TITLE: PHASE II STUDY OF DOCETAXEL CHEMOTHERAPY WITH PEMBROLIZUMAB AND **INTE**RLEUKIN-12 **G**ENE THERAPY IN PATIENTS WITH ANTH**RA**CYCLINE- REFRACTORY TRIP**L**E NEGATIVE BREAST CANCER (INTEGRAL study)

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1.0 TRIAL SUMMARY

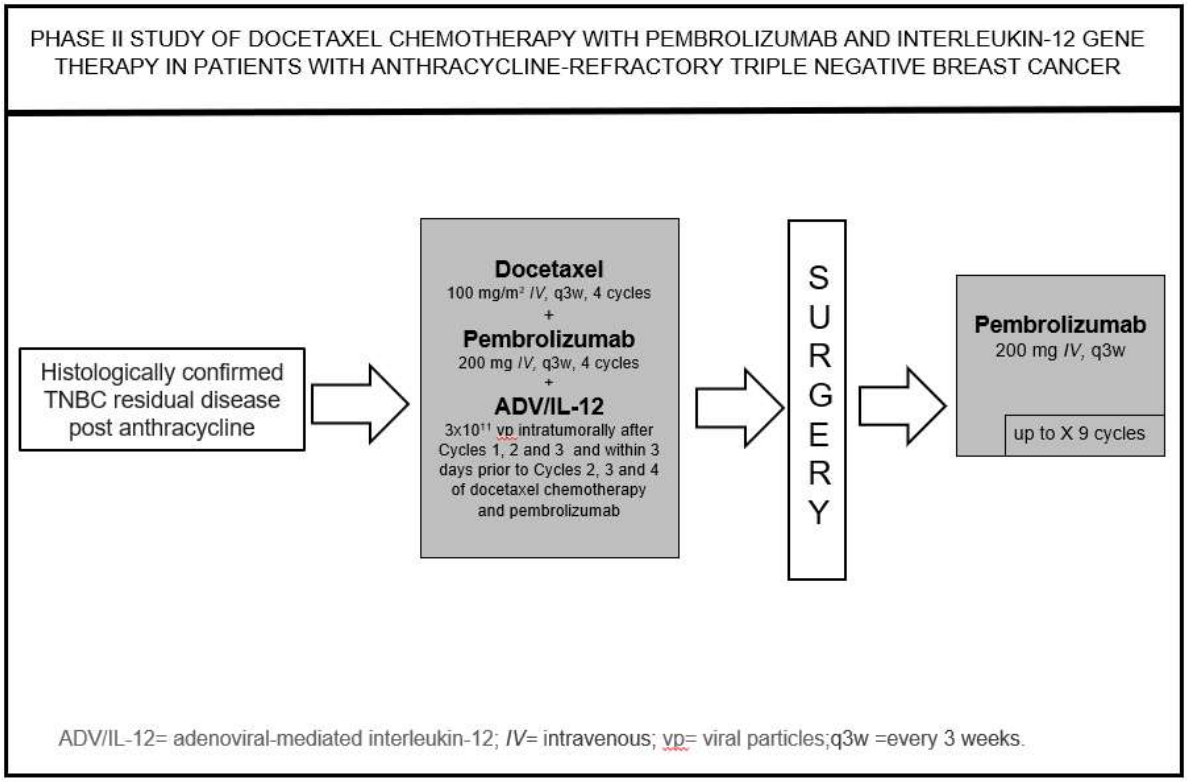
Abbreviated Title	Docetaxel Chemotherapy with Pembrolizumab and Interleukin-12 Gene Therapy in Anthracycline-Refractory Triple Negative Breast Cancer
Trial Phase	II
Clinical Indication	Triple negative breast cancer
Trial Type	Single-arm, open-label
Type of Control	None
Route of Administration	Docetaxel, intravenous infusion; adenovirus-mediated interleukin-12 gene therapy, intratumoral injection; pembrolizumab, intravenous infusion
Trial Blinding	None
Treatment Groups	1
Number of Trial Participants	30
Estimated Enrollment Period	18 months
Estimated Duration of Trial	24 months
Duration of Participation	24 months
Estimated Average Length of Treatment Per Patient	4.5 months

2.0 TRIAL DESIGN

2.1 Trial Design

This is a Phase II trial evaluating the efficacy and safety of docetaxel chemotherapy and pembrolizumab plus adenoviral-mediated interleukin-12 (ADV/IL-12) gene therapy in patients with anthracycline-refractory, triple negative breast cancer (TNBC). Approximately 30 patients will be enrolled. The primary endpoint is pathological complete response (pCR) rate. Exploratory analysis will include tissue and blood-based correlates of response including circulating suppressor and effector immunocyte profiles; intracellular cytokines, enzymes and transcription factors; systemic levels of cytokines and other soluble immune mediators; and tumor immune microenvironment (TIME) composition. All patients will receive 4 cycles of docetaxel chemotherapy and pembrolizumab in combination with ADV/IL-12. Docetaxel chemotherapy (100 mg/m^2 IV), and pembrolizumab (200 mg IV) will be administered every 3 weeks (Q3W) for 4 cycles. ADV/IL-12 at 5×10^{11} virus particles (vp) will be administered intratumorally after Cycle 1 and within 3 days prior to Cycles 2, 3 and 4 of docetaxel chemotherapy and pembrolizumab. All patients will undergo surgery after neoadjuvant treatment completion. After definitive surgery, patients will receive adjuvant pembrolizumab every 3 weeks for up to nine cycles. Further adjuvant therapy will be according to physicians' choice. Patients will be monitored for at least 90 days after treatment discontinuation of ADV/IL-12 for late immune-related toxicities.

