NCI Protocol #: N/A

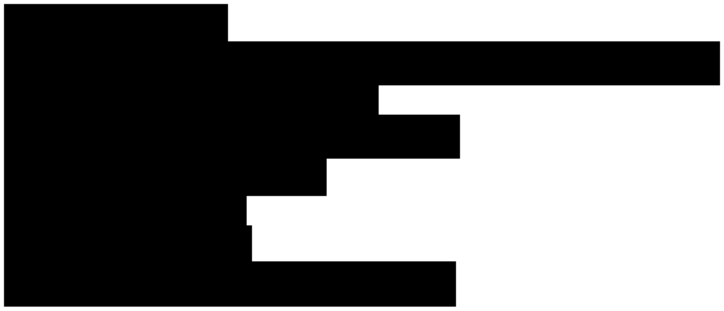
DF/HCC Protocol #: 16-492
TITLE: A Phase 1b study of pembrolizumab in combination with trastuzumab-DM1 in metastatic HER2-positive breast cancer

Coordinating Center: Dana-Farber Cancer Institute

***Principal Investigator (PI):** Sara Tolaney, MD, MPH

Other Investigators:

Statistician:



NCI-Supplied Agent: N/A
Industry-Supplied Agent: Pembrolizumab (MK-3475; Keytruda ™), [redacted]
Commercial Agent: Trastuzumab emtansine (T-DM1; Kadcyla ®), [redacted]

IND #: *132189*
IND Sponsor: *Sara Tolaney, MD MPH*

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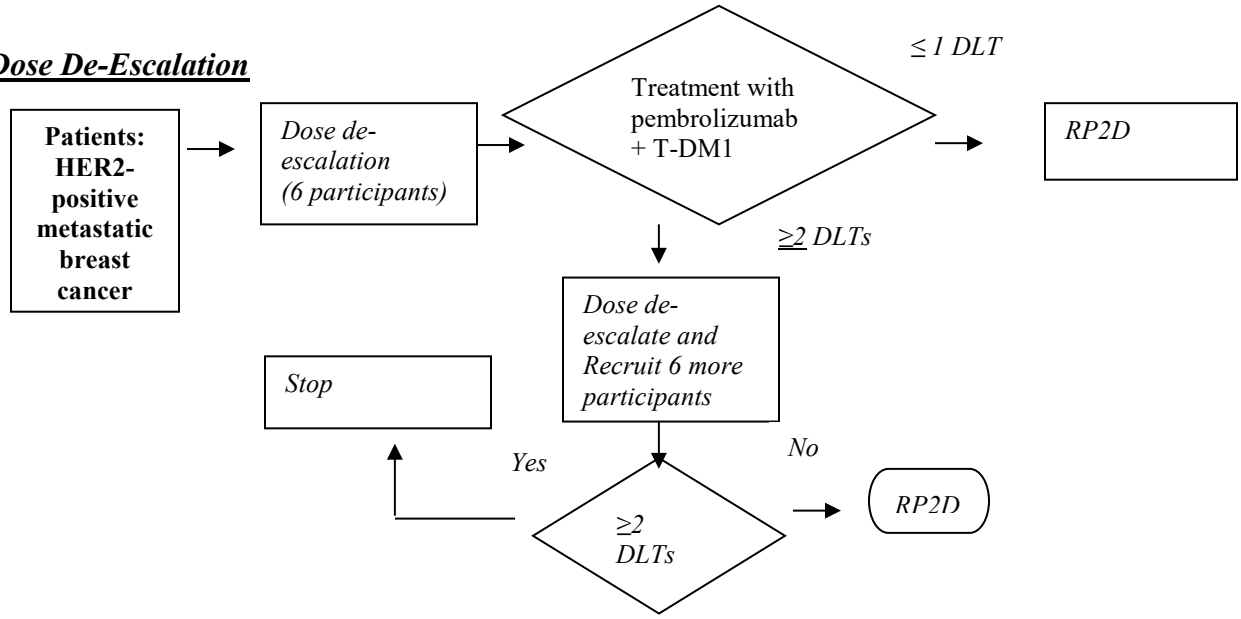
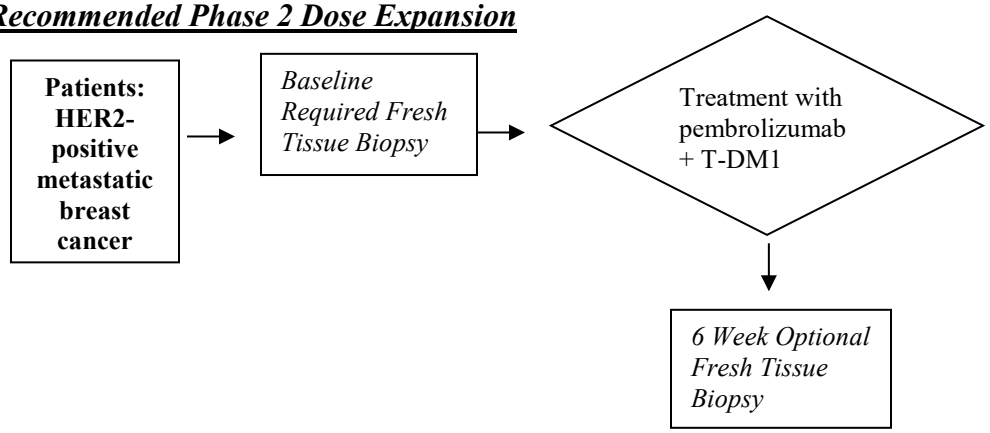
SCHEMA

Dose De-Escalation



Number of participants: 6-12
Cycle Length: 21 days (3 weeks)
Biopsy at baseline and 6 weeks are optional during dose de-escalation
Tumor assessments completed every 6 weeks for the first 6 months and then every 9 weeks.

Recommended Phase 2 Dose Expansion



Number of participants: 15
Cycle Length: 21 days
Biopsy at baseline is required and biopsy at 6 weeks post-treatment initiation is optional
Tumor assessments completed every 6 weeks for the first 6 months and then every 9 weeks.

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1. OBJECTIVES

1.1 Study Design

This is a phase Ib, open label study assessing the safety and tolerability of pembrolizumab in combination with T-DM1. The population to be studied consists of patients with metastatic HER2-positive (HER2+) breast cancer previously treated with a taxane and trastuzumab. The study will be conducted as a dose de-escalation study, as the common toxicities of the two agents are not overlapping. Once the recommended phase 2 dose (RP2D) is declared, 15 additional eligible patients with metastatic HER2+ breast cancer will be entered into an expansion cohort.

1.2 Primary Objectives

To evaluate the safety and tolerability of pembrolizumab in combination with trastuzumab-DM1 (T-DM1) in patients with metastatic HER2+ breast cancer.

1.3 Secondary Objectives

Efficacy objectives:

1. To explore the activity of pembrolizumab in combination with T-DM1, as defined by objective response rate, in patients with metastatic HER2+ breast cancer
2. To explore the activity of pembrolizumab in combination with T-DM1, as defined by progression free survival (PFS), duration of response, disease control rate (defined as partial responses + complete responses + stable disease at 18 weeks), and overall survival (OS) in patients with metastatic HER2+ breast cancer

Correlative science objectives:

1. To characterize a broad array of immune markers in metastatic HER2-positive breast tumors (characterization will be based on histology, protein expression, mRNA expression, and genomic analysis)
   To explore how different immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with disease response to therapy (response assessed by RECIST 1.1 and immune-related RECIST (irRECIST))
2. To characterize changes in immune marker profile in peripheral blood mononuclear cells (PBMCs) from baseline to over the course of trial therapy
3. To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in PBMCs at baseline compared to on-therapy correlates with disease response to therapy (response assessed by RECIST 1.1 and irRECIST)
4. To investigate whether there is an immune marker in circulating PBMCs that corresponds to tumor infiltrating lymphocyte (TIL) percentage in baseline tumor
5. In the cohort of patients who have re-biopsy at week 6 of treatment: To characterize changes in a broad array of immune markers from baseline (as characterized in aim (1)) to 6 weeks on trial therapy, and explore changes in the tumor microenvironment that correlate with disease response to therapy.

6. To characterize the structure and function of the gut microbiome in patients with breast cancer prior to starting this clinical trial.
7. To determine whether pre-treatment characteristics of the structure and function of the gut microbiome in patients with breast cancer is associated with efficacy of pembrolizumab plus T-DM1.
8. To characterize changes in the structure and function of the gut microbiome of patients with breast cancer after two cycles of therapy compared to baseline.
9. To determine whether changes in the overall diversity of the gut microbiome, estimated by the Shannon Index, of patients with breast cancer after two cycles of therapy regimens is associated with efficacy of pembrolizumab plus T-DM1.
10. To determine if the abundance and functional profile of specific gut bacteria are associated with objective response to pembrolizumab plus T-DM1.
11. To evaluate the functional pathways that may play a role as a predictive biomarker of response to pembrolizumab plus T-DM1.
12. To explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel is correlated with patient outcomes (PFS, ORR, and OS). This will be performed on DFCI participants only.

2. BACKGROUND

2.1 Study Disease: Metastatic HER2-Positive Breast Cancer

HER2, also known as *neu* and *c-erbB-2*, is an oncogene encoding a tyrosine kinase growth factor receptor in the family of the epidermal growth factor receptor (EGFR), and is amplified in approximately 20% of all human breast cancers. It is an independent predictor of time to relapse and overall survival in multivariable models, and is a marker of poor prognosis.[1] The functional significance of HER2 amplification in breast cancer prompted development of trastuzumab, a monocloncal anti-HER2 antibody. Addition of trastuzumab to standard chemotherapy produces an overall survival benefit in HER2-amplified metastatic breast cancer.[2] Similarly, trastuzumab plus chemotherapy is superior to chemotherapy alone in multiple large, randomized trials of adjuvant therapy in HER2-positive disease.[3-6]

However, despite significant gains in understanding the biology of HER2-driven cancers and developing highly effective second-generation HER2-targeted agents such as pertuzumab and T-DM1, in the metastatic setting acquired resistance to HER2-directed therapies occurs in virtually all patients. Several mechanisms of resistance have been suggested, including impaired receptor-antibody binding, truncated HER2 protein, and signaling via alternate pathways or through constitutive activation of the PI3K signaling pathway.[7-11] Further therapeutic approaches are therefore needed. As described in more detail below, there is evidence that supports using the anti-tumor immune response as a therapeutic target in HER2+ breast cancer, especially concurrent with antibody-based therapy.

2.2 Pembrolizumab

The PD-1 pathway in cancer

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

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, T regs and Natural Killer 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 subjects with melanoma (MEL). 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

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; MK-3475) has recently been 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. Please refer to the Full Prescribing Information for pembrolizumab for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/.

Clinical data are derived from an ongoing, first-in-human phase I study (PN001, NCT01295827) to evaluate the safety and clinical activity of pembrolizumab as a monotherapy, sponsored by [redacted]. There are five parts to this study (Parts A-D and F) (Investigator's Brochure, 2014).

Part A was a 3+3 dose escalation study in subjects with solid tumors to evaluate safety, tolerability, pharmacokinetics (PK), and pharmacodynamics, and to determine a maximum tolerated dose (MTD) or preliminary recommended phase 2 doses (RP2Ds). Doses were 1, 3, and 10 mg/kg every 2 weeks (Q2W); doses of either 2 mg/kg or 10 mg/kg were also administered every 3 weeks (Q3W). All 3 dose levels were well tolerated and no dose-limiting toxicities (DLTs) were observed; therefore, the MTD was not determined. The RP2D was determined by the sponsor based on safety, PK, and pharmacodynamic measurements, along with the strength of antitumor activity signals observed.

Pharmacokinetics
The half-life ($t_{1/2}$) of pembrolizumab is approximately 4 weeks and there is no indication of dose dependency or half-life in the three dose groups (1,3, and 10 mg/kg) (Investigator's Brochure, 2014). The long $t_{1/2}$ supports a dosing interval of every 2 or 3 weeks.

There was a dose-related increase in exposure from 1 to 10 mg/kg. Serum concentrations of pembrolizumab were lower by a factor of approximately 5 in patients receiving 2 mg/kg Q3W than in those receiving 10 mg/kg Q3W. Steady-state trough concentrations were 20% greater in the patients receiving 10 mg/kg Q2W than in those receiving the same dose Q3W.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the

dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

Anti-Drug Antibodies (ADA) Data
The occurrence of ADA has been observed in less than 1% of the patients screened, indicating a low potential of pembrolizumab to elicit the formation of ADA. No impact of ADA on pembrolizumab exposure has been observed.

Efficacy
When treated with pembrolizumab monotherapy, the overall response rate (ORR) for ipilimumab (IPI)-treated patients with melanoma was 25%/27% according to the Response Evaluation Criteria in Solid Tumors (RECIST)/investigator-assessed immune-related response criteria (irRC), respectively (Investigator's Brochure, 2014). The ORR for IPI-naïve patients with melanoma was 39%/43% by RECIST/investigator-assessed irRC, respectively. The majority of responses were seen in patients with melanoma by 16 weeks of therapy; however, some responses have been reported after 24 weeks or more of therapy with pembrolizumab. Responses can be delayed, and in some patients, a RECIST-defined progression followed by response has been observed.

The preliminary ORR for 38 patients with non-small cell lung cancer was 21%/24% by RECIST/investigator-assessed irRC, respectively (Investigator's Brochure, 2014).

Pharmacodynamics/Biomarkers
Pharmacodynamic data (IL-2 release assay) has suggested that peripheral target engagement is durable (>21 days).

PD-L1 is being investigated as a predictive biomarker for pembrolizumab treatment. At the 15th World Conference on Lung Cancer, Garon et al presented preliminary data on a subset of patients suggesting that higher levels of tumor PD-L1 expression are associated with increased clinical activity.[12] ORR by RECIST 1.1 occurred in 4 out of 7 patients with higher levels of PD-L1 expression (57%, 95% CI 18-90%) versus 2 out of 22 patients with lower levels of PD-L1 expression (9%, 95% CI 1-29%). These data are extremely preliminarily, and PD-L1 is not being used for patient selection.

Biomarkers to evaluate immune modulation and markers in the tumor microenvironment, such as

T-cell infiltration, the baseline expression of markers of T-cell suppression FoxP3 or the immunoregulatory enzyme incoleamine 2,3-dioxygenase (IDO) in tumor biopsies, were associated with a high response rate in patients with advanced melanoma.[13,14]

Safety data
The most frequent treatment-related adverse events (AEs) were fatigue, nausea, cough, pruritis, diarrhea, and rash (Investigator's Brochure, 2014). Most AEs were not considered serious. The most commonly-reported immune-related AEs were rash, pruritis, vitiligo, hypothyroidism, arthralgia, diarrhea, and pneumonitis.

Important identified risks include: pneumonitis, thyroid disorders (hypothyroidism and hyperthyroidism), colitis, diarrhea, hepatitis, nephritis, uveitis, rash/pruritis, and neuropathy.

2.3 T-DM1

T-DM1 mechanism of action
T-DM1 is a promising anti-HER2 agent, with proven efficacy in the metastatic setting. T-DM1 is composed of: trastuzumab, a humanized monoclonal antibody that binds to domain IV on the juxtamembrane region of the extracellular domain (ECD) of HER2 and inhibits tumor growth *in vitro* and *in vivo* via several mechanisms;[15-17] DM1, a potent anti-microtubule agent derived from maytansine; and SMCC, a thioether linker molecule used to conjugate DM1 to trastuzumab. T-DM1 retains all of the modes of action of trastuzumab, which include a binding affinity to the HER2 ECD that is comparable to trastuzumab, inhibition of HER2-ECD shedding, inhibition of the HER2-activated PI3K/Akt signaling pathway, and mediation of antibody-dependent cell-mediated cytotoxicity (ADCC).[18] Linkage of a cytotoxic agent to highly specific monoclonal antibodies targeting tumor-specific and/or overexpressed tumor-associated antigens focuses the delivery of such agents to tumor cells, which creates a more favorable therapeutic window than could be achieved by their administration as free drugs, and may facilitate combinations with different agents. After binding to HER2, T-DM1 undergoes receptor-mediated internalization, followed by intracellular release of DM1 and subsequent cytotoxicity. Activation of cytotoxicity of the conjugate requires that it be internalized into the cell after binding to HER2. The non-internalized conjugate remains inactive, thus limiting the systemic toxicity.

T-DM1 clinical efficacy data
In multiple clinical phase II and III studies, T-DM1 as a single agent has demonstrated robust and clinically meaningful activity, as well as favorable toxicity in patients with HER2+ metastatic breast cancer (MBC). Two phase II studies—TDM4258g and TDM4374g—evaluated the safety and efficacy of T-DM1 administered at a dose of 3.6 mg/kg (maximum tolerated dose [MTD] in Phase I) every 3 weeks until progressive disease (PD) or unacceptable toxicity in HER2+ MBC who had progressed on previous HER2-directed therapy and conventional chemotherapy.[19,20] Patients enrolled onto study TDM4374g were specifically required to have received an antrhacycline, trastuzumab, a taxane, lapatinib, and capecitabine in the neoadjuvant, adjuvant, locally advanced or metastatic setting. Additionally, patients must have been treated with two or more HER2-directed regimens in the locally advanced or metastatic setting, and must have progressed on their most recent treatment.

The clinical activity of T-DM1 was similar in the two studies. In Study TDM4258g, on the basis of the final analysis approximately 12 months after the last patient was enrolled, the overall response rate (ORR) in efficacy evaluable patients was 38.9% (95% confidence interval [CI], 29.7%, 48.5%) by investigator and 26.9% (95% CI, 19.2%, and 35.8%) by independent review. The clinical benefit rate (CBR) (defined as complete response [CR], partial response [PR], or stable disease for > 6 months) was 46.3% by investigator assessment (95% CI, 36.7%, 56.2%) and 40.7% by independent review (95% CI, 31.8%, 50.6%). The median progression-free survival (PFS) was 4.6 months by both the investigators and the independent review facility (IRF) assessment. In Study TDM4374g, on the basis of clinical data collected through 1 January 2010, approximately 9 months after the last patient had enrolled, the ORR among all treated patients was 34.5% (95% CI, 26.1%, 43.9%) by IRF assessment and 32.7% (95% CI, 24.1%, 42.1%) by investigator assessment. The CBR was 48.2% (95% CI, 38.8%, 57.9%) by IRF assessment and 46.4% (95% CI, 37.1%, 56.1%) by investigator assessment. The median duration of response was not reached (95% CI, 4.6 months, not reached) by IRF assessment and 9.7 months (95% CI, 6.6 months, not reached) by investigator assessment. In this study population, there was a median PFS of 6.9 months (95% CI: 4.2, 9.5) as assessed by the IRF and 5.5 months (95% CI: 4.1, 7.5) by investigator review.

The safety profile of T-DM1 was also similar between the two studies. In Study TDM4258g, the five most common adverse events (AEs) were fatigue (65.2%), nausea (50.9%), headache (40.2%), epistaxis (35.7%), and pyrexia (34.8%). Most of these events were Grade 1–2. The three most common Grade 3–4 AEs observed in this trial were hypokalemia (8.9%), thrombocytopenia (TCP) (8.0%), and fatigue (4.5%). In Study TDM4374g, a total of 49 patients (44.5%) experienced at least one Grade ≥ 3 AE. The three most common Grade ≥ 3 adverse events (by Medical Dictionary for Regulatory Activities [MedDRA] preferred terms) were TCP (7.3%), fatigue (4.5%), and cellulitis (3.6%). Serious adverse events (SAEs) were reported in 25 patients (22.7%). No single SAE was reported in more than 4 patients. No Grade ≥ 3 left ventricular systolic dysfunction (LVSD) events (symptomatic congestive heart failure [CHF] and/or left ventricular ejection fraction [LVEF] of < 40%) were reported in either study.

In the randomized phase III EMILIA study, T-DM1 monotherapy proved superior to the approved regimen lapatinib/capecitabine in patients who had progressed on prior trastuzumab and taxane.[21] Median PFS was 9.6 months in the T-DM1 arm compared with 6.4 months in the capecitabine and lapatinib arm (HR = 0.65, 95% CI; 0.55 to 0.77; p <0.001), and median overall survival (OS) at the second interim analysis crossed the stopping boundary for efficacy (30.9 months vs. 25.1; HR = 0.68, 95% CI; 0.55 to 0.85; p <0.001). Overall, fewer Grade ≥ 3 adverse events were observed in the T-DM1 arm (40.8%) than in the lapatinib arm (57%). The most common Grade ≥ 3 adverse events with T-DM1 were thrombocytopenia (12.9%), elevated AST (4.3%) and ALT (2.9%), anemia (2.7%) and fatigue (2.4%). Based on the EMILIA data, T-DM1 was recently approved by the Food and Drug Administration (FDA) for patients with advanced HER2+ breast cancer that has progressed on trastuzumab-based therapy, with superior overall survival (OS) and a better adverse event profile compared to the lapatinib/capecitabine combination.

T-DM1 will be used in this protocol as per standard FDA indications for the single agent. Please refer to the Full Prescribing Information for T-DM1 for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/.

