliance

This study will be conducted in compliance with the clinical trial protocol and with federal regulations, as applicable, including: 21 CFR 50 (Protection of Human Patients/Informed Consent); 21 CFR 56 (Institutional Review Boards); 21 CFR 312 (IND Application); and 45 CFR 46 Subparts A (Common Rule), B (Pregnant Women, Human Fetuses and Neonates), C (Prisoners), and D (Children).

15.3 Institutional Review Board

Each participating site must provide for the review and approval of this protocol and the associated informed consent documents and recruitment material by an appropriate IRB registered with the Office for Human Research Protections (OHRP). Any amendments to the protocol or consent materials must also be approved. Only institutions holding a current US Federalwide Assurance issued by OHRP may participate.

15.4 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Discussion of risks and possible benefits of this therapy will be provided to patients and their families. Consent forms describing the study interventions/study procedures, and risks are given to the patient and written documentation of informed consent is required prior to starting intervention/administering study product.

Consent forms will be IRB-approved and the patient will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the patient and answer any questions that may arise. The patient will sign the informed consent document prior to any procedures being done specifically for the study. Patients should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. Patients may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to patients for their records; the original consent form will be maintained in the research records.

15.5 Patient Confidentiality

Patient confidentiality is strictly held in trust by the Sponsor-Investigator, participating investigators, staff, and the sponsor and its agents. This confidentiality includes the clinical information relating to participating patients, as well as any genetic or biological testing.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor- investigator.

The participating site will allow access to all source data and documents for the purposes of monitoring, audits, IRB review, and regulatory inspections. Source documents provided to the Coordinating Center for the purpose of auditing or monitoring will be de-identified and labeled with the study number, patient ID number, and patient initials.

The study monitor or other authorized representatives of the Sponsor-Investigator may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the patients in this study. The clinical study site will permit access to such records.

16 DATA COLLECTION AND MANAGEMENT

16.1 Data Management Responsibilities

The Sponsor-Investigator is responsible for: (i) reviewing SAE reports; (ii) determining if SARs need to be reported to the FDA, and if so, filing the report; (iii) filing annual IND reports; and (iv) filing IND amendments.

The Sponsor-Investigator is responsible for: (i) the overall conduct of the investigation; (ii) ongoing review of trial data including all safety reports; and (iii) apprising participating sites of any UPs.

The responsible investigator at each site is responsible for: (i) the data management at his or her site; and (ii) reporting SAEs, UPs, and other events requiring expedited reporting as described in Sections 8.10 and 8.11.

Any laboratory conducting correlative studies must maintain the laboratory records and documentation (laboratory notebooks, laboratory protocols, print-outs, recordings, photographs, etc.)

16.2 CRFs and Data Collection

MCC will provide standard electronic CRFs (eCRFs) and create study-specific eCRFs to be able to capture all information required by the protocol. The eCRFs will be approved by the Coordinating Center to ensure the most effective data acquisition.

The investigator(s) and study coordinator(s) must maintain source documents for each patient in the study. All information on eCRFs will be traceable to these source documents, which are generally maintained in the patient's file.

All eCRFs should be completed and available for collection within a timely manner, preferably no more than 5-7 days after the patient's visit.

16.3 OnCore Data Entry

Data will be entered into MCC's OnCore database on an ongoing basis by all participating centers via remote access. Sites are responsible for updating data to allow for data compilation and review. Electronic data submissions will be reviewed periodically for data timeliness and accuracy. Sites will be queried periodically and significant problems with delinquency or accuracy may result in suspension of enrollment at a site.

16.4 Study Record Retention

As applicable, study records will be maintained a minimum of 5 years beyond the publication of any abstract or manuscript reporting the results of the protocol or submission of a final report to clinicaltrials.gov.