2.2 Trial Diagram



3.0 OBJECTIVES

3.1 Primary Objective

(1) To determine the pCR rate of docetaxel chemotherapy and pembrolizumab plus ADV/IL-12 gene therapy in patients with anthracycline-refractory TNBC. pCR is defined as the absence of residual invasive and in situ cancer on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant therapy (i.e., ypT0 ypN0 in the current American Joint Committee on Cancer staging system).

3.2 Secondary Objectives

(1) To determine the safety/toxicity of docetaxel chemotherapy and pembrolizumab plus ADV/IL-12 gene therapy in patients with anthracycline-refractory TNBC, as assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v5.0 (Appendix 1).

3.3 Exploratory Objective

(1) To evaluate the tissue and blood-based correlates of response to docetaxel chemotherapy and pembrolizumab plus ADV/IL-12 gene therapy, including but not limited to expression of

programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1); effector and suppressor immunocyte populations; cytokine profiles; and TIME composition.

4.0 BACKGROUND & RATIONALE

4.1 Background

4.1.1 Programmed Cell Death-1 Targeting

Immune checkpoints are negative regulators of the immune system that are crucial for maintaining self-tolerance, preventing autoimmunity, and protecting tissues from immune collateral damage. Tumors are able to hijack immune checkpoint pathways, thus restraining the ability of the immune system to mount an effective antitumor response. Blockade of immune checkpoints such as cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and PD-1 has proved to be a promising approach for inducing antitumor immunity.

The importance of intact immune surveillance function in controlling the outgrowth of neoplastic transformations has been known for decades.[1] 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 (Tregs) correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, and pancreatic cancers; hepatocellular carcinoma; malignant melanoma; and renal cell carcinoma. TILs can be expanded *ex vivo* and reinfused, inducing durable objective tumor responses in cancers such as melanoma.[2,3]

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 downmodulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an immunoglobulin (Ig) superfamily member related to cluster of differentiation 28 and CTLA-4 that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and programmed cell death-ligand 2 [PD-L2]).[4,5]

The structure of murine PD-1 has been resolved.[6] PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type domain responsible for ligand binding and a cytoplasmic tail 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 and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta, protein kinase C-theta, and zeta-chain-associated protein kinase, which are involved in the CD3 T-cell signaling cascade.[5,7-9] The mechanism by which PD-1 downmodulates T-cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins.[10,11] As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention.

4.1.2 Triple Negative Breast Cancer and Neoadjuvant Therapy

TNBC, which is characterized by the absence of the estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor 2 (HER2) expression, accounts for approximately 15–20% of all breast cancers. TNBC is more aggressive than other breast cancer subtypes, carrying a higher risk of distant recurrence and death in the first 5 years of diagnosis.[12,13] TNBC is a difficult-to-treat disease with poor prognosis and no current targeted therapy options.

Neoadjuvant therapy is standard of care for patients with locally advanced, inflammatory, or inoperable primary breast cancer and is used to downstage tumors, thereby increasing the likelihood of breast-conserving surgery. Anthracycline and taxane-based chemotherapy are routinely used in the neoadjuvant treatment of TNBC, resulting in pCR rates of approximately 30–40%. Current anthracycline and taxane-based neoadjuvant regimens for TNBC include doxorubicin and cyclophosphamide (AC) every two weeks (Q2W; dose dense) or Q3W for 4 cycles; AC followed by paclitaxel Q2W or Q3W or docetaxel Q3W for 4 cycles; and AC followed by weekly paclitaxel for 12 weeks.

pCR rate after neoadjuvant chemotherapy is known to be strongly correlated with survival and prognosis in patients with TNBC.[14-17] In an analysis of a prospectively collected clinical database (n = 255), 3-year overall survival (OS) was found to be significantly higher in TNBC patients who achieved pCR following anthracycline-based neoadjuvant therapy than in those with residual disease (94% vs. 68%).[14] A meta-analysis demonstrated significantly higher 5-year disease-free survival (DFS) and OS rates in TNBC patients who achieved pCR than among those who failed to achieve pCR after anthracycline and taxane-based neoadjuvant therapy (DFS: odds ratio [OR] = 7.42, 95% confidence interval [CI]: 4.09–13.48; OS: OR = 6.74, 95% CI: 3.63–12.52).[15] In a retrospective analysis (n = 94), 2-year locoregional failure rates were 0% and 72.5% (95% CI: 57.4–91.5) and 2-year DFS rates were 82.7% (95% CI: 68.6–99.7) and 44% (95% CI: 30.5–63.4) in patients who did and did not achieve pCR, respectively.[16]