2.4 Rationale

Accumulating evidence suggests that the immune system plays an important role in control of HER2+ breast cancer, and works with HER2-targeted therapies to do so. High levels of immune infiltrates are observed in HER2-overexpressing breast cancers. Recent studies suggest that monoclonal antibodies such as trastuzumab may stimulate adaptive anti-tumor immunity. Two studies in mice demonstrated that anti-HER2 antibody therapy required adaptive CD8-dependent immunity to mediate its optimal effect.[22,23] It is thought that trastuzumab activates toll-like receptor signaling, priming the release of interferon (IFN) and priming IFN-producing CD8+ T cells. In addition, more recent data suggested that each 10% increase in tumor-infiltrating lymphocytes (TILs) was associated with a significant decrease in distant recurrence in patients receiving adjuvant trastuzumab,[24] further suggesting the importance of antitumor immunity in the HER2+ subset of breast cancer. In HER2+ breast cancer patients, therefore, combining cytotoxic chemotherapy (which provokes tumor antigen recognition) with anti-HER2 monoclonal antibodies (which stimulate adaptive immunity) and checkpoint inhibitors (which enhance adaptive antitumor immune responses by preventing T cell exhaustion) is an attractive, rational approach for maximizing the antitumor immune response to advance the treatment of HER2-positive breast cancer.

Trastuzumab emtansine (T-DM1), as described above, is a novel antibody-drug conjugate (ADC) composed of trastuzumab (a humanized antibody directed against the extracellular region of HER2), DM1 (an anti-microtubule cytotoxic agent), and a linker molecule. Pembrolizumab is a checkpoint inhibitor with promising clinical data in melanoma and non-small cell lung cancer, as discussed above, as well as preliminary efficacy data in triple negative breast cancer.[25] The combination of pembrolizumab with T-DM1 has the potential to concurrently: (1) stimulate tumor antigen recognition through use of a cytotoxic; (2) simulate adaptive immunity and ADCC through use of anti-HER2 antibody therapy; and (3) enhance the anti-tumor autoimmune response with an anti-PD-1 antibody. The proposed study will explore the safety and efficacy of this combination in women with advanced HER2+ breast cancer.

2.5 Correlative Studies Background

2.5.1 Blood and Tissue Analysis

There is growing evidence that signatures of tumor-associated immunologic activity are seen in breast cancer and are significant prognostic predictors, particularly in triple negative breast cancer (TNBC) and HER2-positive breast cancer. Many different groups have demonstrated that the amount of tumor-infiltrating lymphocytes (TILs) in a tumor specimen, commonly assessed simply by histological evaluation of a standard hematoxylin and eosin-stained slide by a trained pathologist, is a significant predictor of both response to therapy and overall disease outcomes in the neoadjuvant and adjuvant settings.[26-31] Recently, more in-depth methods of immunologic profiling are being explored in breast cancer, for example mRNA expression of immune-

activating and immunosuppressive factors, and these additional immune profiles also appear to have prognostic significance.[27]

However, the development of immune signatures in breast cancer that go beyond simple histology is at a very early stage. Additionally, much of the work done in this area has been in the adjuvant and neoadjuvant settings, with less known about the immunologic profile of metastatic disease. Given the promise of immune-based therapies in other solid malignancies such as melanoma and non-small cell lung cancer, with preliminary data demonstrating activity of immune checkpoint inhibition in advanced breast cancer patients,[25,32] there is a great need to better characterize the immune profile of breast tumors across multiple disease subtypes, and at different points in the course of therapy. The bulk of our correlative science in this trial focuses on characterizing a broad array of immune markers in metastatic HER2-positive breast cancer, investigating whether those markers predict disease response to therapy, and exploring how markers change from the start T-DM1 plus pembrolizumab treatment to the time of development of progressive disease.

In melanoma, the solid malignancy currently at the forefront of understanding the anti-tumor immune response, investigation into expression of immune mediators in the peripheral blood is at an early phase.[33] As a correlative study to this trial, we will characterize the immune marker profile of peripheral blood mononuclear cells (PBMCs) in enrolled breast cancer patients. Additionally, given the demonstrated clinical significance of TILs in breast cancer specimens, we will investigate whether there is a peripheral marker whose level corresponds to TIL percentage. Lastly, we will evaluate whether there is a correlation between changes in PBMC immune profiles over the course of treatment, and disease response to therapy. Evidence of a correlation would be of significant interest as it would suggest the potential presence of a predictive biomarker in the peripheral blood.

2.5.2 Microbiome Analysis

The gut microbiota has been recognized as a modulator of immune system development[44]. Healthy individuals have microbial populations in their intestinal tract that vary markedly in composition[45,46]. The diversity of intestinal microbiota represents a significant challenge to the host's immune defenses, which must balance immune tolerance of beneficial microbes with inflammatory responses against pathogens. Alterations in the gut microbiota and their resulting interactions with intestinal epithelieum and the host immune system are associated with many disease, including cancer[47]. Recently, two preclinical studies provided to immune checkpoint inhibitors (ICI), raising the possibility that stool microbiota could be used as biomarker predictors of efficacy to immunotherapy[48,49]. Interestingly, postmenopausal women with breast cancer have altered composition and low diversity of their gut microbiota compared to healthy controls[50].

Identification of biomarkers that predict response to ICI-based therapies can spare *de novo* resistant patients from the unnecessary risks of immune-related adverse events. In addition, the identification of bacterial species associated with response could open new strategies to

maximize the clinical benefit of cancer immunotherapy through the modulation of gut microbiota.

This correlative project is made possible by collaboration with the BWH/Harvard Cohorts Biorepository and Microbiome Dx. Further details can be found in Section 9.

2.5.3 Tumor Genomic Profile

In addition to the immune microenvironment, intrinsic tumor factors may be associated with response to immune checkpoint inhibitors. Although some of the mechanisms related to de novo or acquired resistance to ICI have been recently described, including loss of function in beta-2-microglobulin or defects in the interferon signaling pathway[Gao *et al.*, 2016, Zaretsky *et al.*, 2016], the knowledge of immune resistance remains largely unknown. Several gene/pathways have been described as possible candidates of having an immunosuppressive role in different advanced solid tumor, including MYC amplification[Casey *et al.*, 2016], activation in WNT-β-catenin pathway[Spranger *et al.*, 2015], activation in MAPK pathway, loss of PTEN[Li *et al.*, 2016, Peng *et al.*, 2016, George *et al.*, 2017]. On the other hand, few possible biomarkers of response to ICI have emerged, including mutational load[Snyder *et al.*, 2014, Rizvi *et al.*, 2015], tumor aneuploidy[Davoli *et al.*, 2017], mismatch repair defects[Le *et al.*, 2015], and BRCA2 mutation[Hugo *et al.*, 2016]. Notably, there is no data on genomic mechanisms of de novo resistance to anti-PD-1 therapy in patients with breast cancer.

Therefore, as a correlative study to this trial, we will to explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel – OncoPanel - is correlated with patient outcomes (PFS, ORR, and OS). This tool is a cancer genomic assay to detect somatic mutations, copy number variations and structural variants in tumor DNA extracted from fresh, frozen or formalin-fixed paraffin-embedded samples. The OncoPanel assay surveys exonic DNA sequences of 447 cancer genes and 191 regions across 60 genes for rearrangement detection. DNA is isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500 sequencer. The targeted NGS assay (OncoPanel) will be performed at the Center for Advanced Molecular Diagnostics (Department of Pathology, Brigham and Women's Hospital). This assay has been extensively validated and is used as a CLIA-approved clinical molecular test in our institution without any additional sequencing assays to validate the findings [Wagle *et al.*, 2012].

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed invasive breast cancer, with stage IV disease. Patients without pathologic or cytologic confirmation of metastatic disease should have unequivocal evidence of metastasis from physical examination or radiologic evaluation.

3.1.2 Either the primary tumor and/or the metastasis must have been tested for ER, PR and HER2. Patient must have HER2+ breast cancer per ASCO CAP guidelines 2013.

3.1.3 Prior chemotherapy:

- History of prior therapy with trastuzumab and a taxane, separately or in combination, is required.
- Patients must have either received one line of prior therapy for metastatic breast cancer, or have developed a disease recurrence during or within 6 months after completing adjuvant therapy.
- No prior treatment with T-DM1 is allowed.
- Last dose of chemotherapy must be at least 21 days prior to registration.

3.1.4 Prior biologic therapy:

- Patients must have discontinued all biologic or investigational therapy at least 21 days before registration.

3.1.5 Prior radiation therapy:

- Patients may have received prior radiation therapy in either the metastatic or early-stage setting.
- Radiation therapy must be completed at least 14 days prior to registration.

3.1.6 In the dose de-escalation cohort: Subjects must have evaluable disease.
In the expansion cohort: Subjects must have at least one lesion that is not within a previously radiated field that is measurable on computerized tomography (CT) or magnetic resonance imaging (MRI) scan per RECIST version 1.1. Bone lesions are not considered measurable by definition.

3.1.7 Age is ≥18 years.

3.1.8 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A)

3.1.9 Participants must have normal organ and marrow function as defined below:

– absolute neutrophil count	≥1,500/µl
– platelets	≥100,000/µl
– hemoglobin	≥9 g/dL
– total bilirubin	≤1.5mg/dL (≤2.0 in patients with known Gilberts syndrome)
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional ULN. ≤5.0 × institutional ULN for patients with documented liver metastases.
– albumin	>2.5mg/dL
– serum creatinine	≤1.5mg/dL or calculated GFR ≥60 mL/min
– INR/PT	≤1.5 times ULN unless participant is receiving anticoagulant therapy, as long as PT or PTT is within

– aPTT/PTT	therapeutic range of intended use of anticoagulants ≤1.5 times ULN unless participant is receiving anticoagulant therapy, as long as PT or PTT is within therapeutic range of intended use of anticoagulants

3.1.10 Participants enrolling in the dose expansion must have tissue that is amenable to biopsy and be willing to undergo a fresh tissue biopsy at baseline. Participants who undergo an attempted research biopsy procedure for the purpose of this protocol, and in whom inadequate tissue is obtained, are not required to undergo a repeat biopsy in order to continue on protocol.

3.1.11 The effects of pembrolizumab on the developing human fetus are unknown. For this reason and because tratuzumab, a component of T-DM1, is known to be teratogenic, women of child-bearing potential (as defined in Section 5.5.2) and men of childbearing potential must agree to use adequate contraception (as defined in Section 5.5.2) starting with the first dose of study therapy, for the duration of study participation, and for an additional 120 days after the last dose of study medication. Note: abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

- Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, the treating physician and principal investigator should be informed immediately.
- While on the study, women should not breast-feed.
- Subjects of childbearing potential are defined as those who have not been surgically sterilized and/or have had a menstrual period in the past year

3.1.12 Female subject of child-bearing potential, as defined above, must have a negative urine or serum pregnancy within 7 days prior to receiving the first dose of study medication. If a urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

3.1.13 Patients on bisphosphonates may continue receiving bisphosphonate therapy during study treatment.

3.1.14 Left ventricular ejection fraction (LVEF) must be ≥ 50 as assessed by echocardiogram or MUGA documented within 28 days prior to first dose of study drug.

3.1.15 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 The subject has received another investigational agent within 21 days of the first dose of study drug.

3.2.2 The subject has received prior pembrolizumab or any other anti-PD-1 , anti-PD-L1, or anti-PD-L2 therapy, or has participated in any prior studies involving pembrolizumab.

3.2.3 Pre-existing neuropathy greater than or equal to grade 2.

3.2.4 Hypersensitivity to pembrolizumab or T-DM1 or any of their excipients.

3.2.5 The subject has any history or evidence of active, non-infectious pneumonitis or interstitial lung disease.

3.2.6 Known brain metastases that are untreated, symptomatic, or require therapy to control symptoms. Participants with previously diagnosed brain metastases are eligible if they have completed treatment at least one month prior to trial therapy initiation, are neurologically stable with an absence of new neurological symptoms for at least 4 weeks prior to study entry, and have recovered from effects of radiotherapy or surgery. Any corticosteroid use for brain metastases must have been discontinued without the subsequent appearance of symptoms for ≥2 weeks before the first study drug. Treatment for brain metastases may include whole brain radiotherapy, radiosurgery, or a combination as deemed appropriate by the treating physician.

3.2.7 Known carcinomatous meningitis.

3.2.8 The subject has an uncontrolled intercurrent illness including, but not limited to, uncontrolled hypertension, unstable angina pectoris, uncontrolled cardiac arrhythmia, congestive heart failure (New York Heart Association Class III or IV; see Appendix B), active ischemic heart disease, myocardial infarction within the previous six months, uncontrolled diabetes mellitus, chronic liver or renal disease, or severe malnutrition.

3.2.9 Concurrent use of potent CYP3A4 inhibitors (see Appendix C), such as ketoconazole and erythromycin, should be avoided during the study treatment with T-DM1.

3.2.10 Active infection requiring intravenous antibiotics at cycle 1 day 1.

3.2.11 Individuals with a history of a second malignancy are ineligible except for the following circumstances. Individuals with a history of other malignancies are eligible if they have been disease-free for at least 5 years and are deemed by the investigator to be at low risk for recurrence of that malignancy. Individuals with the following cancers are eligible if diagnosed and treated within the past 5 years: cervical cancer *in situ*, and non-melanoma cancer of the skin. Patients with other cancers diagnosed within the past 5 years and felt to be at low risk of recurrence should be discussed with the study sponsor to determine eligibility.

3.2.12 The subject has a medical condition that requires chronic systemic steroid therapy or any other form of immunosuppressive medication including disease modifiying agents. Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

3.2.13 The subject has an active autoimmune disease or a documented history of autoimmune disease or syndrome that requires systemic steroids or immunosuppressive agents.

3.2.14 The participant is positive for Hepatitis B surface antigen, or Hepatitis C RNA.

3.2.15 Known HIV-positive participants. HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with pembrolizumab. In addition, these participants are at increased risk of lethal infections with bone marrow suppressive therapy, i.e. nab-paclitaxel. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.2.16 The subject has received a live vaccine within 28 days of planned start of study therapy. Note: seasonal influenza vaccines for infection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (i.e. Flu-Mist ®) are live attenuated vaccines, and are not allowed.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

N/A

4.4 Registration Process for Other Investigative Sites

N/A

5. TREATMENT PLAN

5.1 Treatment Regimen

The study will be conducted as a dose de-escalation study, as the common toxicities of the two agents are not overlapping. Each cycle will be 21 days in length. Six patients will be entered at dose level 1 (Table 1), consisting of T-DM1 3.6 mg/kg IV every 21 days, in combination with pembrolizumab 200 mg IV every 21 days. If one or fewer dose limiting toxicities (DLTs) are noted within the first six patients, this dose level will be declared the RP2D. If two or more DLTs are found in the first 6 patients, then 6 additional patients will be entered at dose level -1 (Table 1). If one or fewer DLTs are noted at dose level -1, then this will be declared the RP2D. If two or more DLTs are noted at dose level -1, enrollment will be stopped. No dose changes will be explored for pembrolizumab as its toxicity has not been found to be dose-dependent in other clinical contexts.

The DLT period is defined as 21 days from the first dose of therapy (i.e. the first cycle of therapy). All six patients enrolled to the dose level must complete the DLT period before additional patients can be enrolled.

Once the RP2D is declared, 15 eligible patients with metastatic HER2+ breast cancer will be entered into an expansion cohort.

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7.1. Appropriate dose modifications are described in Section 6. Details of the regimen are described in Table 2. No investigational or commercial agents other than those described below may be administered with the intent to treat the participant's malignancy.

Table 1: Dosing levels for dose de-escalation

Dose De-Escalation Schedule		
Dose Level	**Dose**	
	Pembrolizumab (mg)	**T-DM1** (mg/kg)
Level 1: starting dose	200	3.6
Level -1	200	3.0

Table 2: Regimen description

Regimen Description					
Agent	**Premedications**	**Dose**	**Route**	**Schedule**	**Cycle**

	; Precautions				**Length**
Pembrolizumab	Not routinely necessary unless prior infusion reaction.	200 mg, at a final concentration of 1 mg/mL to 10 mg/mL in NS or D5	IV over approximately 30 minutes (range: 25-40 minutes).	Day 1	21 days (3 weeks)
T-DM1	Not routinely necessary unless prior infusion reaction.	Per Table 1*	IV over approximately 90 minutes (cycle 1) and 30 minutes (subsequent cycles). Start T-DM1 infusion after pembrolizumab infusion is complete.	Day 1	
**Doses as appropriate for assigned dose level*					

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1 and Subsequent Cycles, Day 1

Eligibility criteria do not need to be re-met at cycle 1 day 1.

Criteria to treat at cycle 1 day 1:

- **Absolute neutrophil count ≥1500/µl**
- **Platelets ≥100,000/µl**
- **ALT and AST ≤2.5 x ULN in a patient with no documented liver metastases; ALT and AST ≤5.0 x ULN in a patient with documented liver metastases**
- **Total bilirubin ≤1.5 x ULN (2.0 x ULN in a patient with well documented Gilbert's syndrome)**

Criteria to treat at day 1 of subsequent cycles:

- **Absolute neutrophil count ≥1000/µl**
- **Platelets ≥75,000/µl**
- **ALT and AST ≤3.0 x ULN in a patient with no documented liver metastases; ALT and AST ≤5.0 x ULN in a patient with documented liver metastases**
- **Total bilirubin ≤1.5 x ULN (2.0 x ULN in a patient with well documented Gilbert's syndrome)**

5.3 Agent Administration

5.3.1 Pembrolizumab

Pembrolizumab will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through documentation of study treatment administration in the subject's medical record.

Pembrolizumab will be administered in clinic on day 1 of each 3-week cycle (+/- 3 days). It will be administered as a 30 minute IV infusion. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

Pembrolizumab should be administered prior to T-DM1 administration. There should be no overlap in timing of the two administrations; however, there is no required observation period prior to beginning T-DM1 administration.

5.3.2 T-DM1

T-DM1 administration

T-DM1 will be used as per the FDA label. T-DM1 will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through documentation of study treatment administration in the subject's medical record.

T-DM1 will be administered in clinic on day 1 of each 3-week cycle (+/- 3 days). The total

dose of T-DM1 will be calculated according to institutional standards.

The first infusion of T-DM1 will be administered over 90 minutes (+/- 10 minutes). Infusions may be slowed or interrupted for patients experiencing infusion-associated symptoms. Following the initial dose, patients will be observed for at least 60 minutes for fever, chills, or other infusion-associated symptoms. Subsequent doses of T-DM1 may be administered over 30 minutes (+/- 10 minutes) with a 30-minute post-infusion observation if the first infusion was well tolerated. Local health authority guidelines must be followed with regard to further observation and monitoring, if applicable. Dose modifications for T-DM1 toxicity management are listed in Section 6, and Section 6, Table 6.

Premedication for nausea and infusion reactions are not commonly required but may be given at the investigator's discretion.

5.4 Definition of Dose-Limiting Toxicity (DLT)

Dose-limiting toxicity is defined as any of the following events occurring within 21 days (1 cycle) after the first dose of therapy, if judged by the investigator to be possibly, probably, or definitely related to study drug administration:

- Death
- Asymptomatic grade 4 hematologic toxicity lasting ≥14 days unless deemed by the investigator to be clinically insignificant
- Grade 4 thrombocytopenia of any duration
- ≥ Grade 3 Febrile neutropenia
- ≥ Grade 3 Thrombocytopenia if associated with bleeding
- ≥ Grade 3 elevation in AST or ALT associated with a grade 2 elevation in bilirubin that is at least possibly related to study drug (Hy's Law)
- ≥ Grade 3 non-hematologic laboratory value <u>if:</u>
    - Medical intervention is required to treat the patient, or
    - The abnormality leads to hospitalization, or
    - The abnormality persists >7 days

  <u>Excluding:</u>
    - Alkaline phosphatase <u>≤</u>10.0x ULN in a patient with grade 2 alkaline phosphatase elevation at baseline as a result of bone metastasis
    - Any laboratory values deemed by the investigator to be clinically insignificant
- ≥ Grade 3 pneumonitis of any duration
- ≥ Grade 3 Fatigue lasting >5 days
- ≥ Grade 3 other non-laboratory toxicity lasting >3 days despite optimal supportive care, excluding the following:
    - Alopecia of any grade

DLTs will be assessed during the DLT assessment window (lasting for 1 cycle, or 21 days after

initiation of study therapy). The dose de-escalation plan is described in Section 5.1.

Management and dose modifications associated with the above adverse events are outlined in Section 6.

Number of Participants with DLT at a Given Dose Level	Escalation Decision Rule
0 -1 out of 6	Declared as RP2D
≥2 out of 6	6 additional patients will be enrolled at dose level -1. • If 1 or fewer experience DLT at dose level -1, this will be declared as the RP2D. • If 2 or more of the group at dose level -1 experience DLT, enrollment will be stopped.

5.5 General Concomitant Medication and Supportive Care Guidelines

5.5.1 Concomitant Medication Guidelines

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Principle Investigator.

Acceptable Concomitant Medications
All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care.

Prohibited Concomitant Medications
Participants should discontinue the following therapies 21 days prior to enrollment and must remain off them during protocol therapy:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Biologic or targeted agents not specified in this protocol
- Investigational agents other than pembrolizumab

Participants should have completed radiation therapy 14 days prior to enrollment, if radiation therapy to a symptomatic solitary lesion or to the brain is required while on protocol therapy it may be allowed at the principle investigator's discretion.

Participants are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Any systemically active oral, injected, or implanted hormonal method of contraception except for progesterone coated intrauterine devices (IUDs) that had been previously implanted.

- Estrogen replacement therapy.