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APPENDIX 1. PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	**Description**	**Percent**	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (eg, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self; unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about > 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair > 50% of waking hours.	40	Disabled; requires special care and assistance.
		30	Severely disabled; hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX 2. COCKCROFT-GAULT FORMULA

Calculated Creatinine Clearance (Cockcroft-Gault)
Creatinine clearance (mL/min) = [(140 - Age) × Weight in kg ×G] / (Creatinine × 72) G=1 (males); G=0.85 (females)

APPENDIX 3. SUPPORTIVE CARE FOR MANAGEMENT OF ADVERSE EVENTS WITH POTENTIAL IMMUNOLOGIC ETIOLOGY

Appendix 3 provides suggested supportive care measures for the management of AEs with potential immunologic etiology. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. While the steroid dosing/tapering regimen will be at the discretion of the treating physician, the following recommendations may be considered:

- Corticosteroids are given at a dose of 1-2 mg/kg prednisone or equivalent followed by taper.
- Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
- For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid.
- Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

For each disorder, attempts should be made to rule out other causes such as bacterial or viral infection, which might require additional supportive care. It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab. If after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below).

Pneumonitis

- For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

Diarrhea/Colitis

Patients should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

All patients who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.

- For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.
- For **Grade 3 or 4 diarrhea/colitis,** treat with intravenous steroids followed by high-dose oral steroids.

When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)

- Administer anti-hyperglycemic in patients with hyperglycemia
- Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
- Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

Hypophysitis

- For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Initiate hormonal replacements as clinically indicated.
- For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Initiate hormonal replacements as clinically indicated.

When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks

Hyperthyroidism or Hypothyroidism

Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

- **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism)
  - In hyperthyroidism, treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate.
  - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
- **Grade 3-4** hyperthyroidism
  - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate.

When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

Hepatic

- For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values or stabilized (consider weekly); treat with IV or oral corticosteroids.
- For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours; monitor liver function tests more frequently until returned to baseline values or stabilized (consider weekly).

When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

Renal Failure or Nephritis

- For **Grade 2** events, treat with corticosteroids.
- For **Grade 3-4** events, treat with systemic corticosteroids.

When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

Myocarditis

- Based on severity of event, treat with systemic corticosteroids.
- For **Grade 3-4** events, initiate IV corticosteroids followed by oral corticosteroids.

When symptoms improve ≤ Grade 2, steroid taper should be started and continued over no less than 4 weeks

All other immune-related AEs

- For **Grade 2** events that are intolerable or persistent, based on type and severity of event, treat with systemic corticosteroids
- For **Grade 3-4** events, based on type and severity of event, initiate IV corticosteroids followed by oral corticosteroids.

When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks

APPENDIX 4. RECOMMENDED PROCEDURES FOR EVALUATION OF SURGICAL BREAST SPECIMENS

The following procedures used for processing breast specimens following neoadjuvant chemotherapy at MD Anderson Cancer Center are provided as guidelines for participating investigators.

Specimens should be oriented with sutures by the surgeon following removal. The surgeon and breast pathologist should confer to ensure optimal evaluation of the primary tumor site for possible pCR.

- ***In cases showing significant clinical response in the breast***
  - The breast resection specimen is radiographed to identify metallic markers which were placed during or prior to chemotherapy.
  - Each specimen is inked using multiple colors to identify each face of the specimen, and then sectioned into 3-5 mm slices.
  - The sliced specimen is radiographed and a radiologist reviews the films to determine the presence and extent of residual tumor.
  - The pathologist examines the sliced specimen grossly to identify suspicious areas and notes their proximity to margins.
  - The radiographic and pathologic evaluation is discussed with the surgeon who decides whether additional margins should be obtained.
  - Permanent paraffin sections of the suspicious areas and margins are obtained. The number of sections taken is based on the gross inspection, radiologic features, and size of the resection specimen.
  - The entire radiographic abnormality as well as firm and suspicious appearing breast tissue is submitted for histologic evaluation.
  - In general, for non-palpable (clinical complete response) cases at least 10-15 blocks are examined to assess the presence of residual microscopic disease.
- ***In cases with residual palpable mass (partial clinical response or no response in the breast)***
  - The resection specimen is inked and sectioned into 3-5 mm slices.
  - The pathologist examines the slices and determines the tumor size on gross evaluation and confirms the tumor size by microscopic evaluation.
- ***Evaluation of axillary lymph nodes regardless of response***

  All axillary lymph nodes are also carefully evaluated by serial gross sectioning.
  - One or 2 representative histologic sections are evaluated for lymph nodes that contain grossly identifiable metastatic carcinoma.
  - The lymph nodes that do not show grossly identifiable tumor are submitted for histologic evaluation in their entirety. One representative histologic section is evaluated per paraffin block. Immunohistochemical staining for cytokeratin is not routinely performed on negative nodes.