As achieving pCR is a strong predictor of long-term survival in TNBC, there is strong interest in combining existing regimens with other agents to improve pCR rate and outcomes. TNBC has characteristics that may make it potentially amenable to immunotherapy. Several studies have demonstrated that TILs are increased in TNBC and correlated with improved OS and decreased distant recurrence.[18-20] PD-L1 expression has also been shown to be higher in TNBC than in non-TNBC.[21,22] A study mining The Cancer Genome Atlas RNA sequencing data showed significantly greater expression of the *PD-L1* gene in TNBC (n = 120) compared with non-TNBC (n = 716; $P < 0.001$).[23] Microarray analysis revealed PD-L1 expression in 20% of TNBC specimens. Furthermore, PD-L1−positive tumors had greater CD8+ T-cell infiltrate than PD-L1−negative tumors. TILs have been shown to be strongly correlated with pCR to neoadjuvant chemotherapy in TNBC.[24-27] Furthermore, other studies have consistently demonstrated that TNBC patients with high TIL levels have a higher rate of pCR.[28-30] The presence of TILs, particularly high $CD8^+$ TIL levels, in residual tumor has also been shown to be associated with better metastases-free survival and OS in TNBC patients with non-pCR following neoadjuvant chemotherapy.[31-33] Therefore, combinatorial immunotherapy approaches may have value in the neoadjuvant treatment of TNBC.

4.1.3 Pembrolizumab and Triple Negative Breast Cancer

Pembrolizumab (MK-3475; trade name Keytruda) is a potent humanized IgG4 monoclonal antibody with high specificity of binding to PD-1, thus inhibiting its interaction with PD-L1 and PD-L2. Based on preclinical *in vitro* data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an IV immunotherapy for advanced malignancies. Pembrolizumab is indicated for the treatment of patients across a number of indications because of its mechanism of action to bind the PD-1 receptor on the T-cell. Pembrolizumab is approved for the treatment of unresectable or metastatic melanoma, metastatic non-small cell lung cancer (NSCLC), classical Hodgkin lymphoma, primary mediastinal large B-cell lymphoma, recurrent locally advanced or metastatic gastric or gastroesophageal junction adenocarcinoma, recurrent or metastatic head and neck squamous cell carcinoma, locally advanced or metastatic urothelial bladder cancer, microsatellite-high instability cancer, and recurrent or metastatic cervical cancer.

Pembrolizumab monotherapy has demonstrated promising clinical activity in advanced/metastatic TNBC. In the Phase Ib KEYNOTE-012 trial (NCT01848834), pembrolizumab was given at 10 mg/kg Q2W to patients with heavily pretreated, advanced, PD-L1−positive TNBC.[34] Among the 27 patients who were evaluable for antitumor activity, the overall response rate (ORR) was 18.5% (95% CI: 6.3−38.1). Best overall response was complete response (CR) in 1 (3.7%) patient, PR in 4 (14.8%) patients, stable disease (SD) in 7 (25.9%) patients, and progressive disease (PD) in 13 (48.1%) patients. The disease control rate (i.e., percentage of patients with best response of CR, PR, or SD for ≥24 weeks) was 25.9% (95% CI: 11.1−46.3). Common toxicities were mild and included arthralgia, fatigue, myalgia, and nausea. Grade 3 or greater adverse events (AEs) and treatment-related death occurred in 5 (15.6%) patients and 1 patient, respectively.

The multicohort Phase II KEYNOTE-086 study (NCT02447003) evaluated the efficacy and safety of pembrolizumab monotherapy in metastatic TNBC.[35,36] Cohort A (n = 170) included patients with previously treated metastatic TNBC regardless of PD-L1 expression, and cohort B included patients with previously untreated PD-L1−positive metastatic TNBC (tumor PD-L1 combined positive score ≥1%). Pembrolizumab was administered at 200 mg IV Q3W until disease progression, intolerable toxicity, or a maximum of 2 years. The ORR in cohort A was 4.7% (95% CI: 2.3−9.2).[35] Best overall response was CR in 1 (0.6%) patient, PR in 7 (4.1%) patients, SD in 35 (20.6%) patients, and PD in 103 (60.6%) patients. The median duration of response was 6.3 months (range: 1.2+ to 10.3+). PD-L1 status was not associated with response, as the ORR was 4.8% in PD-L1–positive patients and 4.7% in PD-L1–negative patients. The median time to response was 3.0 months (range: 1.9−8.1). The disease control rate (CR + PR + SD ≥24 weeks) was 7.6%. The median progression-free survival (PFS) was 2 months (95% CI: 1.9−2.0) and the median OS was 8.9 months (95% CI: 7.2−11.2). The 6-month PFS and OS rates were 12% and 69%, respectively. All-grade treatment-related AEs occurred in 60% of patients, with 12.4% of patients experiencing Grade 3/4 treatment-related AEs. The most common all-grade AEs were fatigue (20.6%) and nausea (10.6%). All-grade immune-related AEs (irAEs) occurred in 18.8% of patients, only 1.2% of which were Grade 3/4, and none of which led to death. The most common all-grade irAEs were hypothyroidism (11.2%), hyperthyroidism (4.7%), and pneumonitis (3.5%). There were no deaths related to AEs.

Preliminary results from the first 52 patients enrolled in cohort B of KEYNOTE-086 showed an ORR of 23.1% (95% CI: 14−36).[36] Best overall response was CR in 4% of patients, PR in 19% of patients, SD in 17% of patients, and PD in 58% of patients. Median time to response was 8.7 weeks (range: 8.1−17.1). Median PFS was 2.1 months (95% CI: 2.0−3.9); estimated 6-month PFS rate was 29%.