- Live vaccines within 28 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

- Systemic glucocorticoids for any purpose other than to modulate symptoms from an adverse event of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

Concomitant use of strong CYP3A4 inhibitors (e.g., ketoconazole and itraconazole; see Appendix C) with T-DM1 should be avoided. An alternate medication with no or minimal potential to inhibit CYP3A4/5 should be considered. If a strong CYP3A4/5 inhibitor is co-administered with T-DM1, patients should be closely monitored for adverse reactions.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.5.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age not using hormonal contraception or hormonal replacement therapy a high follicle stimulating hormone (FSH) level in the postmenopausal range is required to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a FSH measurement is insufficient.);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) have a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to practice abstinence (if it is consistently employed as the subject's preferred and usual lifestyle; however, periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable) from heterosexual activity or use acceptable contraception (as defined below) during heterosexual activity while receiving protocol therapy and up to 120 days after the last dose of study.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) and up to 120 days after the last dose of protocol therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.5.3 Supportive Care Guidelines – general medications

The following treatments are permitted throughout the duration of the study treatment phase and during follow-up:

- Standard therapies for pre-existing medical conditions unless listed as prohibited therapy below. Any medication intended solely for supportive care (e.g., analgesics, anti-diarrheal, anti-depressants) may be used at the investigator's discretion. Patients on anti-coagulant treatment should have their platelet count monitored closely during treatment with T-DM1. Antiemetics and anti-diarrheal medications should not be administered prophylactically before initial treatment with study drugs. At the discretion of the investigator, prophylactic antiemetic and anti-diarrheal medication(s) may be used as per standard clinical practice before subsequent doses of study drugs.
- Hematopoietic growth factors (e.g., G-CSF, granulocyte macrophage colony stimulating factor) may be used at investigator's discretion for the primary prophylaxis and/or management of treatment-emergent neutropenia and/or for secondary prophylaxis as per NCCN/European Society for Medical Oncology guidelines [34,35] or local standard practice. However, treatment with granulocyte-colony stimulating factors will not permitted in cycle 1 unless the patient has febrile neutropenia and the physician considers its use as clinically indicated. It will be left to the treating physician choice from cycle 2.
- Bisphosphonate or denosumab therapy to be used in accordance with the approved labeled indication and/or nationally recognized treatment guidelines. Participants already receiving bisphosphonate/denosumab at the time of study entry can continue the treatment. Participants with new bone metastases documented as part of study screening procedures may begin a bisphosphonate after study registration but before initiation of protocol therapy.
- Anticoagulants - Anticoagulation with heparin, heparin derivatives, and/or warfarin may be given at the discretion of the treating physician. Coagulation parameters should be checked at least once monthly, or more frequently at discretion of treating physician.
- Pain medications administered per standard clinical practice are acceptable while the patient is enrolled in the study.

Patients who experience toxicities should be treated symptomatically as clinically indicated. Medications that are considered necessary for the subject's welfare and that are not expected to interfere with the evaluation of study treatment or be restricted may be given at the discretion of the investigator. Ancillary treatments will be given as medically indicated.

5.6 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression – in any subject who showed first radiologic evidence of progressive disease (PD) by RECIST 1.1 (see Section 11.1.4) and is deemed clinically stable, it is at the discretion of the investigator to continue treating the subject until progression is confirmed at least 4 weeks from the date of the first radiologic evidence of PD. Further details are described below.

- Participants who have attained a confirmed complete response (CR) that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Concurrent discontinuation of T-DM1 is at the discretion of the investigator. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase (see below) at the discretion of the investigator if no systemic cancer treatment other than T-DM1 was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule as at the time of initial discontinuation.

- Intercurrent illness that prevents further administration of treatment

- Unacceptable adverse event(s)

- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements

- Participant decides to withdraw from the protocol therapy

- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF) and recorded in the clinical trials management system (OnCore). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Sara Tolaney, MD MPH at [redacted]

Confirmation of Progressive Disease

Pembrolizumab, like other immunotherapeutic agents, may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of image responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions.

For any subject who showed first radiologic evidence of progressive disease (PD) by RECIST 1.1 (see Section 11.1.4) and is deemed clinically stable, it is at the discretion of the investigator to continue treating the subject until progression is confirmed at least 4 weeks from the date of the first radiologic evidence of PD. If progression is confirmed, the subject will be discontinued from study treatment. Otherwise, the subject will continue treatment and radiographic scans. Any

subject who had initial radiologic progression and is deemed clinically unstable should be discontinued from both study drugs and no subsequent scan for confirmation is required.

Further details are as below:

For purposes of PFS assessment on this trial, in addition to radiographic assessment of tumor response or progression, the investigator should taking into account the clinical condition/stability of subjects.

Clinically stable is defined by the following criteria:

- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor(s) at critical anatomical sites (eg, cord compression) requiring urgent alternative medical intervention

Any subject who showed first radiologic evidence of progressive disease and is deemed Clinically unstable should be discontinued from both study drugs and is not required to have repeat imaging for confirmation.

For a clinically stable subject with first radiologic evidence of progressive disease (ie,unconfirmed progression of disease), it is at the discretion of the investigator to continue treating the subject with the assigned treatment per protocol until progression of disease is confirmed on a subsequent scan at least 4 weeks later. If progression is not confirmed on the subsequent scan, the subject should continue to receive study treatment and have radiographic scans performed every 6 weeks if the patient has been on study for less than 24 weeks, or every 9 weeks for patients who have been on study greater than 24 weeks to monitor disease status. If radiologic progression is confirmed by subsequent scans, then the subject will be discontinued from study treatment. Exceptions may be considered to continue treatment in the presence of clinically stable or improved condition only after consultation with the principal investigator.

Second Course Phase (Retreatment Period)

Subjects who stop pembrolizumab for CR as described may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR according to RECIST 1.1, and:
    - was treated for at least 24 weeks with pembrolizumab before discontinuing therapy

- received at least two treatments with pembrolizumab beyond the date when the initial CR was declared

AND

- experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
- did not receive any anti-cancer treatment other than T-DM1 since the last dose of pembrolizumab
- meets all other study inclusion/exclusion criteria, as per Section 3.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year. Visit requirements are as outlined for subjects on the initial treatment phase of the trial, in the expansion cohort. Resumption of T-DM1 concurrent with pembrolizumab is at the discretion of the investigator.

5.7 Duration of Follow Up

All participants will be followed for overall survival.
Follow-up for vital status will be accomplished by annual review of the medical record, records/phone calls to outside providers or phone calls to the patient until death is documented.

In patients who come off trial for a reason other than progressive disease, scans should be performed every 6 weeks (if the patient is within 24 weeks of initiation of study treatment) or every 9 weeks (if the patient is greater than 24 weeks from initiation of study treatment) to evaluate for disease progression.

Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.8 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF) and in OnCore.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If there are dosing delays for any reason, all study assessments are to be delayed in the same fashion, such that scans and other assessments occur in conjunction with cycles of treatment.

6.1 Management of toxicities attributable to pembrolizumab

There are two types of uncommon toxicity that have been previously described in patients on both single agent pembrolizumab and single agent T-DM1: (1) hepatotoxicity, specifically manifesting as elevated AST, ALT, or total bilirubin; and (2) pneumonitis. Dose modifications for these toxicities are addressed separately in Section 6.3.

For toxicities likely attributable to pembrolizumab alone, concurrent holding of T-DM1 is at the discretion of the treating physician.

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 4 below.

Table 4: Dose modification guidelines for Pembrolizumab for drug-related adverse events

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to less than or equal to Grade 1 or baseline and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to less than or equal to Grade 1 or baseline and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. 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.

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with 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.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Continue	• Consider administering corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	

Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other cause
	Grade 3 or 4	Permanently discontinue		
Infusion Reaction[2]	Grade 3 or 4	Permanently discontinue	• See Table 5	• See Table 5
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.
2. See Table 5 for further guidance on all grades of pembrolizumab infusion reactions.

NOTE:
For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

Supportive care for pembrolizumab toxicity, particularly suspected immune-mediated toxicity
Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined in Table 4 and Table 5.

Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Table 3 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 5: Infusion Reaction Treatment Guidelines for pembrolizumab

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**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 subject 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 (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 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).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**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 subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

6.2 Management of toxicities attributable to T-DM1

There are two types of uncommon toxicity that have been previously described in patients on both single agent pembrolizumab and single agent T-DM1: (1) hepatotoxicity, specifically manifesting as elevated AST, ALT, or total bilirubin; and (2) pneumonitis. Dose modifications for these toxicities are addressed separately, in Section 6.3.

For toxicities attributable to T-DM1 alone, concurrent holding of pembrolizumab is at the discretion of the treating physician.

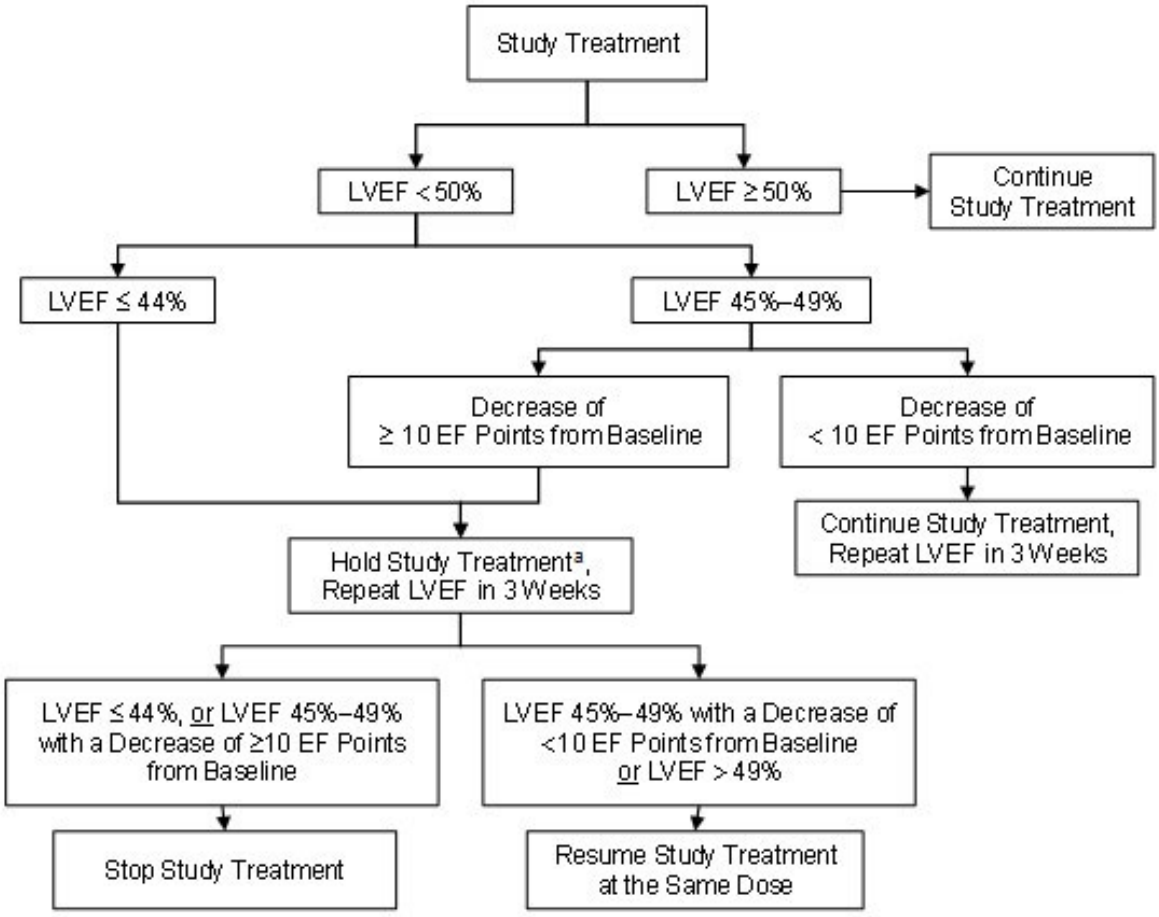
T-DM1 can be held for up to 42 days from the last scheduled dose.

Table 6: Dose modification guidelines for T-DM1 for drug-related adverse events

Event	Grade	Action to Be Taken
Infusion-Related Reactions	Infusion-related symptoms Grades 1–2	Decrease infusion rate by 50% or interrupt infusion for patients who experience any other infusion-related symptoms (e.g., chills, fever). When symptoms have completely resolved, infusion may be restarted at ≤ 50% of prior rate and increased in 50% increments every 30 minutes as tolerated. Infusions may be restarted at the full rate at the next cycle, with appropriate monitoring. In the event of a true hypersensitivity reaction (in which the severity of reaction increases with subsequent infusions) to T-DM1 treatment, the respective agent must be permanently discontinued, and pembrolizumab must be discontinued as well. Supportive care with oxygen, β-agonists, antihistamines, antipyretics, or corticosteroids may be used as appropriate at the investigator's discretion. Premedication with corticosteroids, antihistamines, and antipyretics may be used before subsequent infusions at the investigator's discretion. Patients should be monitored until complete resolution of symptoms.
	Grade ≥ 3 allergic/hypersensitivity reaction	Stop infusion; discontinue all study treatment permanently. Supportive care with oxygen, β-agonists, antihistamines, antipyretics, or corticosteroids may be used, as appropriate, at the investigator's discretion. Patients should be monitored until complete resolution of symptoms.
Paresthesias/ Dysesthesias (Persistent for > 7 Days or Caused the Next Cycle to be Delayed)	Grade 1 or 2	No dose modification
	Grade 3 or 4	Withhold all study therapy dose until neuropathy < Grade 3 Reduce T-DM1 one dose level. Discontinue all study therapy if event does not resolve to Grade < 3 within 42 days.
Ejection fraction decreased	Asymptomatic decrease in LVEF	See Figure 1 for dose modifications
	Grade 3 or 4	Discontinue all study treatment.
Heart Failure	Grade 3 or 4	Discontinue all study treatment.
Heart Failure Accompanied by LVEF <45%	Grade 2-4	Discontinue all study treatment.

Nodular regenerative hyperplasia (NRH)		For any clinical signs of liver dysfunction, discontinue T-DM1 and pembrolizumab and have the patient evaluated by a hepatologist. If there are signs of portal hypertension (e.g., ascites and/or varices) and a cirrhosis-like pattern is seen on CT scan of the liver, the possibility of NRH should be considered. For liver biopsy guidelines, please see Appendix D. All protocol treatment should be discontinued in the event of a diagnosis of NRH.
Neutropenia	Grade 3	Hold all study treatment until recovered to ≥1000K/uL. Assess ANC weekly or as medically indicated until recovery. Resume treatment without dose reduction.
	Grade 4	Hold all study treatment until recovered to ≥1000K/uL. Assess ANC weekly or as medically indicated until recovery. Reduce T-DM1 one dose level.
Thrombocytopenia	Grade 2 or 3 on day of scheduled treatment	Hold all study treatment until recovered to Grade ≤ 1. Assess platelet counts weekly or as medically indicated until recovery. Resume treatment without dose reduction. If a patient requires two delays due to thrombocytopenia, consider reducing dose by one level.
	Grade 4 at any time	Hold all study treatment until recovered to ≥75,000/µl. Assess platelet counts weekly or as medically indicated until recovery. Reduce T-DM1 by one dose level. If event occurs with 3.0 mg/kg dose, discontinue all protocol treatment.

Figure 1: Management of T-DM1 based on LVEF assessments



LVEF = left ventricular ejection fraction; EF = ejection fraction % points

Note: Baseline refers to the screening LVEF.

[a] Three intermittent holds of study treatment will lead to discontinuation.

Dose reduction levels for T-DM1

Dose level reductions for on-protocol toxicity of T-DM1 are listed in Table 7. If participants require a dose reduction during protocol therapy, they will not be allowed to re-escalate the dose of T-DM1.

Table 7: Dose reduction for T-DM1

Dose Level	Dose
1	3.6 mg/kg
– 1	3.0 mg/kg
Indication for further dose reduction	Off study treatment

6.3 Management of toxicities attributable to both pembrolizumab and T-DM1

There are two types of uncommon toxicity that have been previously described in patients on both single agent pembrolizumab and single agent T-DM1: (1) hepatotoxicity, specifically manifesting as elevated AST, ALT, or total bilirubin; and (2) pneumonitis. Dose modifications for these toxicities are addressed in this Section.

Event	Grade	Action To Be Taken
AST/ALT elevation without total bilirubin elevation, **in a patient without liver metastases**	2 – first occurrence	Hold both drugs until return to baseline or ≤Grade 1 and then restart both drugs (at the previous doses). Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1. If toxicity takes ≥12 weeks to resolve, permanently discontinue pembrolizumab.
	2- second occurrence and beyond	Hold both drugs until return to baseline or ≤Grade 1. Restart T-DM1 only (at the previous dose). Permanently discontinue pembrolizumab. Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
	3	Hold both drugs until return to baseline or ≤Grade 1. Restart T-DM1 with dose reduction. Permanently discontinue pembrolizumab. Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
	4	Discontinue all study treatment permanently. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
AST/ALT elevation without total bilirubin elevation, **in a patient with liver**	Same grade as baseline	Maintain dose levels with LFTs[2] monitored per protocol.
	Increase of one grade	Hold both drugs until return to

metastases	from baseline – first occurrence	baseline grade. Restart both drugs (at the same doses). Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1. If toxicity takes ≥12 weeks to resolve, permanently discontinue pembrolizumab.
	Increase of one grade from baseline – second occurrence	Hold both drugs until return to baseline grade and then restart T-DM1 only at the same dose. Permanently discontinue pembrolizumab. Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
	Increase of two grades from baseline	Hold both drugs until return to baseline grade. Restart T-DM1 only with a dose reduction. Permanently discontinue pembrolizumab. Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
	4	Discontinue all study treatment permanently. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
Total bilirubin elevation without transaminase elevation (see legend for special considerations for Gilbert's Syndrome)[1]	2 – first occurrence	Hold both drugs until return to baseline or ≤Grade 1. Restart both drugs (at the previous doses). Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1. If toxicity takes ≥12 weeks to resolve, permanently discontinue pembrolizumab.

	2- second occurrence and beyond	Hold both drugs until return to baseline or ≤Grade 1. Restart T-DM1 only (at the same dose). Permanently discontinue pembrolizumab. Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
	3 and 4	Discontinue all study treatment permanently. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
AST or ALT >3.0x upper limit of normal (ULN), AND total bilirubin >2.0x ULN (regardless of presence of liver metastases)	N/A	Discontinue all study treatment permanently. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
Pneumonitis	2 – first occurrence	Hold both drugs until pneumonitis improves to baseline or ≤Grade 1. Restart T-DM1with a dose reduction. Restart pembrolizumab at same dose unless toxicity does not resolve within 12 weeks of last dose or the corticosteroid dose is unable to be reduced to ≤10 mg of prednisone (or equivalent) per day within 12 weeks.
	2 – second occurrence	Hold both drugs until pneumonitis improves to baseline or ≤Grade 1. Restart T-DM1 with a dose reduction. Permanently discontinue pembrolizumab.
	3 and 4	Discontinue all study treatment permanently. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.

[1]In a patient with known Gilbert's Syndrome, an additional elevation of 0.5 mg/dL in total bilirubin is allowed in each case.
[2]LFTs include ALT, AST, alkaline phosphatase, total bilirubin.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The

following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

7.1.1 Adverse Events Lists

7.1.1.1 Adverse Event List for Pembrolizumab

In the pembrolizumab monotherapy trials (P001/P002, P012, P013, and P028, plus the P011 monotherapy arm), the overall incidence of AEs ranged from 83.0% (73 of 88 subjects in P012) to 100% (10 of 10 subjects in P011). The most commonly reported AEs included fatigue, diarrhea, decreased appetite, nausea, and anemia. The incidence of drug –related AEs (DRAEs) ranged from 39.8% (35 of 88 subjects in P013) to 80.0% (8 of 10 subjects in P011). The most commonly reported DRAEs across all studies were nausea, fatigue, and diarrhea. The incidence of Grade 3-5 DRAEs across studies ranged from 6.8% (6 of 88 in P013) to 12.0% (187 of 1562 subjects) in P001/P002. The most commonly reported Grade 3-5 DRAEs were anemia, alanine aminotransferase elevation, and aspartate aminotransferase elevation. Most subjects who experienced an AE continued in the study, with the incidence of AEs leading to discontinuation ranging from 1.9% (8 of 430 subjects in P028) to 12.3% (192 of 1562 subjects in P001/P002). The majority of AEs leading to discontinuation were not considered drug related. Discontinuations due to a DRAE were infrequent and ranged from 0% (no subjects in P011) to 4.5% (4 of 88 subjects in P013). The most commonly reported DRAEs leading to discontinuation were pneumonitis, alanine aminotransferase elevation, and aspartate aminotransferase elevation.

List of AEs considered expected:

- Endorcine disorders: Adrenal insufficiency, Hyperthyroidism, Hypophysitis, Hypopituitarism, Hypothyroidism, Secondary adrenal insufficiency, Thyroid disorder
- Eye disorders: Uveitis
- Gastrointestinal disorders: Abdominal pain, Colitis, Diarrhea, Pancreatitis
- General disorders and administration site conditions: Asthenia, Pyrexia
- Hepatobiliary disorders: Autoimmune hepatitis, Hepatitis
- Infusion related reaction
- Metabolism and nutrition disorders: Diabetic ketoacidosis, Hyponatremia, Type 1 diabetes mellitus
- Musculoskeletal and connective tissue disorders: Arthralgia, Back pain, Myositis
- Nervous system disorders: Guillain-Barré syndrome
- Renal and urinary disorders: Nephritis
- Respiratory, thoracic and mediastinal disorders: Cough, Pneumonitis
- Skin and subcutaneous tissue disorders: Pruritis, Rash, Severe skin reaction, Vitiligo

7.1.1.2 Adverse Event List(s) for T-DM1

Identified and potential risks of treatment with T-DM1 are based on all available nonclinical and clinical data relating to T-DM1 as well as clinical toxicities related to its components (trastuzumab and maytansine), in addition to other DM1-containing ADCs.