Results of the ongoing adaptively randomized, multicenter, Phase II I-SPY 2 study (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging And molecular Analysis 2; NCT01042379) demonstrated the efficacy of pembrolizumab in the neoadjuvant setting in patients with newly diagnosed, locally advanced breast cancer.[37] Patients were randomized to receive pembrolizumab (200 mg Q3W for 12 weeks) in combination with paclitaxel followed by AC (n = 69) or standard chemotherapy alone (n = 180). The estimated pCR rates for the pembrolizumab with chemotherapy and chemotherapy alone arms were 60% and 20% in TNBC patients, respectively. The safety profile of pembrolizumab was consistent with that observed in previously reported studies across tumors. In the pembrolizumab arm, Grade 3 to 5 treatment-related AEs included diarrhea (n = 5), febrile neutropenia (n = 5), fatigue (n = 4), anemia (n = 3), nausea (n = 3), neutropenia without fever (n = 1), peripheral motor neuropathy (n = 1), peripheral sensory neuropathy (n = 1), and vomiting (n = 1). Grade 3 to 5 irAEs included adrenal insufficiency (n = 5), hepatitis (n = 2), colitis (n = 1), and hypothyroidism (n = 1). Five of 6 patients presented with adrenal insufficiency after completion of AC (21–24 weeks after starting pembrolizumab), and one presented during pembrolizumab treatment (5 weeks after starting pembrolizumab).

Several ongoing studies are evaluating the safety and efficacy of the pembrolizumab and chemotherapy combination in the neoadjuvant setting and pembrolizumab monotherapy in the adjuvant setting (KEYNOTE-173 [], A Phase 1b Study to Evaluate the Safety and Clinical Activity of Pembrolizumab (MK-3475) in Combination With Chemotherapy as Neoadjuvant Treatment for Triple Negative Breast Cancer; KEYNOTE-522 [NCT03036488], A Phase III, Randomized, Double-blind Study to Evaluate Pembrolizumab Plus Chemotherapy vs Placebo Plus Chemotherapy as Neoadjuvant Therapy and Pembrolizumab Versus Placebo as Adjuvant Therapy for Triple Negative Breast Cancer; SWOG S1418/BR006 trial [], A Randomized, Phase III Trial to Evaluate the Efficacy and Safety of MK-3475 [Pembrolizumab] as Adjuvant Therapy for Triple Receptor-Negative Breast Cancer With ≥1 CM Residual Invasive Cancer or Positive Lymph Nodes [ypN+] After Neoadjuvant Chemotherapy).

4.1.3.1 Pembrolizumab Preclinical and Clinical Experience

The preclinical and clinical experience are fully described in the current version of the pembrolizumab investigator's brochure.

4.1.3.1.1 Summary of Clinical Efficacy Data

KEYNOTE-001 is an ongoing Phase I, first-in-human study of pembrolizumab monotherapy in patients with progressive locally advanced or metastatic carcinomas, especially melanoma or NSCLC. The ORR demonstrated the antitumor activity of pembrolizumab in patients with melanoma (ipilimumab-naïve and previously treated with ipilimumab).

KEYNOTE-002 is an ongoing randomized, Phase II pivotal study to evaluate the efficacy of 2 pembrolizumab dose regimens versus standard of care chemotherapy in patients with ipilimumab-refractory melanoma. The study demonstrated superior PFS for both pembrolizumab treatment arms compared with the chemotherapy control arm. Treatment with pembrolizumab lead to an ORR that was >4 fold higher than that of the chemotherapy control arm. This difference was highly statistically significant.

The ORRs for pembrolizumab treatment in KEYNOTE-001 and KEYNOTE-002 compared favorably to historical response rates for available treatments for melanoma, particularly in patients who have progressed after multiple prior therapies.

4.1.3.1.2 Summary of Clinical Adverse Event Data

In the pembrolizumab monotherapy trials (KEYNOTE-001, KEYNOTE-002, KEYNOTE-012, KEYNOTE-013, KEYNOTE-028, and KEYNOTE-011 [monotherapy arm]), the overall incidence of AEs ranged from 83.0% (73 of 88 patients in KEYNOTE-012) to 100% (10 of 10 patients in KEYNOTE-011). 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 patients in KEYNOTE-013) to 80.0% (8 of 10 patients in KEYNOTE-011). 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 patients in KEYNOTE-013) to 12.0% (187 of 1562 patients in KEYNOTE-001/002). The most commonly reported Grade 3–5 DRAEs were anemia, alanine transaminase (ALT) increased, and aspartate transaminase (AST) increased. Most patients who experienced an AE continued in the study, with the incidence of AEs leading to discontinuation ranging from 1.9% (8 of 430 patients in KEYNOTE-028) to 12.3% (192 of 1562 patients in KEYNOTE-001/002). The majority of AEs leading to discontinuation were not considered drug related. Discontinuations due to a DRAE were infrequent and ranged from 0% (no patients in KEYNOTE-011) to 4.5% (4 of 88 patients in KEYNOTE-013). The most commonly reported DRAEs leading to discontinuation were pneumonitis, ALT increased, and AST increased.

The overall pattern of AEs observed in melanoma patients enrolled in KEYNOTE-002 demonstrates the favorable safety profile of pembrolizumab compared with chemotherapy. Consistent with prior observations from randomized comparisons of the 2 mg/kg and 10 mg/kg dose levels when given Q3W, there are no important differences in the safety profile of pembrolizumab at these 2 dose levels, and both doses appear to have a favorable safety profile compared with chemotherapy.

In the combination therapy trials KEYNOTE-021 and KEYNOTE-023, the overall incidence of AEs was 95.4% (62 of 65 patients) and 80% (8 of 10 patients), respectively. In KEYNOTE-021, the most commonly reported AEs across the dose regimens were fatigue (49.2%), constipation and nausea (26.2% each), decreased appetite (23.1%), diarrhea (18.5%), and anemia and alopecia (15.4% each). In KEYNOTE-023, the most commonly reported AEs across the dose regimens were neutropenia and thrombocytopenia (50.0% each), followed by anemia, respiratory tract infection, and back pain (30.0% each).

The incidence of Grade 3–5 DRAEs was 23.1% (15 of 65 patients) in KEYNOTE-021 and 50.0% (5 of 10 patients) in KEYNOTE-023. In KEYNOTE-021, the most common Grade 3–5 DRAEs across dose regimens were AST increased (6.2%) and anemia and ALT increased (4.6% each). In KEYNOTE-023, the only Grade 3–5 DRAEs that occurred in more than 1 patient across dose regimens were neutropenia (40.0%) and anemia (20.0%).

In KEYNOTE-021, most patients continued treatment despite AEs, and only 4.6% of patients discontinued study treatment due to an AE. Only 3.1% of patients discontinued study treatment due to an AE that was considered related to the study treatment by investigators. AEs resulting in discontinuation were reported in 3.1% of patients (2 of 65 patients). Interstitial lung disease, dermatitis allergic, and drug eruption were the only AEs resulting in discontinuation and were reported in 1 patient each (1.5%). In KEYNOTE-023, no patients discontinued due to an AE.

In general, the incidence of drug-related serious adverse events (DRSAEs) was low. Many of the events occurred in 1 patient each and/or <1.0% each. In the pembrolizumab monotherapy trials, the most commonly reported DRSAEs (those that occurred in 3 or more patients overall in at least 1 study) were pneumonitis (range: 0.7–1.3%); colitis (range: 0.3–0.9%); pyrexia (range: 0.3–0.5%); diarrhea (range: 0.2–0.4%); hepatitis (0.7%); nausea, adrenal insufficiency, hyponatremia, hyperthyroidism, hypophysitis, vomiting, and dyspnea (0.3% each); and dehydration, generalized edema, hypothyroidism, renal failure acute, and pericardial effusion (0.2% each). The remaining DRSAEs occurred in 1 or 2 patients each per study.

In the combination therapy trials, all DRSAEs were reported in 1 patient each. In KEYNOTE-021, 8 patients experienced DRSAEs; the DRSAEs were as follows: anemia, febrile neutropenia, atrial fibrillation, colitis, pyrexia, hypersensitivity, ALT increased, AST increased, drug eruption, rash, and urticaria. In KEYNOTE-023, 2 patients experienced DRSAEs; 1 patient had an event of pneumonia and the other had an event of tumor lysis syndrome. Due to the fact that KEYNOTE-021 and KEYNOTE-023 were combination studies and also were of small sample size, comparative evaluation of the AE profile of pembrolizumab combination therapy from those protocols to pembrolizumab monotherapy or to chemotherapy monotherapy in other studies cannot be made.

Among KEYNOTE-001 and KEYNOTE-002 studies, the incidence of AEs of special interest (AEOSIs) was 16.1%. Overall, the most commonly reported AEOSIs included hypothyroidism (7.2%), pneumonitis (2.9%), infusion reaction (2.5%), and hyperthyroidism (2.2%). The incidences of the remaining AEOSIs were low (range: 0.1–1.3%). The overall incidence of drug-related AEOSIs was 14.3%. Overall, 2.6% of patients discontinued treatment due to an AEOSI, and the most commonly reported drug-related AEOSI leading to discontinuation was pneumonitis (1.3%). Only one patient died of an AEOSI (pneumonitis, 0.1%). Corticosteroids were not used to manage myositis, pericarditis, thyroiditis, type 1 diabetes mellitus, uveitis, or vasculitis.

In the KEYNOTE-522 study, the incidence of treatment-related adverse events of grade 3 or higher was 78.0% in the pembrolizumab-chemotherapy group and 73.0% in the placebo-chemotherapy group, including death in 0.4% (3 patients) and 0.3% (1 patient), respectively). Of note, at the first interim analysis, among the first 602 patients who underwent

randomization, the percentage of patients with a pathological complete response was 64.8% versus 51.2% (estimated treatment difference, 13.6 percentage points; P<0.001) in the pembrolizumab compared to placebo group, respectively.[38]