Pulmonary toxicity, hepatotoxicity, cardiac toxicity (left ventricular dysfunction), infusion-related reaction/hypersensitivity, thrombocytopenia (including thrombocytopenia associated with severe hemorrhage), and peripheral neuropathy are important identified risks with T-DM1 and are detailed in the subsections below. Fetal harm and impaired fertility are important potential risks with T-DM1. Guidance on dose modifications are provided in Section 6.

Please refer to the Investigator's Brochure for a full description of the T-DM1 safety profile, warnings, precautions, and guidance.

Pulmonary Toxicity
Cases of interstitial lung disease (ILD), including pneumonitis, some leading to acute respiratory distress syndrome or fatal outcome, have been reported in clinical trials with T-DM1. Signs and symptoms include dyspnea, cough, fatigue, and pulmonary infiltrates. These events may or may not occur as sequelae of infusion reactions. Patients with dyspnea at rest as a result of complications of advanced malignancy and/or comorbidities may be at increased risk of pulmonary events. Treatment has included administration of steroids and oxygen, as well as study drug discontinuation.

Hepatotoxicity
Hepatotoxicity, predominantly in the form of asymptomatic increases in the concentrations of serum transaminases (Grade 1–4 transaminitis), has been observed in patients treated with T-DM1. Transaminase elevations were generally transient with peak elevation at Day 8 after therapy administration and subsequent recovery to Grade 1 or less prior to the next cycle. The incidence of increased AST was substantially higher than that for ALT. A cumulative effect of T-DM1 on transaminases has been observed: the proportion of patients with Grade 1 or 2 elevated transaminases increases with successive cycles; however, no increase in the proportion of Grade 3 abnormalities over time was observed. The majority of patients with elevated transaminases improved to Grade 1 or normal within 30 days of the last dose of T-DM1.

Rare cases of severe hepatotoxicity, including death due to drug-induced liver injury (DILI) and associated hepatic encephalopathy, have been observed in patients treated with T-DM1. While there is evidence of drug induced liver toxicity in patients treated with T-DM1, its potential to cause acute severe liver injury with clinically meaningful changes in liver function is unclear, as the observed cases may have been confounded by concomitant medications with known hepatotoxic potential and/or underlying conditions. A contributory role of T-DM1 in these cases cannot be excluded so acute severe liver injury (Hy's law) remains an important potential risk with T-DM1. A Hy's law case has the following components:

1. Aminotransferase enzymes are greater than 3 × ULN with concurrent elevation of serum TBILI to > 2 × ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase).
2. No other reason can be found to explain the combination of increased aminotransferases and serum TBILI, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury.
3. Gross jaundice, clinical disability, or the need for hospital care and should be at least probably drug-induced (by T-DM1).

Cases of nodular regenerative hyperplasia (NRH) of the liver have been identified from liver biopsies in patients treated with T-DM1 and presenting with signs and symptoms of portal hypertension. NRH is a rare liver condition characterized by widespread benign transformation of hepatic parenchyma into small regenerative nodules; NRH may lead to non-cirrhotic portal hypertension and also may be fatal.[36] NRH should be considered in patients who develop clinical symptoms of portal hypertension and/or a cirrhosis-like pattern seen on CT scan of the liver but with normal transaminases and no other manifestations of cirrhosis or liver failure following long-term treatment with T-DM1. Diagnosis of NRH can only be confirmed by histopathology.

Cardiac Toxicity
Patients treated with T-DM1 are at increased risk of developing left ventricular dysfunction. LVEF < 40% has been observed in patients treated with T-DM1.

Infusion-Related Reactions (IRRs)/Hypersensitivity
Infusion-related reactions (anaphylactoid/cytokine release reactions) and hypersensitivity (anaphylactic/allergic reactions) may occur with the administration of monoclonal antibodies and have been reported with T-DM1. Treatment with T-DM1 has not been studied in patients who had trastuzumab permanently discontinued due to an IRR/hypersensitivity; treatment with T-DM1 is not recommended for these patients.

Infusion-related reactions (IRRs), characterized by one or more of the following symptoms-flushing, chills, pyrexia, dyspnea, hypotension, wheezing, bronchospasm, and tachycardia-have been reported in clinical trials of T-DM1. In general, these symptoms were not severe. In most patients, these reactions resolved over the course of several hours to a day after the infusion was terminated. Serious hypersensitivity (anaphylactic-like reactions) has been observed in clinical trials of T-DM1.

Patients will be observed closely for infusion related/hypersensitivity reactions during and after each T-DM1 infusion as detailed in Section 5.3.2. Pre-medication is allowed according to standard practice guidelines. In the event of a true hypersensitivity reaction (in which severity of reaction increases with subsequent infusions), T-DM1 treatment must be permanently discontinued.

Thrombocytopenia
Thrombocytopenia, or decreased platelet count, was reported in patients in clinical trials of T-

DM1. The majority of these patients had Grade 1 or 2 events (≥ 50,000/mm3), with the nadir occurring by Day 8 and generally improving to Grade 0 or 1 (≥ 75,000/mm3) by the next scheduled dose. In clinical trials, the incidence and severity of thrombocytopenia were higher in Asian patients. In Study TDM4370g, the incidence of thrombocytopenia in Asian patients was higher (52.5%) compared with the overall population (30.4%). However, the incidence rate of Grade ≥ 2 hemorrhage did not increase in Asian patients compared to the overall population. Rare cases of bleeding events with a fatal outcome have been observed. Cases of severe hemorrhagic events, including central nervous system hemorrhage, have been reported in clinical trials with T-DM1. In some of the observed cases the patients were also receiving anti-coagulation therapy. Patients on anti-coagulant treatment have to be monitored closely during treatment with T-DM1. Platelet counts will need to be monitored prior to each T-DM1 dose.

Peripheral neuropathy
Peripheral neuropathy, mainly Grade 1 and predominantly sensory, has been reported in clinical trials of T-DM1. These events were most apparent in patients with preexisting neuropathy. There have been no serious events of neuropathy, but there has been 1 patient to date who experienced a Grade 3 peripheral neuropathy. Once the AE resolved, the patient continued on study drug. One additional patient required a dose reduction for a Grade 2 peripheral neuropathy. Patients should be clinically monitored on an ongoing basis for signs/symptoms of neurotoxicity.

Dose delay and modification guidelines can be found in Section 6.

Please refer to the Full Prescribing Information for T-DM1 for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Attribution** of the AE:
  - 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.

7.3 Expedited Adverse Event Reporting

7.3.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.3.2 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.4 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.5 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.6 Expedited Reporting to [redacted]

A serious adverse event is any adverse event occurring at any dose or during any use of [redacted] product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is an other important medical event

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to [redacted] product, must be reported within 24 hours to the Sponsor and within 2 working days to [redacted]

Non-serious Events of Clinical Interest will be forwarded to [redacted] and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to [redacted] product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to [redacted]

SAE reports and any other relevant safety information are to be forwarded to the [redacted] facsimile number: [redacted]

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the [redacted] Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to [redacted]. (Attn: Worldwide Product Safety; [redacted]) at the time of submission to FDA.

All subjects with serious adverse events must be followed up for outcome.

7.6.1 Events of Clinical Interest (ECIs)

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to [redacted]

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to [redacted] if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to [redacted] product, must be reported within 24 hours to the Sponsor and within 24 hours to [redacted]

Events of clinical interest for this trial include:

1. Overdose of [redacted] product, as defined in Section 7.6.2 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.
2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, 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.

7.6.2 Definition of an Overdose of Pembrolizumab for This Protocol and Reporting of Overdose to [redacted]

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater. No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a [redacted] product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of [redacted]'s product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to [redacted].

7.6.3 Reporting of Pregnancy and Lactation to [redacted]

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the 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 (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to

[redacted] Global Safety. ([redacted]

7.7 Routine Adverse Event Reporting

All Grade 2 or higher Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agents administered in this study can be found in Section 7.1.

8.1 Pembrolizumab

8.1.1 Description

Pembrolizumab is a humanized monoclonal antibody of the IgG4/kappa isotype. Other names: MK-3475, Keytruda™. Pembrolizumab blocks negative immune regulatory signaling by binding to the PD-1 receptor, inhibiting the interaction between PD-1 and its ligands.

The molecular weight of pembrolizumab is 148.9-149.5 KDa.

8.1.2 Form

Clinical supplies will be manufactured and provided by [redacted] as summarized in Table 7.

Table 7: Product Description

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/ 4mL	Solution for Injection

8.1.3 Storage and Stability

Store intact vials between 2°C-8°C (36°F-46°F). Do not freeze. Protect from light by storing in the original box.

Stability testing of the intact vials is ongoing.

Administer prepared solutions immediately after preparation. If not administered immediately, prepared solutions may be stored refrigerated for a total cumulative storage time at room temperature and refrigeration of 24 hours. Pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of liquid drug product solution in vials, room temperature storage of

infusion solution in the IV bag, and the duration of infusion.

8.1.4 **Compatibility**

Compatible IV bag materials: PVC plasticized with DEHP, non-PVC (polyolefin), EVA, or PE lined polyolefin.

8.1.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.1.6 **Availability**

Pembrolizumab is an investigational agent and will be supplied free of charge from [redacted]

8.1.7 **Preparation**

Pembrolizumab solution for infusion must be diluted prior to administration. Allow the required number of vials to equilibrate to room temperature. Do not shake the vials. Do not use if opaque or extraneous particulate matter other than translucent to white proteinaceous particles is observed. Do not use if discolored. To prepare the infusion solution add the dose volume of pembrolizumab to an infusion bag containing 0.9% Sodium Chloride Injection, USP of 5% Dextrose Injection, USP. Gently invert the bag 10-15 times to mix the solution. The final concentration must be between **1 mg/mL to 10 mg/mL.**

8.1.8 **Administration**

Route of administration: IV infusion only. Do not administer as an IV push or bolus injection.

Method of administration: Infuse over approximately 30 minutes (range: 25-40 minutes) using an infusion set containing a low-protein binding 0.2 to 5 m in-line filter make of polyethersulfone or polysulfone. Infusion rate should not exceed 6.7 mL/min. A central line is not required however if a subject has a central venous catheter in place, it is recommended that it be used for the infusion. Do not co-administer drugs through the same infusion line. Following the infusion, flush the IV line with normal saline.

8.1.9 **Ordering**

Pembrolizumab will be obtained directly from [redacted] the study sponsor.

8.1.10 Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.11 Destruction and Return

At the end of the study, unused supplies of pembrolizumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.2 T-DM1

Please refer to the Investigator's Brochure for detailed agent information, and to the FDA label for additional information.

8.2.1 Formulation, Preparation, and Storage

Trastuzumab-MCC-DM1 (trastuzumab emtansine, T-DM1) is provided as a single-use lyophilized formulation in a colorless 20-mL Type I glass vial closed by means of a FluroTec-coated stopper and an overseal with flip-off cap. Upon receipt of T-DM1 vials should be refrigerated at 2°C–8°C. All vials of T-DM1 should be handled by appropriately trained site staff wearing gloves and visually inspected upon receipt to ensure they are intact without exterior contamination. Drug from any vials that appear abnormal upon inspection should not be administered to patients.

The lyophilized product should be reconstituted using Sterile Water for Injection (SWFI). Using a new syringe, 8mL SWFI should be added to the vial and the vial swirled gently until the product is completely dissolved. The vial should not be shaken. The resulting product contains 20 mg/mL T-DM1, 10 mM sodium succinate, pH 5.0, 60 mg/mL sucrose, and 0.02% (w/v) polysorbate 20. Each 20 mL vial contains enough T-DM1 to allow delivery of 160 mg T-DM1. The reconstituted product contains no preservative and is intended for single use only. The vial should be inspected to ensure the reconstituted product is a clear colorless solution, and is free of particulates before proceeding. Drug from any vial that appears abnormal upon inspection should not be administered to patients. Using a new syringe, the indicated volume of T-DM1 solution should be removed from the vial(s) and added to the IV bag containing at least 250 mL of 0.45% sodium chloride (preferred) or 0.9% sodium chloride injection and gently inverted to mix the solution. A 0.22 micron non-protein adsorptive polyethersulfone (PES) in-line filter is recommended when using 0.45% sodium chloride and required when using 0.9% sodium chloride injection. The solution of T-DM1 should not be shaken.

The solution of T-DM1 for infusion should be used immediately. If not used immediately, storage times should not be longer than 24 hours at 2°C−8°C (36°F−46°F) for solutions of

T-DM1 diluted in polyvinyl chloride (PVC) or latex-free PVC-free polyolefin, polypropylene, or polyethylene bags containing 0.45% or 0.9% Sodium Chloride Injection, USP.

For additional details, please refer to the current version of the T-DM1 Investigator Brochure.

8.2.2 **Compatibility**

T-DM1 has no known compatibility problems. For additional details, see the T-DM1 Investigator Brochure.

8.2.3 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.2.4 **Availability**

T-DM1 will be obtained from commercial supply, as it is being used per the FDA label.

8.2.5 **Administration**

The first infusion of T-DM1 will be administered over 90 minutes (± 10 minutes). Infusions may be slowed or interrupted for patients experiencing infusion-associated symptoms. Vital signs must be assessed before and after dose administration. Following the initial dose, patients will be observed for at least 60 minutes for fever, chills, or other infusion-associated symptoms. If prior infusions were well tolerated (without any signs or symptoms of infusion reactions), subsequent doses of T-DM1 may be administered over 30 minutes (± 10 minutes), with a minimum 30-minute observation period after infusion.

8.2.6 **Ordering**

T-DM1 will be ordered and supply maintained as per standard practices of the Dana-Farber pharmacy.

8.2.7 **Drug Accountability**

The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form.

8.2.8 **Destruction and Return**

T-DM1 should be destroyed according to institutional policies. Destruction will be

documented according to institutional policies.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

All patients will be asked to provide archival tumor tissue (either paraffin blocks or 15 unstained slides, ideally 4 micron thickness). However, if archival tissue is not available or not evaluable, that will not be a basis to exclude the patient from any portion of the trial or the planned analysis. Archival tissue will be used for immune profiling assays to ascertain baseline values in patients who choose not to undergo baseline research fresh tissue biopsy (in the dose de-escalation cohort) or in whom there is insufficient tissue or failed testing of any of the planned assays from baseline fresh tissue biopsy.

A baseline research biopsy prior to study therapy is required in the expansion cohort of this trial, and optional in the dose de-escalation cohort. We plan to use baseline biopsy tissue to perform a number of immune profiling assays. On baseline tumor biopsies, we will perform characterization based on histology (TILs), protein expression, and mRNA expression.

An optional research biopsy after 6 weeks on protocol therapy will be collected for patients who consent and have biopsy accessible tumor. These biopsies will undergo the same characterization testing as described for baseline biopsies.

Serial blood draws for correlative science are required on this trial; blood draws will be obtained every 3 weeks on treatment days, prior to the infusion of study drugs, at the end-of-treatment visit in patients who go off study for progressive disease, and all efforts will be made to obtain a sample at the time of progressive disease from participants who went off study for anything other than progressive disease. We will perform flow cytometry on these samples to characterize protein expression of immune mediators. Circulating free DNA will be assessed as well.

If participants agree, a portion of these specimens will be banked for possible future genomic analysis, and other further testing.

All patients will additionally be asked to provide a stool sample at three separate timepoints: prior to treatment, during treatment, and at the time of disease progression. A fourth collection may be requested from patients who experience grade ≥ 2 diarrhea after discussion with the PI. This collection is not required, but is strongly encouraged. These samples will be analyzed for microbiota content. Please refer to the separate laboratory manual for stool details including specific collection, processing, and shipping instructions.

9.1 Summary table: research tissue and blood specimen collection

Research Sampling	Time point	Contents
Blood	Cycle 1 Day 1	1-10 mL Streck Tube

		5- 10mL green top tubes*
	Every Cycle Day 1	5- 10mL green top tubes*
	Restaging Visits Only	1-10mL Streck Tube
	Off Treatment for PD	1-10 mL Streck Tube 5- 10mL green top tubes*
	Time of PD, in patients who came off-study for a reason other than PD (optional)	1-10 mL Streck Tube 5- 10mL green top tubes*
Fresh Tissue	Pre-treatment - Optional for dose de-escalation participants - Required for dose expansion participants	5-7cores
	Cycle 3 Day 1 (Optional)	5-7cores
Archival Tissue	Anytime	1 block or 15, 4 micron unstained slides
Stool Sample	Pre-Treatment (within 28 days of starting therapy)	Home Stool Kit (DNA Genotek)
	C3D1 (within 14 days prior)	
	Disease Progression	
	At the time of grade ≥ 2 diarrhea (Optional)	

* Purple top EDTA tubes or CPT tubes may be substituted for green tops

9.2 Fresh Tissue Collection

9.2.1 Collection and handling of biopsy specimens

Research core biopsies of an accessible lesion will be obtained from all participants on the dose expansion cohort prior to initiating protocol therapy. These biopsies will be optional in participants on the dose de-escalation cohort. Additional biopsies after 6 weeks on protocol therapy will be optional in all participants. Guidelines for biopsy from various metastatic sites can be found in Appendix E.

Biopsies should not be performed on Friday afternoons, as there may not be time for processing of the fresh tissue. If a biopsy must be performed on Friday morning, the lab of [redacted] must be notified in advance to ensure that there will be adequate time for processing fresh tissue, since fresh tissue cannot be stored over the weekend (contacts: [redacted] pecimens in RNA Later and formalin should be stored at room temperature. Specimens in RNALater and formalin may be stored over the weekend and delivered on Monday.

Ideally five core biopsies will be obtained:

- Two cores should be placed in 10% neutral buffered formalin
- One core should be placed in RNAlater

- Two cores should be placed in sterile DMEM

The order of specimen collection should be:

- First core: 10% neutral buffered formalin
- Second core: Sterile DMEM
- Third core: RNAlater
- Fourth core: Sterile DMEM
- Fifth core: 10% neutral buffered formalin

If additional cores are obtained, they should be processed as follows:

- Sixth core: RNAlater
- Seventh core: 10% neutral buffered formalin

If a skin punch is performed, the goal of tissue collection would be 2, 5mm punches. The processing instructions should be as follows:

- First punch: 10% neutral buffered formalin
- Second punch: RNAlater

After being obtained, processing of the cores is as follows:

- All samples should be de-identified and labeled with the protocol number, participant initials, participant study ID number and date of procedure.
- Cores in sterile DMEM should be brought as fresh tissue immediately to the lab of [redacted] at:

  [redacted]

  This core must arrive to the lab to be processed for TILs (as described below) **within 1.5 hours of its collection,** though an additional 2 hour window is allowed. In addition, a small piece of this core will be immediately frozen in liquid nitrogen upon arrival to [redacted] for later use for RNA sequencing. Please notify the lab of expected specimen collection approximately one week in advance of specimen drop-off (contacts: [redacted]
- Cores in formalin should be brought to the Brigham and Women's Specialized Histopathology Lab on the 6th floor of the Thorn building (with the appropriate work order submitted and printed), where a block will be made. An email will be sent to the CRC within 2-3 days to confirm that the block has been made. The block should then be picked up from the SHL lab and brought to Dr. Scott Rodig on the 6th floor of the Thorn building.

- Cores in RNAlater should be delivered or shipped to the DF/HCC Clinical Trial Core Laboratory at the address provided here:

  Dana-Farber Cancer Institute
  Attn: Lynda Chichester
  Smith 9th Floor, Rm 948
  450 Brookline Avenue
  Boston, MA 02215
  dfcibreastbank@partners.org

  Please email the DF/HCC Clinical Trials Core Laboratory (dfcibreastbank@partners.org) with protocol number, participant name, study ID, date of collection, approximate time of collection, and study time point the day prior to collection. Any tissue remaining after study-specific protocol testing occurs will be banked in the DF/HCC Clinical Trial Core Laboratory and may be used for additional or future analyses as needed.

9.3 Procedures for obtaining blood specimens for study

Research blood collection is mandatory for all participants for flow cytometry and DNA isolation. The samples will be banked in the DF/HCC Clinical Trials Core Laboratory for these and future research purposes. These specimens will become the property of the DF/HCC.

Blood draws should not be performed on Friday afternoons, as there may not be time for processing of the blood. If a blood draw must be performed on Friday morning, the lab of [redacted] must be notified ahead of time to ensure that there will be adequate time for processing the blood, since it cannot be stored over the weekend [redacted]

The following research blood samples are required:

Cycle 1 Day 1:

- 1-10 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood*

Every Cycle Day 1:

- 5- 10mL green top tubes for whole blood*

Every Restaging Visit:

- 1-10 mL Streck Tube for whole blood

Off Treatment (if for progressive disease):

- 1-10 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood*

The following Time of Progression research blood samples are optional for patients who came off treatment for a reason other than progressive disease:

- 1-10 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood*

*If green top tubes are not available, purple top or CPT tubes may be substituted.

9.3.1 Handling of Blood Specimens

All samples should be de-identified and labeled with the protocol number, participant initials, participant study ID number, date of collection and time point (e.g., "Baseline" or "Cycle 1" or "Progressive Disease").

- Green Top tubes:

  Green top tubes must be processed within 3-4hrs of its being drawn and need to be delivered at ambient temperature to the lab of [redacted] Please contact the lab approximately 1 week ahead of time to notify them of upcoming specimen drop off (contacts: [redacted]

  Lab address: [redacted]

- Streck tubes:

  Fill the Streck tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in accurate results.

  Tube precautions:
  - DO NOT FREEZE OR REFRIGERATE TUBES as this could result in cfDNA breakage. Blood collected in the Streck tube can be stored for 14 days between 6-37 degrees Celsius.
  - Do not use tubes after expiration date.
  - Fill the tube completely; overfilling or underfilling of tubes will result in an incorrect blood-to-additive ratio and may lead to incorrect analytical results.

Deliver to:

Dana-Farber Cancer Institute
Attn: Lynda Chichester
Smith 9th Floor, Rm 930
450 Brookline Avenue
Boston, MA 02215
dfcibreastbank@partners.org

Email the blood bank (dfcibreastbank@partners.org) and the current Dana-Farber CRC with the sample information and tracking information the day before transporting specimens.

9.3.2 Sites Performing Correlative Studies

DFCI Center for Immuno-Oncology
DFCI Clinical Trials Core Laboratory

9.3.3 Blood and tissue banking

Any leftover blood or tissue will be banked in the lab of [redacted] and/or the DF/HCC Clinical Trials Core Laboratory such that it can be used for additional or future analyses as needed.

9.4 Stool Collection

9.4.1 Handling and Shipping

All stool samples will be collected by each patient at home using a home-based kit with a pre-paid mailer that provides nearly equivalent metagenomic and metatranscriptomic data to state-of-the-art fresh-frozen sample-collection protocol. Patients will be asked to provide samples at the following timepoints:

- Baseline
- After two cycles of therapy
- At the time of disease progression
- Optional collection at the time of grade ≥ 2 diarrhea

Most kits will be provided to the patients at their clinic visits. If the study team is unable to provide the kits to the patients in clinic, they may be mailed to patients by members of the study team. All kits will contain a questionnaire for patients to complete and return with their samples regarding timing and conditions surrounding their stool sample.

Please refer to the separate lab manual for collection and processing instructions.

Samples will be stored at the BWH/Harvard Cohorts Repository and will be shipped in batches

by the biorepository to an external lab vendor, Microbiome Dx, who will perform the analysis of the samples.

9.4.2 Sites performing correlative studies

- BWH/Harvard Cohorts Biorepository
- Microbiome Dx

9.5 Hypotheses for correlative objectives

- We hypothesize that all breast tumors can be placed in a baseline category of general "immune activity" level based on histologic, protein-based, and genomic analyses.
- We hypothesize that breast tumors with a higher level of "immune activity" at baseline, as assessed by the mentioned analyses, will have a higher likelihood of responding to treatment with T-DM1 plus pembrolizumab.
- We hypothesize that tumors with a more significant change in "immune activity" over the course of 6 weeks of therapy with T-DM1 plus pembrolizumab will have a higher likelihood of benefitting from the treatment.
- We hypothesize that the immune marker profile in the peripheral blood will change over the course of T-DM1 plus pembrolizumab therapy.
- We hypothesize that a larger increase in markers of immune activity in the peripheral blood will correlate with a better disease response as assessed on concurrent restaging scans and in terms of best radiographic response at any time on trial.
- We hypothesize that an immune marker or composite of markers in the peripheral blood at baseline will correspond to TIL percentage in baseline tumor biopsies.
- We hypothesize that the structure and function of the gut microbiome differs among patients with breast cancer.
- We hypothesize that the structure and function of the gut microbiome before starting pembrolizumab and T-DM1 can be predictive of efficacy of this therapy, with a greater microbial diversity, estimated by Shannon index, being predictive of efficacy.
- We hypothesize that the structure and function of gut microbiome changes in response pembrolizumab-based therapy and can predict the likelihood of response to pembrolizumab plus T-DM1.
- We hypothesize that the abundance and functional profile of specific gut bacteria is associated with response to pembrolizumab plus T-DM1.
-

9.6 Planned assays for correlative objectives

All of the below-mentioned analyses may be altered based on novel developments in the field of cancer immune profiling at the time of correlative science. Additional markers or alternative technologies (based on scientific developments and/or novel technologies) may also be used, to explore potential prognostic or predictive candidate markers/panels or markers related to treatment benefit and/or safety, to improve diagnostic tests, or to understand breast cancer

biology. Planned collaborators include: Dana-Farber Cancer Institute, The Broad Institute of MIT, Brigham and Women's Hospital, Harvard Medical School, Beth Israel Deaconness Medical Center, Adaptive Technologies, and Microbiome Dx. Any samples or data shared with collaborators external to the study team will not contain any PHI or identifying information. See section 12.4 for more information.

9.6.1 Tumor infiltrating lymphocyte (TIL) percentage and determination of lymphocyte predominant breast cancer (LPBC)

Paraffinized, hematoxylin and eosin-stained slides taken from two tissue planes will be derived from each biopsy and will be reviewed by certified pathologists. The extent of lymphocytic infiltrate in tumor tissue will be assessed and stromal TIL percentage will be determined. More detailed guidelines for the quantification of stromal TILs in breast cancer can be found in the recommendations from the International TILs Working Group 2014.[31]

After assessment of the TIL percentage, the specimen may be categorized as lymphocyte predominant breast cancer (LPBC), defined as a tumor that contains >60% stromal lymphocytes, or non-LPBC.

9.6.2 Immunohistochemistry

Tissue will be collected and fixed by 10% neutral buffered formalin overnight, dehydrated, and paraffin embedded. Four micrometer-thick sections will be cut. The paraffin blocks and unstained slides will be stored at room temperature. All immunohistochemical staining will be performed in the Center for Immuno-Oncology Pathology Core at Dana-Farber/Harvard Cancer Center (DF/HCC) Specialized Histopathology Core.

Formalin fixed-paraffin embedded (FFPE) tumor slides will be prepared and H&E stained for assessment of TIL in pre- and post-treatment tumor samples. To identify subsets of different immune populations (effector/memory CD8 cells, T regulatory cells, dendritic cells, tumor associated macrophages, and Tie-2 expressing monocytes (TEM)), immunohistochemical (IHC) staining will be performed on FFPE tumor slices using some or all of the following antibodies:
Core set: CD8, PD-1, PD-L1, PD-L2
Others: CD3, CD4, CD25, FoxP3, Indoleamine 2,3 deoxygenase-1 (IDO1), CD11c, CD83, CD86, CD56, CD14, CD16, Tie2 (See also Appendix E)

Chen et al[37] describe a semi-quantitative scoring method, which is in accordance with typical biomarker scoring in anatomic and surgical pathology. Briefly, staining is scored according to intensity (0=no staining, 1=weak staining, 2=moderate staining, 3=strong staining), staining patter (M=predominantly cell membrane; C=predominantly cell cytoplasm), and the percentage of cells showing positive staining (0-100%). The semi-quantitative scoring is performed for: 1) the neoplastic tumor cells and 2) the non-neoplastic infiltrating immune cells. Significant discordant results have been rare during case evaluations.[37]

It should be noted that the above staining protocols are based on standard methods used at the time of protocol writing. It is possible that at the time protein expression assays are conducted,

novel and improved methods for staining will exist. In this case, we plan to use the best available, best validated experimental method available at the time.

9.6.3 Flow cytometry, genomic analysis of biopsy stissue

TILs will be isolated from the biopsy specimen and assessed by surface staining.

Messenger RNA (mRNA) expression within tumor biopsy specimens will be assessed using the most current and informative methodologies at the time that correlative science is performed on all specimens. NanoString signatures and comprehensive RNA sequencing may be used. Potential genes of interest, based on prior immune profiling of breast tumors,[27] include CXCL9, CCL5, CD8ACD80, CXCL13, IGKC, CD21, IDO1, PD-1, PD-L1, PD-L2, CTLA4, and FOXP3. Additional DNA analysis, for example to assess mutational load and neoantigen burden, may also be performed.

9.6.4 Analysis of PBMCs

PBMCs will be and used to assess immune cell populations by flow cytometry.

9.6.5 Analysis of cell-free DNA

Blood will be collected at baseline, restaging visits and at time of progression for evaluation of cell-free DNA (cfDNA). The cfDNA will be banked in the DF/HCC Clinical Trials Core laboratory for future research purposes. The banked samples will be used to analyze DNA, RNA and protein in future studies.

9.6.6 Analysis of DNA extraction from stool samples

Microbial DNA is extracted using the Mag-Bind Universal Pathogen DNA Kit (Omega Bio-Tek). Briefly, 250 mg of the specimen is transferred to a deep-well plate for bead beating followed by DNA precipitation and purification following the manufacturer's instructions. Finally, DNA is eluted in 100 uls of Elution Buffer and stored at -80°C until further use. 16S sequencing libraries are generated by amplifying the v3-v4 hypervariable regions of the 16S gene in a polymerase chain reaction using primers F341and R785. Resulting amplicons are tagged with unique molecular barcodes that are later used to demultiplex sequencing reads into individual sample buckets. Libraries are loaded on a MiSeq flowcell and sequenced following Illumina's loading instructions. Sequence data are retrieved from the instrument by converting base call format files into fastq files for data processing purposes.

MicrobiomeDX uses BacPro™, a proprietary algorithm, to inspect and validate sequencing files by employing demultiplexing, trimming, merging, and quality filtering steps. Paired sequencing reads are merged using an overlap of 25 bp allowing for 10 base mismatches. Merged sequences are dereplicated and clustered in a de-novo fashion using VSEARCH, while filtering out sequence chimeras and singletons. Representative sequences from each cluster are mapped against the SILVA database at 99% sequence identity to obtain accurate taxonomic classifications and relative abundances. In parallel, feature tables are constructed to derive alpha

diversity indices, and distance matrices are built to derive beta diversity indices. The BacPro™ pipeline generates a comprehensive report that includes alpha diversity scores describing community richness and evenness, taxonomic composition with relative abundances, and beta diversity metrics to determine the in-between sample differences based on the bacterial communities identified.

9.6.7 Shotgun sequencing and metabolic pathway reconstruction of stool samples

Stool samples from patients included in the trial will be subjected to whole genome shotgun sequencing. Libraries will be constructed with Illumina barcodes from the TruSeq DNA Sample Prep kit (Illumina) and reagents from KAPA Library Preparation kit (Kapa Biosystems), and then sequenced on an Illumina MiSeq platform using 2_250 nucleotide paired-end sequencing, according to the manufacturer's instructions. Sequencing reads will be converted into relative abundances of microbial metabolic modules using HUMAnN35, the Human Microbiome Project metabolic reconstruction pipeline and mapped to the KEGG36. Relative species abundances will be calculated by the MetaPhlAn pipeline37.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within **14 days** prior to start of protocol therapy (unless otherwise specified). If these screening assessments occur within 3 days before start of study treatment, then they may serve as the baseline Cycle 1 Day 1 values. Scans must be done within **28 days** prior to the start of therapy.

As detailed in the Study Calendar, a negative pregnancy test in women of child-bearing potential must be documented within **7 days** before the first dose of study medication.

A baseline tumor biopsy, obtained within **7 days** before starting protocol therapy, is also required in patients in the expansion cohort, and optional in patients in the dose de-escalation cohort.

In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within ± 3 days of the protocol-specified date, unless otherwise noted.

	Screening	Cycle 1 Day 1	Cycle 1 Day 8[a]	Cycle 1 Day 15[a]	Cycle 2 Day 1	Cycle 3 Day 1	Cycle 4+ Day 1	Off-Treatment[p]	Follow-Up[p]
Informed consent	X								
Demographics	X								
Medical history	X								
Concurrent medications	X	X----------						----------X	
Adverse event evaluation[r]		X----------						----------X	
Physical exam	X	X	X	X	X	X	X	X	
Vital signs[b]	X	X	X	X	X	X	X	X	
Weight	X	X			X	X	X	X	
Performance status	X	X	X	X	X	X	X	X	
NYHA classification	X								
CBC with differential	X	X	X	X	X	X	X	X	
COMP[c]	X	X	X	X	X	X	X	X	
TSH, free T4	X				X	X	X		
Cortisol[s]	X				X	X	X		
Coagulation panel[d]	X								
Hepatitis Screen[e]	X								
Pregnancy test[f]	X								
Single, 12-lead EKG	X								
Echocardiogram or MUGA[g]	X						X[g]		
Tumor Assessments[h]	X					X	X	X	X[i]
Survival Assessments[q]									X
Tumor biopsy	X[j]					X[k]			
Research Blood Collection[l]		X			X	X	X	X[m]	X[n]
Research Stool Collection[t]	X					X		X	
Stool Questionnaire[u]	X					X		X	
Archival tumor tissue	X[o]								
Pembrolizumab: 200mg given IV over 30 minutes (-5, +10 minute window) q3weeks on Day 1 of each cycle **T-DM1:** dose level dependent, given IV over 90 minutes (+/- 10 minute window) on Cycle 1Day 1. T-DM1 will be administered q 3 weeks on Day 1 over 30 minutes (+/- 10 minute window) for subsequent cycles if no infusion reaction occurs.									

a. Visits are required on Cycle 1 Days 8 and 15 only for participants in the dose de-escalation cohort.
b. Vitals should include: diastolic and systolic blood pressure, heart rate, and temperature. Height only required on Cycle 1 Day 1.
c. COMP should include: sodium, potassium, chloride, bicarbonate, BUN, serum creatinine, glucose, calcium, albumin, total protein, alkaline phosphatase, ALT, AST, total bilirubin (NOTE: the frequency of checking magnesium and phosphorus levels is left up to the treating provider)
d. Coagulation Panel: PT/INR and PTT
e. Hepatitis B surface Antibody, Hepatitis B surface Antigen, and Hepatitis C Antibody
f. Female subjects of child-bearing potential, within **7 days** before the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, then a serum test is required.
g. Echocardiogram or MUGA should be performed within 28 days of registration and then at the end of every 4 cycles (every 12 weeks). After 24 weeks on study, echocardiogram or MUGA may be spaced to every 18 weeks.
h. Baseline assessments must be done within 28 days prior to the start of therapy. Tumor assessments will be performed every 2 cycles (6 weeks days 15-21 for the first 24 weeks. If at 24 weeks, a participant has SD or better by RECIST the frequency of assessments may be reduced to every 3 cycles (9 weeks) days 15-21. Effort should be made to use the same type of imaging to assess measurable lesions at baseline and in follow-up.
i. During follow-up of patients who went off study for reasons other than PD, tumor assessments should continue every 6 to 9 weeks from the date of the last assessment until progressive disease is documented
j. Baseline biopsy should be obtained within 7 days of initiating protocol therapy. This biopsy is optional for participants in the dose-descalation cohort but mandatory in the dose expansion cohort. See Section 9.1 for biopsy handling and processing instructions.
k. Optional tumor biopsy after 6 weeks on treatment therapy (i.e. at cycle 3 day 1, or within 5 days prior).
l. Research blood collection for immune cells and cfDNA. See Section 9 for additional blood handling and processing instructions
m. Research blood should only be collected on patients who come off treatment for progressive disease.
n. Optional blood collection for immune cells/cfDNA may be offered to patients who develop progressive disease during follow-up.
o. All patients will also be asked to provide archival tumor tissue (either paraffin blocks or 15 unstained slides). However, if archival tissue is not available or not evaluable, that will not be a basis to exclude the patient from any portion of the trial or the planned analysis
p. Off-treatment visit is to occur within 21 days of last dose. End of treatment assessments do not have to be repeated if the same assessments were performed within 7 days prior to the visit (28 days for tumor assessments)). If study treatment is discontinued for PD, then tumor assessments are not required for that study participant.
q. Survival status should be collected annually, either by review of the medical record or by phone call (see Section 5.7).
r. SAEs and ECIs need to be collected and reported up to 30 days post treatment to meet the DF/HCC reporting requirements and for [redacted] they need to be reported from the time consent is signed through 90 days following cessation of treatment (120 days for pregnancy and lactations), or the initiation of new anti-cancer therapy, whichever is earlier.
s. Cortisol may be drawn at any time of day, at baseline and on every Day 1.
t. Baseline stool collection should be obtained within 28 days before starting protocol therapy. The C3D1 stool collection should be performed as close to C3D1 as possible, but may be collected up to 14 days prior. A sample will additionally be collected at the time of disease progression. An optional stool sample may be collected at the time of grade ≥ 2 diarrhea after discussions with the PI. As these collections are for exploratory correlative purposes, failure to provide a sample at these timepoints will not constitute a protocol violation. See section 9 and/or lab manual for stool collection and processing instructions.
u. Each stool collection kit will contain a questionnaire for the patients to complete regarding the conditions surrounding their collection. These will be a part of the kit and are not to be administered in clinic. Failure to complete these questionnaires at the required or optional timepoints will not constitute a protocol violation.

11. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, participants with measurable disease will be assessed by standard criteria. For the purposes of this study, participants should be re-evaluated every 6 weeks for the first 24 weeks and then every 9 weeks until progression.

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 6 weeks for the first 24 weeks and then every 9 weeks until progression. In addition to a baseline scan, confirmatory scans should also be obtained 9 weeks (+/- 7 days) following initial documentation of objective response. In all participants, the date of progression will be documented as the first

date progression was observed.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered

non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by

color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake

greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

MIBG (meta-iodobenzylguanidine).The following is recommended, to assure high quality images are obtained.

Patient preparation: Iodides, usually SSKI (saturated solution of potassium iodide), are administered to reduce thyroidal accumulation of free radioiodine, preferably beginning the day prior to injection and continuing for 3 additional days (4 days total). For infants and children, one drop t.i.d. is sufficient, for adolescents 2 drops t.i.d., and for adults 3 drops t.i.d. Participants and/or parents are asked about exposure to potential interfering agents. If none is noted, an indwelling intravenous line is established. The dose of MIBG is administered by slow intravenous injection over 90 seconds.

Images from the head to the distal lower extremities should be obtained.

I-123MIBG scintigraphy is performed to obtain both planar and tomographic images.

Planar: Anterior and posterior views from the top of the head to the proximal lower extremities are obtained for 10 minutes at 24 hours and occasionally at 48 hours following injection of 10 mCi/1.7 square meters of body surface area (~150 μCi/kg, maximum 10 mCi). Anterior views of the distal lower extremities are adequate. A large field of view dual head gamma camera with low energy collimators is preferred.

SPECT: Most participants receiving I-123 MIBG also undergo SPECT at 24 hours, using a single or multi-headed camera with a low energy collimator. The camera is rotated through 360 degrees, 120 projections at 25 seconds per stop. Data are reconstructed using filtered back projections with a Butterworth filter and a cut off frequency of 0.2-0.5. SPECT/CT may be performed at institutions with this capacity.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the

study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on

study.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.4.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.6 Progression-Free Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.1.7 Response Review

N/A

11.2 Antitumor Effect – Hematologic Tumors

N/A

11.3 Other Response Parameters: Immune-Related Response Criteria (irRECIST)

11.3.1 Definition of Tumor Response Using Immune-Related Response Criteria (irRECIST)

irRECIST is RECIST 1.1 adapted as described below to account for the unique tumor response seen with immuno-therapeutics.

irRECIST will be used by site investigators and local radiology review to assess tumor response and progression, and may be used to make treatment decisions. This data will be collected in the clinical database.

irRECIST will be used by the central imaging vendor, however, this evaluation will be done retrospectively.

irRECIST takes into account the clinical condition/stability of subjects, as described in the table below, in addition to response or progression via tumor imaging.

Clinically stable is defined by the following criteria:

- Absence of symptoms and signs indicating clinically significant progression of disease, including worsening of laboratory values.
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention.

Table: irRECIST: Tumor Imaging and Treatment after 1st Radiologic Evidence of PD **or** SD, CR, or PR

	Clinically Stable		**Clinically Unstable**	
	Tumor Imaging	**Treatment**	**Tumor Imaging**	**Treatment**
1st radiologic evidence of PD	Repeat tumor imaging at ≥ 4 weeks at site to confirm PD	May continue study treatment at the site Investigator's discretion while awaiting confirmatory scan by site	Repeat tumor imaging at ≥ 4 weeks to confirm PD per physician discretion only	Discontinue treatment
Repeat tumor imaging confirms PD	No additional tumor imaging required	Discontinue treatment (exception is possible upon consultation with Sponsor).	No additional tumor imaging required	N/A
Repeat tumor imaging shows SD, PR or CR	Continue regularly scheduled tumor imaging assessments	Continue study treatment at the siteInvestigator's discretion	Continue regularly scheduled tumor imaging assessments	May restart study treatment if condition has improved and/or clinically stable per site Investigator's discretion. Next tumor imaging should occur according to the every 9 week (63 ± 7 days) imaging schedule in the first year or every 12 weeks after one year.

In determining whether or not the tumor burden has increased, decreased or stayed stable, site investigators should consider all target lesions a well as non-target lesions.

Any subject deemed **clinically unstable** should be discontinued from trial treatment at first evidence of progressive disease by tumor imaging and is not required to have repeat tumor imaging for confirmation.

For a **clinically stable** subject with first radiologic evidence of progressive disease (i.e. unconfirmed progression of disease), it is at the discretion of the site investigator to continue treating the subject with the assigned treatment per protocol until progression of disease is confirmed at least 28 days from the date of the tumor imaging first suggestiong PD. If progression is not confirmed on the subsequent tumor imaging, the subject should continue to receive study therapy and have tumor imaging preformed per protocol, or sooner if clincially

indicated, to monitor disease status. If radiologic progression is confirmed by subsequent tumor imaging, then the subject will be discontinued from trial treatment.