4.1.4 Tumor Immune Microenvironment and Immune Checkpoint Inhibitor Response

Immune checkpoint inhibitors such as pembrolizumab have shown great promise for cancer treatment. However, a majority of patients do not respond to this type of treatment. The TIME has emerged as a critical determinant of immune checkpoint inhibitor response, with responses correlated with an immunologically active, T-cell-inflamed microenvironment.[39] Accumulation of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and Tregs and insufficient accumulation of effector T-cells and other TILs in the TIME have been implicated in the resistance to immune checkpoint therapy. Under healthy conditions, MDSCs and Tregs play critical roles in immune homeostasis. However, tumor-induced recruitment and activation of MDSCs and Tregs is an important mechanism of tumor-mediated immunosuppression, leading to impaired efficacy of cancer immunotherapy. *In vivo* studies have shown that elevated MDSCs cause resistance to anti-PD-1 immune checkpoint therapy, whereas MDSC suppression eradicates metastatic mouse cancers resistant to anti-PD-1 immune checkpoint therapy.[40] Comparison of *in vivo* anti-PD-1−sensitive and anti-PD-1−resistant tumors has suggested that intratumoral Tregs might be responsible for limiting anti-PD-1 therapeutic efficacy.[41] MDSCs and Tregs in the TIME represent a significant obstacle to immune checkpoint blockade therapies via suppression of immune checkpoint blockade-mediated antitumor T-cell responses. Immune checkpoint blockade has been shown to be most effective in so-called “inflamed” tumors populated with tumor-specific $CD8^+$ TILs.[42] Agents capable of increasing the number and activity of T-cells or TILs in the tumor microenvironment may enhance the response to anti-PD-1 therapy.

4.1.5 ADV/IL-12 and the Tumor Immune Microenvironment

IL-12 is an immunostimulatory cytokine produced by innate immune cells such as dendritic cells, macrophages, and neutrophils and has been shown to stimulate cytotoxic T-cell and natural killer (NK) cell proliferation and activation, induce cytokine production (especially interferon-γ), inhibit T-helper 2-type responses, and inhibit MDSCs.[59-64] Despite its promise as an anticancer therapeutic agent, severe toxicity associated with the systemic administration of recombinant IL-12 limited its clinical application. In an effort to overcome the systemic toxicity of IL-12, local delivery approaches have been developed, including ADV/IL-12 gene therapy. We and others have shown intratumoral delivery of ADV/IL-12 gene therapy to be effective in inducing antitumor immune responses, tumor regression, long-term survival, and long-lasting immunity in various preclinical models including prostate cancer, laryngeal squamous cell carcinoma, breast cancer, glioma, and colon cancer.[65-72] We demonstrated that a single intratumoral injection of an adenovirus expressing murine IL-12 (AdmIL-12) reduced tumor weight by more than 50% and increased mean survival time (23.4 vs. 28.9 days) compared with virus vector (adenoviral vector carrying β-galactosidase [AdLacZ]) and saline controls in an orthotopic mouse prostate cancer model.[67] Injection of AdmIL-12 into the primary tumor also suppressed the growth of established lung metastases. Tumor growth suppression was associated with significant elevations in cytolytic NK cell activity and intratumoral infiltration of CD4+ and CD8+ T-cells. In a subsequent study, we found that AdmIL-12-transduced macrophages injected into orthotopic prostate tumors *in vivo*

significantly suppressed primary tumor growth and spontaneous lung metastases, prolonged survival, and increased intratumoral CD4+ and CD8+ T-cell infiltration and cytotoxic NK cell activity compared with control AdLacZ-transduced macrophages.[68] Comparable antitumor and antimetastatic effects were obtained with direct orthotopic injection of AdmIL-12. In a murine glioma model, AdmIL-12 reduced mean tumor volume and prolonged survival compared with virus vector (AdLacZ) and normal saline controls.[70] Sixty-day survival rates were 50%, 10%, and 10% for the AdmIL-12, AdLacZ, and saline-treated groups, respectively. The antitumor effects of AdmIL-12 were mediated by an increased intracranial infiltration of CD4+ and CD8+ T-cells. Furthermore, tumor rechallenge experiments demonstrated the long-lasting tumor-specific immunity conferred by AdmIL-12. Intratumoral AdmIL-12 induced complete tumor regression in 26 of 34 (76%) mice in an established colon cancer model.[72] This antitumor effect was mediated primarily by CD8+ T-cells, as demonstrated by *in vivo* T-cell depletion studies using anti-CD4 and anti-CD8 antibodies. CD8+-depleted mice had a similar tumor growth rate to that of untreated controls, whereas complete tumor regression was observed in 66% of CD4+ T-cell−depleted mice.

4.1.5.1 ADV/IL-12 Preclinical and Clinical Experience

Intratumoral AdmIL-12 administration at doses found to be therapeutically effective in an orthotopic murine model of established hepatic metastases (2×10^8 and 4×10^8 plaque-forming units [pfu] per mouse) was not associated with organ toxicity, significant systemic vector dissemination, elevations in serum proinflammatory cytokines, sustained elevations in serum transaminases, changes in serum total bilirubin and creatinine, or changes in white blood cell (WBC) and platelet counts.[73] AdmIL-12 vector DNA was not detected in the lungs, heart, kidney, brain, or ovary and a variable amount was detected in the spleen. Control vector (AdDL-312) DNA was not detectable in any of the organs tested. Grade 0 toxicities were seen in the liver, heart, kidney, ovary, and brain of AdmIL-12−treated mice. Dose-dependent elevations in serum ALT and AST were observed in the AdmIL-12−treated groups. Differences in ALT between the AdmIL-12−treated groups and the control group at all tested time points were not significant. In the AdmIL-12 group treated at 4×10^8 pfu, serum AST was significantly higher on Day 8 compared with the control group. Median serum AST decreased by Day 17 and did not significantly differ from the Day 17 median serum AST of the control group. No elevations in serum total bilirubin or creatinine occurred in either of the AdmIL-12−treated groups. AdmIL-12 treatment showed a nonsignificant dose-dependent trend toward decreased WBC and platelet counts relative to the control group.