NOTE: If a subject with confirmed progression by tumor imaging (i.e. 2 scans at least 28 days apart demonstrating progressive disease) is clinically stable or clinically improved, and there is no further increase in the tumor burden at the confirmatory scan, an exception may be considered to continue treatment upon consultation with the principal investigator.

The same imaging modality (i.e., CT or MRI), acquisition and technical parameters should be used throughout the study for a given subject.

11.3.1.1 Immune-Related Best Overall Response Using irRECIST (irBOR)

irBOR is the best confirmed overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual subject in the study. For the assessment of irBOR, all available assessments per subject are considered.

irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The ODQ will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the ODQ according to the schedule set by the ODQ.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study

team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multicenter Guidelines

N/A

12.4 Collaborative Research Future Use of Data and Biospecimens

Tissue, blood, stool, bodily fluids, and other materials derived from these will be collected in this study to analyze genes, DNA, RNA, proteins and cells for the study's correlative endpoints and potential future research, utilizing new types of biomarker testing as it becomes available.

These samples and any data generated as a part of these clinical trials may be used for future research studies and may be provided to collaborating investigators both within and outside of the DF/HCC for either correlative endpoints or secondary use. Samples and data may be shared with outside non-profit academic investigators, as well as with for-profit pharmaceutical investigators or commercial entitites, with whom we collaborate. When samples or data are sent to collaborators and when any research is performed on them, all information will be identified with a code, and will not contain any PHI, such as name, birthday, or MRNs.

In order to allow the greatest amount of research to be performed on the specimens and information generated as a part of this trial, researchers in this study may share results of genetic sequencing with other scientists. De-identified specimen or genetic data may be placed into one of more publicly-accessible scientific databases, such as the National Institutes of Health's Database for Genotypes and Phenotypes (dbGaP). The results from the correlative research on this study will be shared with these public databases. Through such databases, researchers from around the world will have access to de-identified samples or data for future research. More detailed information, beyond the public database, may only be accessed by scientists at other research centers who have received special permission to review de-identified data.

13. STATISTICAL CONSIDERATIONS

All statistics for both primary and secondary endpoints will be descriptive in nature.

13.1 Study Design/Endpoints

This is a phase Ib dose de-escalation study with a primary objective of evaluating the safety and

tolerability of pembrolizumab in combination with T-DM1 in patients with metastatic HER2+ breast cancer. A cohort of 6 patients will initially be enrolled at dose level 1. If 2 or more patients experience a DLT, 6 additional patients will be treated at dose level -1. If 2 or more patients experience a DLT at dose level -1, the study will be terminated. If 0 or 1 of 6 patients experience a DLT at dose level 1 or dose level -1, that dose level will be considered as RP2D. Once the RP2D is determined, 15 additional patients will be enrolled in an expansion cohort.

Primary Endpoint

- Safety and tolerability profile.
    - Assessment of DLTs during the first 21 days of treatment, as defined in Section 5.4
    - Determination of the RP2D
    - Maximum grade of all treatment-related adverse events using CTCAE v4.0

Secondary Endpoints include:

- Objective response rate (ORR) using RECIST 1.1 and irRECIST
- Progression-free survival (PFS) using RECIST 1.1 and irRECIST
- Duration of response, in patients who achieve an objective response
- Disease control rate (CR+PR+SD) at 18 weeks using RECIST 1.1 and irRECIST
- Overall survival

Blood and tissue correlative science objectives include:

- To characterize a broad array of immune markers in metastatic HER2-positive breast tumors (characterization will be based on histology, protein expression, mRNA expression, and genomic analysis)
- To explore how different immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with disease response to therapy (response assessed by RECIST 1.1 and irRECIST)
- To characterize changes in immune marker profile in peripheral blood mononuclear cells (PBMCs) from baseline (pre-trial therapy) to on-treatment assessments
- To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in PBMCs between baseline and on-treatment assessments correlates with disease response to therapy (response assessed by RECIST 1.1 and irRECIST)
    - In terms of response as assessed on serial scans
    - In terms of best response achieved at any time on trial
- To investigate whether there is an immune marker in circulating PBMCs that corresponds to tumor infiltrating lymphocyte (TIL) percentage in baseline tumor
- In the cohort of patients who have re-biopsy at week 6 of treatment: To characterize changes in a broad array of immune markers from baseline (as characterized above) to 6 weeks on trial therapy, and explore changes in the tumor microenvironment that correlate with disease response to therapy

13.2 Sample Size, Accrual Rate and Study Duration

Sample size for this trial will depend on the outcome of the dose de-escalation phase. Six to 12 patients will be enrolled in the dose de-escalation phase, and once RP2D is determined, 15 more patients will be enrolled in an expansion cohort. The operating characteristics of the dose de-escalation portion of this study are shown below, which provides the probability of declaring the RP2D for a range of underlying true DLT rates. For example, for a toxicity that occurs in 60% of patients, there is less than 5% probability (0.04) of declaring the RP2D.

True underlying DLT rate	10%	20%	30%	40%	50%	60%	70%	80%	90%
Probability of declaring RP2D	89%	66%	42%	23%	11%	4%	1%	0.2%	<0.01%

With 6 patients treated at the RP2D during dose de-escalation, and 15 patients in the dose expansion cohort (n = 21), the probability of observing at least one serious toxicity event is 89% if the true toxicity rate is 10%, and 66% if the true toxicity rate is 5%.

Objective response rate will be estimated among 15 patients in the expansion cohort and patients with measurable disease at screening who were treated at RP2D in the dose de-escalation phase. A total of 15 to 21 patients will be evaluable for ORR. The table below gives the 95% CI (calculated using the exact binomial method) for the ORR estimate in a cohort size of 15 patients, given various true underlying ORR. For example, if the true underlying response rate is 40% among 15 patients, the 95% CI would be 19-64%.

True underlying ORR	20%	27%	33%	40%	47%	53%
95% CI (n=15)	4-48%	8-55%	12-62%	16-68%	21-73%	27-79%

We estimate accrual to be 2 per month, and estimate accrual will take 12-18 months to complete.

13.3 Stratification Factors

N/A

13.4 Interim Monitoring Plan

During the dose de-escalation phase, the first 6 patients within a dosing cohort need to complete 21 days of treatment and the safety data will be reviewed before enrolling additional patients.

13.5 Analysis of Primary Endpoints

The determination of the RP2D under the dose de-escalation scheme is defined in Section 13.1. Treatment-related toxicities will be summarized by maximum grade and by term using CTCAE v4.0 and reported with 90% binomial exact confidence intervals.

13.6 Analysis of Secondary Endpoints

Best overall response will be assessed among patients who received at least one dose of both study drugs at the RP2D and have measurable disease at screening. Radiographic response will be assessed using RECIST 1.1 criteria as defined in section 11.1.4, and irRECIST will be assessed as defined in section 11.3. The objective response rate by RECIST 1.1 (CR + PR) and immune-related response (irCR + irPR) will be reported with 95% exact confidence intervals.

PFS will be described using the method of Kaplan-Meier, and it will be presented with a 95% confidence interval. PFS is defined as the time from study registration to radiographic evidence of disease progression (as defined in Section 5.6) or death due to any cause, whichever occurred first. Patients alive without disease progression are censored at the date of last disease evaluation.

Correlative objectives will be descriptive-only, using summary statistics (such as mean, sd, median, IQR and range) to give the empirical distribution of immune markers detected in the study population. With 15 patients evaluable for immune markers and under a Gaussian assumption to a continuous measure, a 95% confidence interval around a mean will have a width of approximately one standardized unit. Correlation to responses will be reported as odds-ratios with 95% confidence intervals. Specifically, as an example, we plan to measure PD-L1 expression by immunohistochemistry in baseline biopsies as a continuous variable, and correlate baseline PD-L1 expression and change in PD-L1 expression with response (by RECIST and irRECIST) as an exploratory analysis. All analysis of Oncopanel in correlation with patient outcomes are exploratory and hypothesis-generating. Any promising findings will be explored in future studies.

13.7 Reporting and Exclusions

13.7.1 Evaluation of Toxicity

All patients who receive any amount of either study drug will be evaluable for safety and tolerability, even if there are major protocol treatment deviations or if they are later found to be ineligible.

13.7.2 Evaluation of the Primary Efficacy Endpoint

All participants who have received at least one dose of study medication at the RP2D will be assessed for PFS, and the subset of patients with measurable disease will be assessed for objective response.

14. PUBLICATION PLAN

The Primary Investigator will be the final arbiter of the manuscript content.

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	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 (*e.g.*, 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 more than 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 more than 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 B NEW YORK HEART ASSOCIATION (NYHA) CLASSIFICATIONS

The New York Heart Association (NYHA) Cardiac Disease Classification provides a functional and therapeutic classification for the prescription of physical activity for cardiac subjects. Based on NYHA definitions, subjects are to be classified as follows:

Class	Definition
Class I	Subjects with no limitation of activities; they suffer no symptoms from ordinary activities.
Class II	Subjects with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.
Class III	Subjects with marked limitation of activity; they are comfortable only at rest.
Class IV	Subjects who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

APPENDIX C STRONG CYP3A INDUCERS/INHIBITORS

The list provided below is not exhaustive. For a more comprehensive, frequently updated list, please visit: http://medicine.iupui.edu/clinpharm/ddis/table.aspx.

Medications that strongly inhibit CYP3A:
Amprenavir
Atazanavir
Boceprevir
Clarithromycin
Conivaptan
Delavirdine
Diltiazem
Erythromycin
Fosamprenavir
Indinavir
Itraconazole
Ketoconazole
Lopinavir
Mibefradil
Miconazole
Nefazodone
Nelfinavir
Posaconazole
Ritonavir
Saquinavir
Telaprevir
Telithromycin
Verapamil
Voriconazole
Grapefruit, grapefruit juice, or any product containing grapefruit

Medications that strongly induce CYP3A:
Carbamazepine
Felbamate
Nevirapine
Phenobarbital
Phenytoin
Primidone
Rifabutin
Rifampin
Rifapentin
St. John's wort

APPENDIX D GUIDELINES FOR LIVER BIOPSY

As nodular regenerative hyperplasia (NRH) can be a very subtle diagnosis to make on liver biopsy, every attempt should be made to maximize the amount of tissue obtained. A minimum size of an 18 gauge needle and percutaneous biopsies of at least 1.5 cm in length are recommended, if clinically appropriate. In order to diagnose NRH, reticulin and trichrome stains are necessary. Smaller biopsies obtained via a transjugular approach, as well as smaller biopsy gun needle biopsies, are discouraged. Small wedge biopsies are also discouraged.

APPENDIX E GUIDELINES FOR COLLECTING RESEARCH BIOPSY TISSUE

Tissue specimens will be collected from metastatic lesions using standard institutional procedures. The amount of tissue collected may follow the guidelines listed below.

Skin/chest wall: A goal of 2 5-mm punch biopsies will be obtained.

Lymph node: A goal of 5-7 core biopsy specimens will be obtained using an 18-gauge needle.

Liver: A goal of 5-7 core biopsy specimens will be obtained using an 18-gauge needle.

Lung: Because of the risk of pneumothorax associated with core needle biopsies of lung nodules, no core biopsies of lung nodules are mandated on this protocol, unless they are clinically indicated.
Bone: Because the yield of malignant tissue from bone biopsies tends to be relatively low, if a patient has another accessible site of disease (i.e. skin, lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 3-6 core biopsy specimens will be obtained using an 11-13 gauge needle.

Please note that the above are guidelines for the amount of tissue to be obtained, and are not meant to replace clinical judgment at the time the procedure is performed. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure. Alternative amounts of tissue collected, based on best judgment of the physician performing the procedure, are allowed per protocol.

If a patient is undergoing resection of a lesion for clinical reasons (i.e. wedge resection of a new lung lesion for confirmation of diagnosis or re-testing of hormone receptor or HER2 status; or, a resection of a chest wall lesion; or, resection of a lymph node), then the patient may opt to have a portion of that tissue (roughly equivalent to the goal amount of tissue listed in the guidelines above, i.e. the equivalent of two 5-mm punch biopsies of the skin, or 4-6 18-gauge core biopsies) stored for research at the time of the procedure (provided that the tissue is processed as specified), in which case, the patient would not be required to undergo a separate research biopsy at baseline on this protocol.

2DANA-FARBER CANCER INSTITUTE
Nursing Protocol Education Sheet

Protocol Number:	**16-492**
Protocol Name:	**A Phase 1b Study of Pembrolizumab in combination with Trastuzumab-DM1 in Metastatic HER2-Positive Breast Cancer**
DFCI Site PI:	Sara Tolaney, MD
DFCI Research Nurse:	Margaret Campbell, Elizabeth Kasparian, Kathleen Roche, Jaclyn Lehnus, Kelly O'Neil, Kelly Marchetti, Morgan Marx

Page the DFCI research nurse or DFCI site PI if there are any questions/concerns about the protocol.
Please also refer to ONC 15: Oncology Nursing Protocol Education Policy
***** Remember to check the ALERT PAGE*****

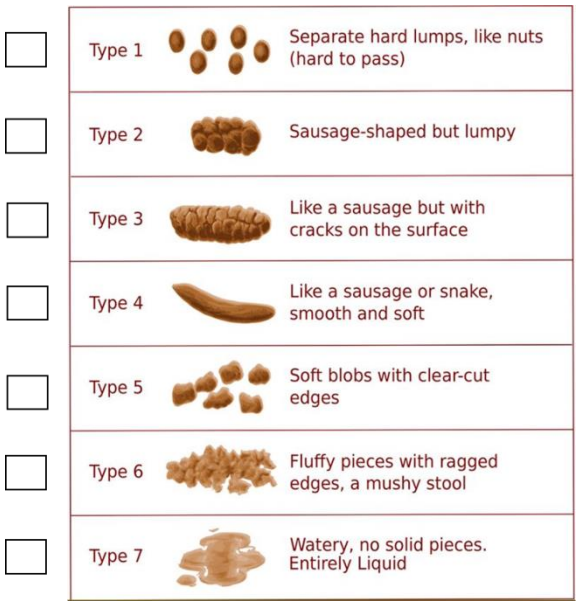
SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Design	• **Pembrolizumab** is a monoclonal antibody of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. (Section 2.2) • **T-DM1** is an anti-HER2 agent (Section 2.3) • A cycle is defined as 21 days. (Section 5.1)
Dose Calc.	• Pembrolizumab is dosed as a fixed dose. (Section 5.1) • T-DM1 is dosed in mg/kg. (Section 5.1) • The total dose of T-DM1 will be calculated according to institutional standards. (Section 5.3.2)
Study Drug Administration	Agent *Administration* Guidelines are found in Section 5.3. • Pembrolizumab will be administered as a 30 minute (-5/+10 minutes) IV infusion on day 1(+/- 3 days) of each cycle. (Section 5.3.1) • T-DM1 will be administered on day 1 (+/- 3 days) of each cycle. The first infusion of T-DM1 will be administered over 90 minutes (+/- 10 minutes) with a 60 minute observation period. If well tolerated, subsequent doses of T-DM1 may be administered over 30 minutes (+/- 10 minutes), with a minimum 30-minute observation period after infusion. (Section 5.3.2) • Pembrolizumab should be administered prior to T-DM1 administration. There should be no overlap in timing of the two administrations. (Section 5.3.1) • Premedication for nausea and infusion reactions are not commonly required but may be given at the investigator's discretion. (Section 5.3.2) • Pre-treatment Criteria is found in section 5.2. • Following Pembrolizumab infusion, flush the IV line with normal saline. (Section 8.1.8) • The solution of T-DM1 should not be shaken. (Section 8.2.1)
Dose Mods & Toxicity	*Criteria to Treat, Dose Modifications/Dosing Delays for Toxicities* are outlined in Section 6.0. • This protocol uses NCI CTCAE criteria, version 4.0. (Section 6.0) • The definition of a DLT is found in Section 5.4. • T-DM1 can be held for a maximum of 42 days from the time of the last scheduled dose for resolution of toxicities. (Section 6.2)
Con Meds	*Concomitant Therapy* Guidelines are in Section 5.5 • Please review the cited sections for permitted, prohibited, and "use with caution" medications/therapies/foods
Required Data	*Study Calendar and Assessment Required data* are outlined in Section 10.0. • The study calendar is in Section 10.0. • **Vital signs:** The time points are in Section 10.0/Footnote b. • **ECGs**: Single 12-lead EKG at screening visit only. (Section 10.0) • **Biomarkers**: The time points are in Section 10.0.
Tips	**All study drugs require documentation of exact administration time.**

Breast Cancer Stool Collection Questionnaire

Subject ID:	Date collected _____/_____/_____ Month Day Year	Hour collected _____ ○ am ○ pm

1. Based on the small chart included in the postcard, what did the stool you put into the tube look like? (Choose one or two answers).



2. Prior to this collection, when was your last bowel movement?

Earlier today, in the last 6 hours	Earlier today, more than 6 hours ago	Yesterday	Two days ago	More than two days ago
☐	☐	☐	☐	☐

3. In the past 2 months, how often have you had a bowel movement and what was the frequency of stool with the following textures?

	More than twice per day	Twice per day	Once per day	Every other day	Every 3-6 days	Once a week or less	Never
Any bowel movement	☐	☐	☐	☐	☐	☐	
Stool texture (The answers can be different from the above)							
Hard / lumpy	☐	☐	☐	☐	☐	☐	☐
Soft / smooth	☐	☐	☐	☐	☐	☐	☐
Watery liquid	☐	☐	☐	☐	☐	☐	☐

4. In the past 2 months, have you used any of the following medications?

	No	Yes, occasionally	Yes, regularly
Oral antibiotics	☐	☐	☐
If yes, what is the name (list of used)?			
Injected antibiotics	☐	☐	☐
If yes, what is the name (list of used)?			
Prilosec, Nexium, Prevacid (lansoprazole), Protonix, Aciphex	☐	☐	☐
H2 blocker: Pepcid, Tagamet, Zantac, Axid	☐	☐	☐

5. In the past 2 months, have you used any medications modifying bile production, including (but not limited to): Cholesytramine (e.g. Questran, Prevalite, Locholest), colestipol (e.g, colestid), colesevelam (e.g. Welchol), chenodeoxycholic acids (e.g. CDCA), or ursodeoxycholic acid (e.g. UDCA, Ursodiol, Actigall)?

☐No ☐Yes, occasionally ☐Yes, regularly

6. In the past 2 months, have you undergone a colonoscopy or other procedure requiring bowel preparation? ☐No ☐Yes

7. In the past week, have you taken (or eaten) any of the following? If so, please specify the frequency.

	Not used	Once or twice	Three to six times	Daily	More than once per day
Fiber substitute, such as Metamucil, Konsyl or Citracel	☐	☐	☐	☐	☐
Laxatives, such as Ex-lax, Dulcolax, MiraLax, Senna, or enema	☐	☐	☐	☐	☐
Stool softener, such as Colace	☐	☐	☐	☐	☐
Probiotic supplements	☐	☐	☐	☐	☐
Yogurt or kefir	☐	☐	☐	☐	☐
Other fermented foods, such as sauerkraut or kombucha	☐	☐	☐	☐	☐

8. In the past 6 months, did you gain or lose weight?

No	Gained ≤5 lbs.	Gained >5 lbs.	Lost ≤5 lbs.	Lost >5 lbs.
☐	☐	☐	☐	☐

9. Did you have any problems or concerns with the stool sample collection, for example the solution spilled out of the tube or you had problems with catching stool in the toilet accessory? (Please describe)

_____

_____

PHARMACY MANUAL

PEMBROLIZUMAB (MK-3475)

DF/HCC Protocol: 16-492

[redacted]

SUMMARY OF REVISIONS

The following are a list of revisions to the Pharmacy Manual for pembrolizumab (MK-3475):

Revision Date	Revisions to Document	New Version #:
10-Dec-14	Global change: updated MK-3475 to pembrolizumab	2.0
10-Dec-14	Global change: Inserted header Pembrolizumab (MK-3475) Pharmacy Manual for Investigational Studies	2.0
10-Dec-14	Expanded table of contents	2.0
10-Dec-14	Removed trailing zeros after decimal points	2.0
10-Dec-14	Section 2: Revised footnote 1 in trial treatment table	2.0
10-Dec-14	Section 3.1: Removed text, "The pH is maintained using a 10 mM histidine buffer"	2.0
10-Dec-14	Section 3.2: Added text to emphasize normal saline as preferred diluent Insert cautionary statement regarding drug transport and delivery Inserted text that any deviation from the guidance listed in this manual, must be discussed with sponsor	2.0
10-Dec-14	Section 3.3: Clarified weight based dosing calculation for changes in weight (10% rule) Removed calculation for 200 mg fixed dosing	2.0
10-Dec-14	Section 3.4: Clarified preferred method of dose preparation as volumetric reconstitution Clarified reconstitution technique.	2.0
10-Dec-14	Section 3.6: Inserted text stating infusion rates may differ for	2.0

	infusion reactions Inserted text that entire bag needs to be dosed during infusion Removed text regarding excess volume preparation Added text to document volume administered per DEG instructions	
10-Dec-14	Section 4.2 Added text to emphasize normal saline as preferred diluent Insert cautionary statement regarding drug transport and delivery Inserted text that any deviation from the guidance listed in this manual, must be discussed with sponsor	2.0
10-Dec-14	Section 4.3: Clarified weight based dosing calculation for changes in weight (10% rule) Removed calculation for 200 mg fixed dosing	2.0
10-Dec-14	Section 4.4: Clarified preferred method of dose preparation as volumetric method	2.0
10-Dec-14	Section 4.5: Inserted text stating infusion rates may differ for infusion reactions Inserted text that entire bag needs to be dosed during infusion Removed text regarding excess volume preparation Added text to document volume administered per DEG instructions	2.0
21-Oct-15	Section 2:	3.0