Intratumoral ADV/IL-12 therapy has been shown to be a feasible and safe procedure in the clinical setting.[74,75] In a Phase I trial, patients with advanced digestive tumors (pancreatic, colorectal, or primary liver tumors; n = 21) received intratumoral ADV/IL-12 at doses ranging from 2.5×10^{10} to 3×10^{12} vp.[74] Patients with SD and no serious adverse reactions were allowed to receive up to 3 monthly doses of ADV/IL-12. PR and SD were observed in 1 patient and 10 patients, respectively. In 4 of 10 assessable patients, a significant increase in intratumoral infiltration of CD4+ and CD8+ T-cells was observed, with increases in CD8+ T-cells ranging from 193% to 336%.

Overall, ADV/IL-12 administration was well tolerated, and dose-limiting toxicity was not observed. A total of 319 AEs were recorded; the majority of which were related to disease

progression. Mild to moderate fever responsive to common antipyretics was observed 24 to 48 hours after ADV/IL-12 injection in nearly 60% of patients, regardless of ADV/IL-12 dose, and was occasionally associated with profuse sweating and malaise. Four patients (19%) experienced pain at the injection site lasting 1 to 3 days after treatment, and a painless, transient erythema around the injection site occurred in 1 patient. On the day of treatment, 5 patients (24%) had nausea and/or vomiting, which responded to antiemetics. One patient developed a transudative pleural effusion 9 days after ADV/IL-12 injection that required thoracentesis and did not recur. As there was no obvious explanation for this effusion, a relation with ADV/IL-12 injection could not be ruled out. One patient with a colostomy experienced an intense edema of the intestinal mucosa that protruded through the colostomy. This event that occurred 3 days after injection of ADV/IL-12 into a liver metastasis lasted for 8 days and recurred after a second injection of ADV/IL-12. Lymphopenia was the most frequent AE, occurring in 85% of patients. Most patients had Grade 1 or 2 lymphopenia before treatment, but lymphocyte count almost invariably decreased at Day 1 and returned to basal values by Day 7. There was an apparent direct relationship between adenoviral dose and lymphopenia intensity. Mild, transient thrombocytopenia also occurred in 3 patients receiving the highest dose. Regarding liver toxicity, most patients had altered liver function tests of varying degrees before treatment, but relevant changes consistent with a reaction to ADV/IL-12 were not observed. However, 4 patients had a transient, modest rise in serum bilirubin after treatment. Notably, none of the 6 cirrhotic patients with hepatocellular carcinoma experienced significant liver toxicity, even at the highest dose level. Among patients receiving multiple doses, side effects usually recurred, but cumulative toxicity was not observed. Also, there was no indication of long-term toxicity among patients observed for more than 6 months. No clinical manifestations of autoimmune reactions were observed, despite the fact that serum autoantibodies became detectable frequently or showed an increase of their preexisting titer irrespective of adenoviral dose.

In a Phase I/II study, we found intratumoral ADV/IL-12 to have a favorable safety profile and induce positive clinical responses in patients with hormone refractory prostate cancer (n = 9).[75] ADV/IL-12 was injected directly into the prostate (or metastatic lesion in patients who had undergone prostatectomy) in escalating doses from 1.0×10^{10} to 5.0×10^{12} vp. Each patient received a total of 3 injections every 4 weeks. AEs included the following: Grade 1 urinary retention, Grade 3 transaminase elevation, and Grade 3 fever. These AEs quickly resolved with proper treatment. One patient receiving the highest dose (5.0×10^{11} vp) showed a decrease in prostate-specific antigen and shrinkage of lymph node metastasis.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Patient Population

TNBC is an aggressive, difficult-to-treat disease with poor prognosis and no current targeted therapy options. More than half of TNBC patients do not achieve pCR after neoadjuvant chemotherapy. TNBC patients with residual disease after neoadjuvant chemotherapy have a significant risk of distant recurrence (65% rate) and death, especially in the first 2-3 years after treatment.[77] There are no FDA-approved current treatment options for these patients with chemoresistant disease and historically, the anticipated pCR rate is close to 0-5%. Strategies to increase pCR rate and improve outcomes in patients with residual disease remains a major therapeutic challenge. TNBC is characterized by increased levels of TILs and high expression levels of PD-L1[18-23] and therefore, is considered to be more amenable to immune-based

treatments than other types of breast cancer. TILs have been shown to be strongly correlated with 1) pCR to neoadjuvant chemotherapy in TNBC[24-27] and 2) better metastasis-free survival and OS in TNBC patients with non-pCR following neoadjuvant chemotherapy.[31-33] Therefore, combinatorial immunotherapy approaches may have value in the neoadjuvant treatment of TNBC. In the I-SPY 2 study, addition of pembrolizumab to standard neoadjuvant chemotherapy increased estimated pCR rate by 40% in TNBC patients.[37] An immunologically active, T-cell-inflamed microenvironment is central to immune checkpoint inhibitor response.[39,42] We hypothesize that ADV/IL-12 will both dramatically reshape the TIME to increase the efficacy of pembrolizumab to improve pCR rate following standard neoadjuvant chemotherapy in patients with TNBC. In support of our hypothesis, we have preliminary data showing that ADV/IL-12 decreased tumor growth (Fig. 1A), increased survival (Fig. 1B), and increased PD-1−positive T-cell infiltration (Fig. 2) *in vivo* in a TNBC xenograft model.