	Text added to footnote 2 for sourcing and recording of lot number, manufacturer, and expiry date.	
21-Oct-15	Section 3.2: Added guidance for collection of the following diluent information (manufacturer, lot, and expiry). Removed the following text, " unless instructed by the sponsor in writing" in the following sentence : Pembrolizumab (MK-3475) SHOULD NOT BE MIXED WITH OTHER DILUENTS unless instructed by the SPONSOR in writing. Added diluted drug product in the following sentence: Sites should follow their SOPs for drug transport and delivery, with all possible effort to minimize agitation of the reconstituted and diluted drug product between the pharmacy and the clinic	3.0
21-Oct-15	Section 3.3: Clarified re-calculation of weight based dosing guidance.	3.0
21-Oct-15	Section 3.5 Additional text added for concentration range requirements.	3.0
21-Oct-15	Section 3.7: Removed chemotherapeutic waste designation for solution remaining in vials that must be discarded.	3.0

21-Oct-15	Section 4.1: Text added about cap color.	3.0
21-Oct-15	Section 4.2: Added guidance for collection of the following diluent information (manufacturer, lot, and expiry).	3.0
21-Oct-15	Section 4.3: Clarified re-calculation of weight based dosing guidance.	3.0
21-Oct-15	Section 4.4: Additional text added for concentration range requirements.	3.0
21-Oct-15	Section 4.5: Added the following text regarding infusion set materials: *Contact Sponsor for materials not listed above	3.0
21-Oct-15	Section 4.6: Added text for discarding used vials.	3.0
28-Feb-17	Section 2.0: Updated footnote in trial treatment table to include SmPC and guidance regarding locally sourced drug.	4.0
28-Feb-17	Section 3.1	4.0

	Added text stating formulation is latex free	
28-Feb-17	Section 3.2 Added rounding guidance. Added guidance on temperature excursions. Clarified 4 hour room temperature time limitation. Updated language around particulates.	4.0
28-Feb-17	Section 3.3 Updated units from lb to kg to align with weight based dosing examples.	4.0
28-Feb-17	Section 3.4 Clarified use of biosafety cabinets. Updated gravimetric dosing guidance. Added statement for use of spikes. Updated text for potential for foaming.	4.0
28-Feb-17	Section 3.5 Deleted duplicate text regarding use of biosafety cabinets. Updated text regarding formation of particulates.	4.0
28-Feb-17	Section 3.6 Added guidance for preparation of placebo.	4.0
28-Feb-17	Section 3.7 Added instructional text that states 250mL volume is only applicable to weight based studies.	4.0
28-Feb-17	Section 3.8	4.0

	Clarified instructions for return of un-used vials.	
28-Feb-17	Section 4.1 Added text stating formulation is latex free. Updated cap color for liquid formulation. Added text regarding overfill volume.	4.0
28-Feb-17	Section 4.2 Added rounding guidance. Added guidance on temperature excursions. Clarified 4 hour room temperature time limitation. Updated language around particulates.	4.0
28-Feb-17	Section 4.3 Updated units from lb to kg to align with weight based dosing examples.	4.0
28-Feb-17	Section 4.4 Clarified use of biosafety cabinets. Updated gravimetric dosing guidance. Added statement for use of spikes. Updated text for potential for foaming.	4.0
28-Feb-17	Section 4.5 Added guidance for preparation of placebo.	4.0
28-Feb-17	Section 4.6 Added instructional text that states 250mL volume is only applicable to weight based studies. Additional text added regarding formation of	4.0

	foam.	
28-Feb-17	Section 4.7 Clarified instructions for return of un-used vials.	4.0
21-Mar-2018	Section 1.0: Added unblinded clinical scientist to contact list Updated CDS title to CS Updated IVRS to IRT throughout document	5.0
21-Mar-2018	Section 3.2 Added text for temperature excursions that temperature data needs to be included in clinical complaint. Clarified for blinded studies that uCRA should be contacted for temperature excursions. Updated the room temperature allowance from 4 hours to 6 hours and clarified fridge time allowance. Clarified the start of room temperature time.	5.0
21-Mar-2018	Section 3.6 Revised flushing statement. Section 3.7 Updated drug destruction instructions.	5.0
21-Mar-2018	Section 4.2 Added text for temperature excursions that temperature data needs to be included in clinical complaint. Clarified for blinded studies that uCRA should be	5.0

	contacted for temperature excursions. Updated the room temperature allowance from 4 hours to 6 hours and clarified fridge time allowance. Clarified the start of room temperature time.	
21-Mar-2018	Section 4.4 Updated room temperature time allowance to 6 hours and clarified cumulative storage time.	5.0
21-Mar 2018	Section 4.5: Revised flushing statement. Section 4.6: Updated drug destruction instructions.	5.0

TABLE OF CONTENTS

Contents

1. Contact List

[redacted] (Pharmacy Manual questions only)

For questions regarding the details outlined within this Pharmacy Manual, please contact your clinical scientist (CS):

[redacted]

Principal Investigator

Sara Tolaney, MD, MPH

[redacted]

2. Trial Treatment

Table 2: Regimen description

Regimen Description					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Pembrolizumab	Not routinely necessary unless prior infusion reaction.	200 mg, at a final concentration of 1 mg/mL to 10 mg/mL in NS or D5	IV over approximately 30 minutes (range: 25-40 minutes).	Day 1	21 days (3 weeks)
T-DM1	Not routinely necessary unless prior infusion reaction.	Per Table 1*	IV over approximately 90 minutes (cycle 1) and 30 minutes (subsequent cycles). Start T-DM1 infusion after pembrolizumab infusion is complete.	Day 1	
**Doses as appropriate for assigned dose level*					

[1] Refer to protocol for specific study drug administration sequence for combination studies of pembrolizumab (MK-3475) and chemo/immunotherapy

[2] Refer to local product label/SmPC for drug preparation and administration instructions; For any commercially available product that is provided by the trial site, subsidiary or designee, every attempt will be made to source these supplies from a single lot/batch number Local guidelines should be followed for collection of locally sourced product information such as manufacturer, lot and expiry, unless otherwise instructed by sponsor When the product is provided by [redacted] drug accountability log should be used for collection of this information

3. Drug Preparation – Pembrolizumab (MK-3475) Powder for Solution for Infusion, 50 mg/vial

3.1 DRUG PRODUCT

- Pembrolizumab **(MK-3475) Powder for Solution for Infusion, 50 mg/vial**

- Pembrolizumab (MK-3475) Powder for Solution for Infusion is a sterile, non-pyrogenic lyophilized powder for intravenous infusion supplied in single-use Type I glass vial containing 50 mg of pembrolizumab (MK-3475). The product is preservative-free, latex free, white to off-white powder and free from visible foreign matter.

- Pembrolizumab (MK-3475) Powder for Solution for Infusion is reconstituted with 2.3 mL sterile water for injection (WFI) to yield a 2.4 mL solution containing 25 mg/mL of pembrolizumab (MK-3475) at pH 5.2 – 5.8.

- Pembrolizumab (MK-3475) Powder for Solution for Infusion vial contains an excess fill of 10 mg (equivalent to 0.4 mL of reconstituted solution) to ensure the recovery of label claim of 50 mg pembrolizumab (MK-3475) per vial (equivalent to 2 mL of reconstituted solution).

3.2 STABILITY AND HANDLING OF DRUG PRODUCT

- Pembrolizumab (MK-3475) Powder for Solution for Infusion vials should be stored at refrigerated conditions 2 – 8 °C (36 - 46 ºF). Prior to reconstitution, the vial of lyophilized powder can be out of refrigeration (temperatures at or below 25°C (77°F)) for up to 24 hours.

- To determine whether to report a temperature excursion, the temperature values should be rounded to whole numbers.
- Rounding:
  1. Decimal values from 0.1 to 0.4 round down to the nearest whole number (e.g., 8.3 = 8)
  2. Decimal values from 0.5 to 0.9 round up to the nearest whole number (e.g., 8.7 = 9)
- Then compare the rounded values to the required temperature range to determine if there's an excursion.

- All temperature excursions, however small, must be reported by the site to the Clinical Complaint Intake mailbox [redacted] for investigation within 1 business day using the Clinical Supply Complaint & GCP Inquiry Form (excel version) and attached temperature data. Please also notify your CRA. All Clinical Supply stock that is subject to an investigation must be placed in quarantine and remain unavailable to dispense to patients until disposition has been determined.

- Please note temperature excursions after drug product is prepared are out of scope of the clinical complaint process. Please contact HQ clinical study team for further guidance.

- Following reconstitution with **sterile water for injection**, Pembrolizumab (MK-3475) infusion solutions should be prepared in **0.9% Sodium Chloride Injection, USP** (normal saline) or regional equivalent and the final concentration of pembrolizumab (MK-3475) in the infusion solutions should be between 1 mg/mL and 10 mg/mL.

- If normal saline is not available, 5% Dextrose Injection, USP or regional equivalent (5% dextrose) is permissible, Please note, the preferred diluent is 0.9% Sodium Chloride and 5% dextrose is only permissible if normal saline is not available.

- Local guidelines should be followed for collection of diluent information such as manufacturer, lot and expiry. When the diluent is provided by [redacted], the drug accountability log should be used for collection of diluent information.

- Pembrolizumab (MK-3475) SHOULD **NOT** BE MIXED WITH OTHER DILUENTS

- Pembrolizumab (MK-3475) solutions may be stored at room temperature for a cumulative time of up to 6 hours. The 6 hour countdown begins when the vial is pierced, and includes room temperature storage of reconstituted drug product solution in vials, room temperature storage of admixture solutions in the IV bags and the duration of infusion. (Please note this 6 hour timeframe is to provide a microbial control strategy. The microbial clock only starts when the product stopper is pierced and not when the vial is removed from the refrigerator.)

- In addition, reconstituted vials and/or IV bags may be stored under refrigeration at 2 °C to 8 °C (36 °F to 46 °F), total cumulative storage time at room temperature and refrigeration should not exceed 24 hours.

- If refrigerated, allow the vials and/or IV bags to come to room temperature prior to use.
- Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Discard the drug product vial if visible particles are observed.
- Sites should follow their SOPs for drug transport and delivery, with all possible effort to minimize agitation of the reconstituted and diluted drug product between the pharmacy and the clinic
- **DO NOT USE PEMBROLIZUMAB (MK-3475) IF DISCOLORATION IS OBSERVED.**
- **DO NOT SHAKE OR FREEZE THE VIAL(S).**
- **DO NOT ADMINISTER THE PRODUCT AS AN INTRAVENOUS (IV) PUSH OR BOLUS.**
- **DO NOT COMBINE, DILUTE OR ADMINISTER IT AS AN INFUSION WITH OTHER MEDICINAL PRODUCTS**.
- **DO NOT CO-ADMINISTER OTHER DRUGS THROUGH THE SAME INFUSION LINE.**
- **Any departure from the guidance listed in this manual, must be discussed with sponsor**

3.3 DOSE CALCULATION

Follow directions applicable to the dose level (mg/kg) of the study

2 mg/kg Dose

- The preparation of infusion solutions should be based on the dose level and the subject's actual body weight in kilograms (kg) on the day of dosing. However if preparation of the infusion is required to be completed prior to the subject's visit

(e.g., per local SOP, logistical issues, etc.), then the solution may be prepared in advance in accordance with the instructions in this manual.

- If infusion solution is prepared in advance, weight from the most recent scheduled or unscheduled visit should be used.

- If the weight from the most recent scheduled or unscheduled visit is used, the dose amount should be recalculated and a new preparation of the infusion solution created if the subject's weight on the day of dosing changes by more than 10% from the last weight measurement.

    - Ex: 100 kg. at previous visit but current visit is less than 90 kg. or greater than 110 kg. then recalculation of the dose and a new preparation of infusion solution is required

- Required dose amount (mg) = dose level (mg/kg) * subject weight (kg)

- Calculate the required number of pembrolizumab (MK-3475) vials by dividing the required dose amount (mg) by 50 (mg) and rounding to the next whole number.

Example 1 (2 mg/kg dose)

Dose level = 2 mg/kg, subject weight = 69 kg
Required dose amount (mg) = 2 mg/kg * 69 kg = 138 mg
Required number of pembrolizumab (MK-3475) vials = 138/50 = 2.76
Rounded to next whole number = 3 vials

10 mg/kg Dose

- The preparation of infusion solutions should be based on the dose level and the subject's actual body weight in kilograms (kg) on the day of dosing. However if preparation of the infusion is required to be completed prior to the subject's visit (e.g., per local SOP, logistical issues, etc.), then the solution may be prepared in advance in accordance with the instructions in this manual.

- If infusion solution is prepared in advance, weight from the most recent scheduled or unscheduled visit should be used.

- If the weight from the most recent scheduled or unscheduled visit is used, the dose amount should be recalculated and a new preparation of the infusion solution created if

the subject's weight on the day of dosing changes by more than 10% from the last weight measurement.

- Ex: 100 kg. at previous visit but current visit is less 90 kg. or greater 110 kg. then recalculation of the dose and a new preparation of infusion solution is required

- Required dose amount (mg) = dose level (mg/kg) * subject weight (kg)

- Calculate the required number of pembrolizumab (MK-3475) vials by dividing the required dose amount (mg) by 50 (mg) and rounding to the next whole number.

Example 2 (10 mg/kg dose)
Dose level = 10 mg/kg, subject weight = 69 kg
Required dose amount (mg) = 10 mg/kg * 69 kg = 690 mg
Required number of pembrolizumab (MK-3475) vials = 690/50 = 13.8
Rounded to next whole number = 14 vials

- Preference is for sites to prepare study medication on day of infusion. For sites that must prepare dose in advance, weight from most recent scheduled or unscheduled visit can be used for advanced preparation. Then on day of dosing, reweigh subject for actual weight.

  - If the weight on day of dosing is within 10% of weight used for advanced dose calculation, you can proceed with dosing what was prepared during advanced preparations
  - If a patient's weight on day of dosing has fluctuated by more than 10% compared to weight used for advanced study prep, then the dose should be recalculated using the new weight measurement.

<u>200 mg Fixed Dose</u>

- **Required Number of vials = 4 vials (50 mg/vial)**
- **8 mLs Total**

3.4 RECONSTITUTION OF DRUG PRODUCT (POWDER FOR SOLUTION FOR INFUSION, 50 MG/VIAL)

- Aseptic technique must be strictly observed throughout the preparation procedure.
- Use of a biosafety cabinet is preferred since no anti-microbial preservative is present in the product; however, it is not mandatory unless specified by site standard operating procedure.
- Equilibrate required number of pembrolizumab (MK-3475) vials to room temperature.
- The preferred method of dose preparation is the volumetric method.
- Sponsor recommends reconstitution and administration of pembrolizumab (MK-3475) that follows the parameters in this manual, however if use of gravimetric preparation is mandatory due to local site procedures, the following requirements must be satisfied and documented:
    - Draw the required volume up to 2.0 mL (50 mg) of pembrolizumab from each vial
    - Limit the number of punctures of each vial to two (one for reconstitution, one for withdrawal)
- For gravimetric preparation method using density of reconstituted pembrolizumab solution, a value of 1.03 g/mL should be used
- For gravimetric preparation method using the total solid content information, the following information on the total solid and active contents per vial of pembrolizumab lyophilized product should be used:
    - Total calculated weight of pembrolizumab per vial = 60.0 mg
    - Total calculated weight of solids per vial (including pembrolizumab) = 232.2 mg
- [redacted] does not support methods of preparation of [redacted] agents beyond what is stated in the product literature. Sites should reference the SmPCs or packaging inserts for preparation instructions.

- Remove the cap from the seal. **Do not decrimp the vial**.

- Insert a needle through the stopper of the pembrolizumab (MK-3475) Powder for Solution for Infusion vial(s) to release vacuum (if any). Leave the needle inserted in the stopper. If local standard operating procedures (SOPs) prohibit leaving a needle inserted in the stopper, this step can be skipped.

- If the site procedures require use of spikes or other closed system transfer devices (CSTDs), please contact sponsor for approval.

- If one WFI bottle is used to reconstitute one pembrolizumab (MK-3475) vial: Attach a 3 mL syringe to a needle. Insert the needle through the stopper of the sterile water for injection (WFI) bottle. Draw excess of 2.3 mL of WFI in the syringe and remove the syringe-needle assembly from the vial.

- If one WFI bottle is used to reconstitute more than one pembrolizumab (MK-3475) vials: Insert a needle through the stopper of the sterile WFI bottle. Attach a 3 mL syringe to the needle inserted in the sterile WFI bottle and draw excess of 2.3 mL of WFI in the syringe. Carefully detach the syringe without removing the needle from the WFI bottle. Repeat the process to fill additional syringes while keeping the needle inserted in sterile WFI bottle to minimize particle shedding from stopper. Use a new sterile WFI bottle after filling approximately 10 syringes.

- Attach a new needle to the filled syringe (if applicable). Remove excess air and WFI from the syringe-needle assembly while ensuring that there is 2.3 mL WFI still remaining in it.

- Aseptically add 2.3 mL of sterile water for injection to yield a 25 mg/mL (pH 5.2-5.8) solution of pembrolizumab (MK-3475).

Caution: To avoid foaming, ensure that water is delivered along the walls of the vial and not directly squirted on the lyophilized powder.

- Remove the needle(s) from the stopper of pembrolizumab (MK-3475) vial.

- Slowly swirl the vial to allow reconstitution of the lyophilized powder. Allow up to 5 minutes for the bubbles to clear.

Caution: Do not shake the vials otherwise this may result in formation of foam. If foam is noticed in either vial or bag, the drug product will need to be discarded. A new preparation should be made, taking care not to shake or agitate the product.

3.5 PREPARATION OF INFUSION SOLUTION

- Aseptic technique must be strictly observed throughout the preparation procedure.
- Reconstitute the required number of vials to prepare the infusion solution.
- Choose a suitable infusion bag size so that the following conditions are met:
    - Concentration of pembrolizumab (MK-3475) is between 1 mg/mL and 10 mg/mL
    - The infusion volume to bag capacity ratio should not be less than 0.3. In other words, the bag must be filled to at least 30% of its capacity.
- Choose a suitable infusion bag material. The bag may be empty or it may contain normal saline. The following infusion bag materials are compatible with pembrolizumab (MK-3475):
    - PVC plasticized with DEHP
    - Non-PVC (polyolefin)
    - EVA
    - PE lined polyolefin

    *Contact Sponsor for materials not listed above
- Calculate the volume of pembrolizumb (MK-3475) and normal saline required to prepare the infusion (admixture) bag

    Volume of reconstituted pembrolizumab (MK-3475) (mL) = required dose amount (mg) / 25 (mg/mL)

    Volume of normal saline = total infusion volume – volume of pembrolizumab (MK-3475) from above
- If a bag pre-filled with normal saline is being used, remove the excess volume of normal saline using a sterile syringe (Polypropylene, latex-free) attached to a suitable needle. Keep in consideration the excess bag fill volume as well as the volume of reconstituted

pembrolizumab (MK-3475) to be added to the bag to prepare the infusion solution. This helps ensure that the concentration in the bag can be accurately calculated and falls within the acceptable range of 1 mg/mL to 10 mg/mL. If the site would like to proceed without removing excess saline they must ensure that the concentration of MK-3475 would still fall within acceptable range.

- If an empty bag is being used, withdraw the necessary volume of normal saline from another appropriate bag and inject into the empty bag. Keep in consideration the volume of reconstituted pembrolizumab (MK-3475) to be added to the bag to prepare the infusion solution.

- Withdraw the required volume of pembrolizumab (MK-3475) from the vial(s) (up to 2 mL from each vial) using a sterile syringe attached to a suitable needle. The vial(s) may need to be inverted to remove solution.

  Volume of pembrolizumab (MK-3475) (mL) = required dose amount (mg) / 25 (mg/mL)

  Note: If it is necessary to use several vials, it is advisable to withdraw from several vials into a suitable size single use syringe using a new needle for each vial.

- Add the required pembrolizumab (MK-3475) (reconstituted solution) into the infusion IV bag containing normal saline and gently invert the bag 10-15 times to mix the solution.
- If the infusion bag is excessively handled or shaken, particulates may form. If this occurs, discard the bag and create a new bag. Please contact your HQ clinical study team if particulates are noticed for further instructions. Be prepared to provide the following information:
  - IV bag manufacture, lot and expiry
  - Target volume of admixture solution in the IV bag (e.g. 100 mL, 200 mL etc.)
  - Amount of drug product (mL or mg) added to the bag
  - Drug product lot
  - Brief description of the nature of visible particles (color, shape, size, numbers etc.)

- **DO NOT FREEZE THE PEMBROLIZUMAB (MK-3475) INFUSION SOLUTION.**

- Discard any unused portion left in the vial as the product contains no preservative

3.6 ADMINISTRATION

- Pembrolizumab (MK-3475) infusions should be administered in 30 minutes, with a window of -5 and +10 minutes, using an infusion pump. A central catheter is not required for infusion; however if a subject has a central venous catheter in place, it is recommended that it be used for the infusion.

- The following infusion set materials are compatible with pembrolizumab (MK-3475):

    - PVC Infusion set that is plasticized using DEHP
    - PVC and tri-(2-ethylhexyl) trimellitate (TOTM) infusion set
    - Polyethylene lined PVC infusion set
    - PVC Infusion set that is plasticized using Di-2-ethylhexyl Terephthalate (DEHT)
    - Polyurethane set

    *Contact Sponsor for materials not listed above

- A sterile, non-pyrogenic, low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone (PES) must be used during administration to remove any adventitious particles. If the infusion set does not contain 0.2 to 5 µm in-line filter, it is recommended to use 0.2 to 5 µm add-on filter which may contain an extension line (Note: the materials of the extension line and filter should be as mentioned above).

- Attach the infusion line to the pump and prime the line, either with normal saline (at least 25 mL) or with infusion solution as per local SOP, before starting the infusion.

- Infuse pembrolizumab (MK-3475) over approximately 30 minutes, with a window of -5 and +10 minutes, through a peripheral line or indwelling catheter.

- Ensure the entire contents of the bag are dosed and all remaining drug solution in the line is administered through saline flushing.

- Document volume administered according to data entry guidelines.

In case of infusion reactions, infusion rate may differ; refer to protocol for specific instructions.

- Whenever possible, the lowest infusion rate should be used that will allow completion of the infusion within the 30 minutes

- Maximum rate of infusion should not exceed 6.7 mL/min through a peripheral line or indwelling catheter.

- **DO NOT CO-ADMINISTER OTHER DRUGS THROUGH THE SAME INFUSION LINE.**

- **UNUSED INFUSION SOLUTION FOR INJECTION SHOULD NOT BE USED FOR ANOTHER INFUSION OF THE SAME SUBJECT OR DIFFERENT SUBJECT.**

3.7 RETURN AND DISCARDING OF PEMBROLIZUMAB (MK-3475) VIALS

- Unused pembrolizumab (MK-3475) Powder for Solution for Infusion or Solution for Infusion vial(s) shall be returned to the designated facility for destruction after CRA has performed full accountability of un-used vials.

    - For US clinical sites, return to the central depot that shipped supplies to the site:
        - [redacted]
        - [redacted]

    - For ex-US clinical sites, consult with local [redacted] subsidiary for facility address.

    - For clinical sites that are not able to return to designated facility for destruction, please work with your CRA and refer to SOP 104_ER5_Return and Destruction of Clinical Supplies Procedure for guidance to follow for destruction of unused vials at the local site.

- For Interactive response technology (IRT) studies, the IRT Clinical Drug Supply Return Forms generated in the system must be used

- Solution remaining in a used vial should be discarded according to your local procedures.

- Any information on the label identifying the subject should be redacted prior to returning the study medication.

4. Drug Preparation – Pembrolizumab (MK-3475) Solution for Infusion

4.1 DRUG PRODUCT

Pembrolizumab (MK-3475) Solution for Infusion, 100 mg/ 4 mL vial

- Pembrolizumab (MK-3475) Solution for Infusion is a sterile, non-pyrogenic aqueous solution supplied in single-use Type I glass vial containing 100 mg/4 mL of pembrolizumab (MK-3475). The product is preservative-free, latex free solution which is essentially free of extraneous particulates.

- Cap color of MK-3475 (Pembrolizumab) 100 mg vials:

    - Both red, salmon, and blue color caps may be used. Though the cap color may be different, the product inside the vial is the same MK-3475 drug product.

- Pembrolizumab (MK-3475) Solution for Infusion vials are filled to a target of 4.25mL (106.25mg) to ensure recovery of 4.0mL (100mg).

4.2 STABILITY AND HANDLING OF DRUG PRODUCT

- **Pembrolizumab (MK-3475) Solution for Infusion, 100 mg/ 4 mL vial**: pembrolizumab (MK-3475) Solution for Infusion vials should be stored at refrigerated conditions 2 – 8 °C (36 - 46 °F) and protected from light.

- To determine whether to report a temperature excursion, the temperature values should be rounded to whole numbers.

- Rounding:

    - Decimal values from 0.1 to 0.4 round down to the nearest whole number (e.g., 8.3 = 8)
    - Decimal values from 0.5 to 0.9 round up to the nearest whole number (e.g., 8.7 = 9)

- Then compare the rounded values to the required temperature range to determine if there's an excursion.
- All temperature excursions, however small, must be reported by the site to the Clinical Complaint Intake mailbox [redacted] for investigation within 1 business day using the Clinical Supply Complaint & GCP Inquiry Form (excel version) and attached temperature data. Please also notify your CRA. All Clinical Supply stock that is subject to an investigation must be placed in quarantine and remain unavailable to dispense to patients until disposition has been determined.
- Please note temperature excursions after drug product is prepared are out of scope of the clinical complaint process. Please contact HQ clinical study team for further guidance. **Note**: vials should be stored in the original box to ensure the drug product is protected from light.

- Pembrolizumab (MK-3475) infusion solutions should be prepared in **0.9% Sodium Chloride Injection, USP** (normal saline) or regional equivalent or 5% Dextrose Injection, USP (5% dextrose) or regional equivalent and the final concentration of pembrolizumab (MK-3475) in the infusion solutions should be between 1 mg/mL and 10 mg/mL.

- Please note, the preferred diluent is 0.9% Sodium Chloride and 5% dextrose is only permissible if normal saline is not available.

- Local guidelines should be followed for collection of diluent information such as manufacturer, lot and expiry. When the diluent is provided by [redacted] the drug accountability log should be used for collection of diluent information.

- Pembrolizumab (MK-3475) SHOULD **NOT** BE MIXED WITH OTHER DILUENTS.

- Pembrolizumab (MK-3475) solutions may be stored at room temperature for a cumulative time of up to 6 hours. The 6 hour countdown begins when the vial is pierced, and includes room temperature storage of admixture solutions in the IV bags

and the duration of infusion. (Please note this 6 hour timeframe is to provide a microbial control strategy. The microbial clock only starts when the product stopper is pierced and not when the vial is removed from the refrigerator.)

- In addition, IV bags may be stored under refrigeration at 2 °C to 8 °C (36 °F to 46 °F), total cumulative storage time at room temperature and refrigeration should not exceed 24 hours.

- If refrigerated, allow the IV bags to come to room temperature prior to use.

- Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Discard the drug product vial if visible particles are observed.

- Sites should follow their SOPs for drug transport and delivery, with all possible effort to minimize agitation of the drug product between the pharmacy and the clinic

- **DO NOT USE PEMBROLIZUMAB (MK-3475) IF DISCOLORATION IS OBSERVED**.

- **DO NOT SHAKE OR FREEZE THE VIAL(S).**

- **DO NOT ADMINISTER THE PRODUCT AS AN INTRAVENOUS (IV) PUSH OR BOLUS.**

- **DO NOT COMBINE, DILUTE OR ADMINISTER IT AS AN INFUSION WITH OTHER MEDICINAL PRODUCTS.**

- **Any departure from the guidance listed in this manual, must be discussed with sponsor**

4.3 DOSE CALCULATION

Follow directions applicable to the dose level (mg/kg) of the study.

2 mg/kg Dose

- The preparation of infusion solutions should be based on the dose level and the subject's actual body weight in kilograms (kg) on the day of dosing. However if preparation of the infusion is required to be completed prior to the subject's visit (e.g., per local SOP, logistical issues, etc.), then the solution may be prepared in advance in accordance with the instructions in this manual.

  - If infusion solution is prepared in advance, weight from the most recent scheduled or unscheduled visit should be used.

  - If the weight from the most recent scheduled or unscheduled visit is used, the dose amount should be recalculated and a new preparation of the infusion solution created if the subject's weight on the day of dosing changes by more than 10% from the last weight measurement.

    - Ex: 100 kg at previous visit but current visit is less than 90 kg or greater than 110 kg, then re-calculation of the dose and a new preparation of infusion solution is required.

  - Required dose amount (mg) = dose level (mg/kg) * subject weight (kg)

- Calculate the required number of **pembrolizumab** (MK-3475) vials by dividing the required dose amount (mg) by 100 (mg).

  Example 1 (2 mg/kg dose):
  Dose level = 2 mg/kg, subject weight = 69 kg
  Required dose amount (mg) = 2 mg/kg * 69 kg = 138 mg
  Required number of **pembrolizumab** (MK-3475) vials = 138/100 = 1.38
  Rounded to next whole number = 2 vials

10 mg/kg Dose

- The preparation of infusion solutions should be based on the dose level and the subject's actual body weight in kilograms (kg) on the day of dosing. However if preparation of the infusion is required to be completed prior to the subject's visit (e.g., per local SOP, logistical issues, etc.), then the solution may be prepared in advance in accordance with the instructions in this manual.

- If infusion solution is prepared in advance, weight from the most recent scheduled or unscheduled visit should be used.

- If the weight from the most recent scheduled or unscheduled visit is used, the dose amount should be recalculated and a new preparation of the infusion solution created if the subject's weight on the day of dosing changes by more than 10% from the last weight measurement.

    - Ex: 100 kg at previous visit but current visit is less than 90 kg or greater than 110 kg, then recalculation of the dose and a new preparation of infusion solution is required.

- Required dose amount (mg) = dose level (mg/kg) * subject weight (kg)

- Calculate the required number of **pembrolizumab** MK-3475 vials by dividing the required dose amount (mg) by 100 (mg).

Example 2 (10 mg/kg dose):
Dose level = 10 mg/kg, subject weight = 69 kg
Required dose amount (mg) = 10 mg/kg * 69 kg = 690 mg
Required number of **pembrolizumab** (MK-3475) vials = 690/100 = 6.9
Rounded to next whole number = 7 vials

- Preference is for sites to prepare study medication on day of infusion. For sites that must prepare dose in advance, weight from most recent scheduled or unscheduled visit can be used for advanced preparation. Then on day of dosing, reweigh subject for actual weight.

    - If the weight on day of dosing is within 10% of weight used for advanced dose calculation, you can proceed with dosing what was prepared during advanced preparations

    - If a patient's weight on day of dosing has fluctuated by more than 10% compared to weight used for advanced study prep, then the dose should be recalculated using the new weight measurement.

<u>200 mg Fixed Dose</u>

- **2 vials (100 mg/4 mL)**
- **8 mL total**

4.4 PREPARATION OF INFUSION SOLUTION

- Aseptic technique must be strictly observed throughout the preparation procedure
- Use of a biosafety cabinet is preferred since no anti-microbial preservative is present in the product; however, it is not mandatory unless specified by site standard operating procedure.
- Equilibrate required number of pembrolizumab MK-3475 vials to room temperature
- The preferred method of dose preparation is the volumetric method
- Sponsor recommends reconstitution and administration of pembrolizumab (MK-3475) that follows the parameters in this manual, however if use of gravimetric preparation is mandatory due to local site procedures, the following requirements must be satisfied and documented:
    - Draw the required volume up to 4.0 mL (100 mg) of pembrolizumab from each vial
    - Limit the number of punctures of each vial to one
- For gravimetric preparation method using density of pembrolizumab solution, a value of 1.03 g/mL should be used
- [redacted] does not support methods of preparation of [redacted] agents beyond what is stated in the product literature. Sites should reference the SmPCs or packaging inserts for preparation instructions
- If the site procedures require use of spikes or other closed system transfer devices (CSTDs), please contact sponsor for approval
- Choose a suitable infusion bag size so that the following conditions are met:
    - Concentration of pembrolizumab MK-3475 is between 1 mg/mL and 10 mg/mL
    - The infusion volume to bag capacity ratio should not be less than 0.3. In other words, the bag must be filled to at least 30% of its capacity.
- Choose a suitable infusion bag material. The bag may be empty or it may contain normal saline The following infusion bag materials are compatible with pembrolizumab (MK-3475):

- PVC plasticized with DEHP
- Non-PVC (polyolefin)
- EVA
- PE lined polyolefin

*Contact Sponsor for materials not listed above

- Calculate the volume of pembrolizumab (MK-3475) and normal saline required to prepare the infusion (admixture) bag

  Volume of pembrolizumab (MK-3475) (mL) = required dose amount (mg) / 25 (mg/mL)

  Volume of normal saline = total infusion volume – volume of pembrolizumab (MK-3475) from above

- If a bag pre-filled with normal saline is being used, remove the excess volume of normal saline using a sterile syringe (Polypropylene, latex-free) attached to a suitable needle. Keep in consideration the excess bag fill volume as well as the volume of pembrolizumab (MK-3475) to be added to the bag to prepare the infusion solution. This helps ensure that the concentration in the bag can be accurately calculated and falls within the acceptable range of 1 mg/mL to 10 mg/mL. If the site would like to proceed without removing excess saline they must ensure that the concentration of MK-3475 would still fall within acceptable range.

- If an empty bag is being used, withdraw the necessary volume of normal saline from another appropriate bag and inject into the empty bag. Keep in consideration the volume of pembrolizumab (MK-3475) to be added to the bag to prepare the infusion solution.

- Withdraw the required volume of pembrolizumab (MK-3475) from the vial(s) (up to 4 mL from each vial) using a sterile syringe attached to a suitable needle. The vial(s) may need to be inverted to remove solution.

  Volume of pembrolizumab (MK-3475) (mL) = required dose amount (mg) / 25 (mg/mL)

  Note: If it is necessary to use several vials, it is advisable to withdraw from several vials into a suitable size single use syringe using a new needle for each vial.

- Add the required pembrolizumab (MK-3475) into the infusion IV bag containing normal saline and gently invert the bag 10-15 times to mix the solution.

- Pembrolizumab (MK-3475) solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of admixture solutions in the IV bags and the duration of infusion.

- In addition, IV bags may be stored under refrigeration at 2 °C to 8 °C (36 °F to 46 °F), total cumulative storage time at room temperature and refrigeration should not exceed 24 hours.

- If refrigerated, allow the IV bags to come to room temperature prior to use.

- If the infusion bag is excessively handled or shaken, particulates may form. If this occurs discard the bag and create a new bag taking care not to shake. Please contact your HQ clinical study team if particulates are noticed for further instructions. Be prepared to provide the following information:
    - IV bag manufacture, lot and expiry
    - Target volume of admixture solution in the IV bag (e.g. 100 mL, 200 mL etc.)
    - Amount of drug product (mL or mg) added to the bag
    - Drug product lot
    - Brief description of the nature of visible particles (color, shape, size, numbers etc.).

- **DO NOT FREEZE THE PEMBROLIZUMAB (MK-3475) INFUSION SOLUTION.**

- Discard any unused portion left in the vial as the product contains no preservative

4.5 ADMINISTRATION

- Pembrolizumab (MK-3475) infusions should be administered in 30 minutes, with a window of -5 and +10 minutes, using an infusion pump. A central catheter is not required for infusion; however if a subject has a central venous catheter in place, it is recommended that it be used for the infusion.

- The following infusion set materials are compatible with (pembrolizumab) MK-3475:

    - PVC Infusion set that is plasticized using DEHP

- PVC and tri-(2-ethylhexyl) trimellitate (TOTM) infusion set
- Polyethylene lined PVC infusion set
- PVC Infusion set that is plasticized using Di-2-ethylhexyl Terephthalate (DEHT)
- Polyurethane set

*Contact Sponsor for materials not listed above

- A sterile, non-pyrogenic, low-protein binding 0.2 to 5 µm in-linefilter made of polyethersulfone (PES) must be used during administration to remove any adventitious particles. If the infusion set does not contain 0.2 to 5 µm in-line filter, it is recommended to use 0.2 to 5 µm add-on filter which may contain an extension line (Note: the materials of the extension line and filter should be as mentioned above).

- Attach the infusion line to the pump and prime the line, either with normal saline (at least 25 mL) or with infusion solution as per local SOP, before starting the infusion.

- Infuse pembrolizumab (MK-3475) over approximately 30 minutes, with a window of -5 and +10 minutes, through a peripheral line or indwelling catheter.

- Ensure the entire contents of the bag are dosed and all remaining drug solution in the line is administered through saline flushing.

- Document volume administered according to data entry guidelines.

- *In case of infusion reactions, infusion rate may differ; refer to protocol for specific instructions.*

- Whenever possible, the lowest infusion rate should be used that will allow completion of the infusion within the 30 minutes.

- Maximum rate of infusion should not exceed 6.7 mL/min. through a peripheral line or indwelling catheter.

- **DO NOT CO-ADMINISTER OTHER DRUGS THROUGH THE SAME INFUSION LINE.**

- **UNUSED INFUSION SOLUTION FOR INJECTION SHOULD NOT BE USED FOR ANOTHER INFUSION OF THE SAME SUBJECT OR DIFFERENT SUBJECT.**

- **Caution: Do not shake the vials/bags otherwise this may result in formation of foam. If foam is noticed in either vial or bag, the drug product will need to be discarded. A new preparation should be made, taking care not to shake or agitate the product.**

4.6 RETURN AND DISCARDING OF PEMBROLIZUMAB (MK-3475) VIALS

- Unused pembrolizumab (MK-3475) Solution for Infusion vial(s) shall be returned to the designated facility for destruction after CRA has performed full accountability of un-used vials

    - For US clinical sites, return to the central depot that shipped supplies to the site:
        - [redacted]
        - [redacted]

    - For ex-US clinical sites, consult with local [redacted] subsidiary for facility address.

    - For clinical sites that are not able to return to designated facility for destruction, please work with your CRA and refer to SOP 104_ER5_Return and Destruction of Clinical Supplies Procedure for guidance to follow for destruction of unused vials at the local site.

- For Interactive response technology (IRT) studies, the IRT Clinical Drug Supply Return Forms generated in the system must be used

- Solution remaining in a used vial should be discarded according to your local procedures.

- Any information on the label identifying the subject should be redacted prior to returning the study medication.

DANA-FARBER/PARTNERS CANCERCARE

MASSACHUSETTS GENERAL HOSPITAL | DANA-FARBER CANCER INSTITUTE | BRIGHAM AND WOMEN'S HOSPITAL

[Date]

Dear Research Participant:

You are receiving this letter because you participated in the research study called, "A phase Icancer Study of Pembrolizumab in Combination with Trastuzumab-DM1 in Metastatic HER2-Positive Breast Cancer," and we would like to share some recent changes with you.

WHAT HAS HAPPENED?

The overall study plan has not changed, however the consent form for this study has recently been updated to reflect changes in the regulations regarding the way that biological specimens (blood, tissue, and stool (which were collected for research purposes)) and data may be handled. These changes are as follows:

1. **Biobanking—biological specimens previously collected from you may be shared with an outside lab or collaborator and will be banked for future research.**

   Biological specimens (such as blood, tissue, bodily fluids, other specimens collected as a part of the study, and any materials that may be derived from these) will be collected and shared with an outside lab or collaborator for analysis. The specimens will not be identifiable. The specimens will be banked for future use.

   As a part of this study, you previously agreed to provide researchers with a sample of your tumor tissue from a prior biopsy, surgery, or procedure. There is a risk associated with submitting this type of tissue for research studies and biobanking. Generally, hospitals will keep some of your tissue from a prior biopsy, surgery, or procedure. This tissue may be used to help treat your cancer in the future. There is a small risk that when the tissue sample is submitted to the study or the biobank that your tissue could be used up.

2. **Data Collection—Your data may be shared with an outside collaborator or stored for future use.**

   Data will be collected and shared with an outside collaborator for analysis. The data will not be identifiable. The data will be banked for future use.

3. **Future use of data and specimens—Your de-identifiable data and/or biospecimens which were previously collected during this study may be stored and used for future research.**

Your personal information and/or biospecimens collected during this study may be stored and used for future research. If so, any personal identifiers will be removed so that the information or samples cannot be linked back to you. As a result, we will no longer be able to identify and destroy them.

Investigators, including investigators from collaborating institutions, can request this data and samples for new research. Samples and data may also be shared with outside non-profit academic investigators as well as with for-profit pharmaceutical investigators or commercial entities, with whom we collaborate.

You will not be asked to provide additional informed consent for the use of your de-identified information or samples in future research.

Future research studies may include genetic research. Your genes are unique to you. At this time, you cannot be identified through this research. There is a risk that you might be reidentified in the future as genetic research progresses.

NEXT STEPS?

After reviewing this information, we ask that you sign below acknowledging receipt of this information and your continued consent to participation in this study.

Documentation of Re-Consent – 16-492 "A Phase 1b Study of Pembrolizumab in Combination with Trastuzumab-DM1 in Metastatic HER2-Positive Breast Cancer"

My signature below indicates:

- I have had enough time to read this letter and think about its contents
- I have had all of my questions answered to my satisfaction
- I am willing to participate in this study
- I have been informed that my participation is voluntary, and I can withdraw at any time

_____ _____

Signature of Participant
Or Legally Authorized Representative

Date

_____

Relationship of Legally Authorized Representative to Participant

Please return this letter to us at your earliest convenience using one of the following methods and contact information below: scan and email, fax, or mail (using the enclosed mailer).

[If DFCI participant, add DFCI CRC contact information. If BIDMC participant, add BIDMC CRC contact information]

If we do not receive your signed acknowledgement within approximately 30 days, a member of the study team will contact you by phone or email or will meet with you at your next clinic visit.

In closing, it is important that you understand that your participation in this research study is purely voluntary. It is up to you to decide whether your biospecimens or data may be shared or banked for future research. You have the right to choose not to continue your participation in this study, and to not sign this letter.

We hope that by sharing your biospecimens and data with internal and external collaborators, we will increase the number of research opportunities available to us to learn more about breast cancer. We hope to use the lessons learned from this trial to develop other research studies and novel approaches to better the lives of people living with this disease. We greatly appreciate your participation in this important study and thank you for your continued support.

For any questions about the content of this letter you may contact a member of the study team using the contact information provided above.

Sincerely,

<Insert Consenting/Treating Physician Signature>