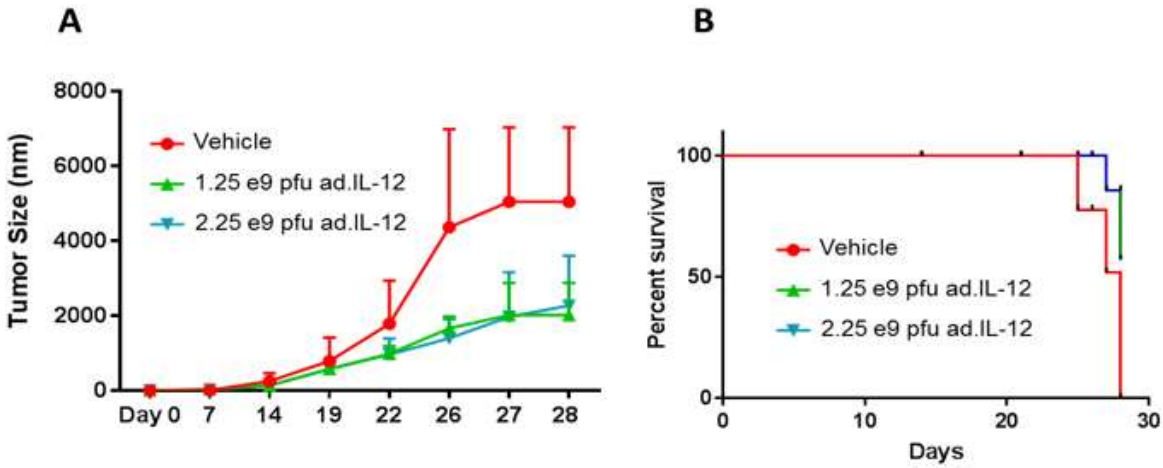


Figure 1. ADV/IL-12 decreases tumor growth and increases survival *in vivo* in a TNBC xenograft model. A, Immunocompetent C57 black mice were implanted with 2E5 mouse E0071 TNBC cells on Day 0. On Day 19, mice (n = 5 per treatment group) were treated with a single intratumoral injection of vehicle or ADV/IL-12 at 1.25e9 or 2.25e9 plaque-forming units (pfu). After treatment, tumor size was measured at the indicated time points. **B,** Survival analysis of ADV/IL-12-treated and vehicle-treated control mice.

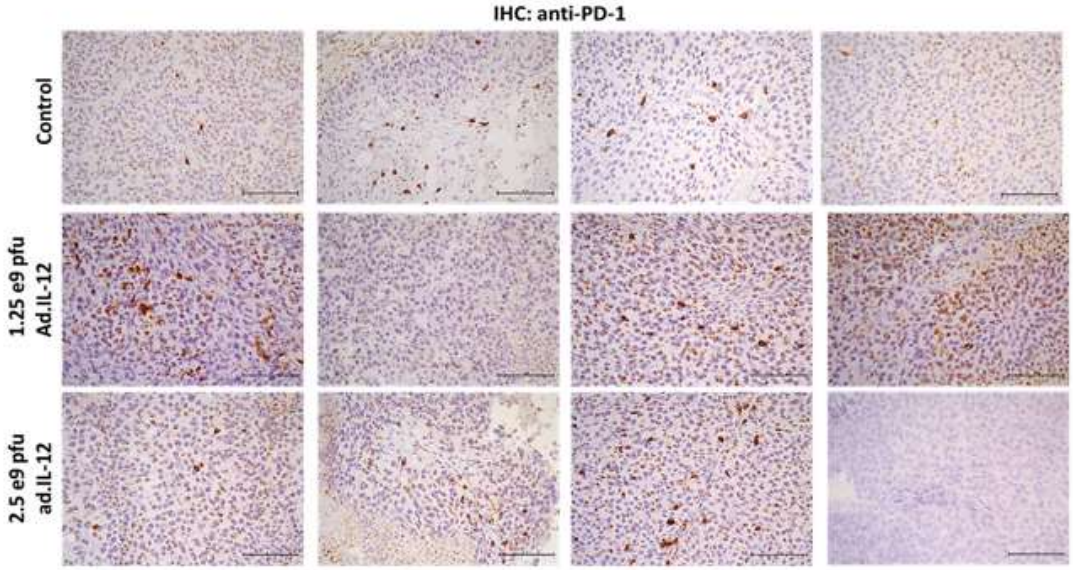


Figure 2. ADV/IL-12 increases PD-1-positive T-cell infiltration in *vivo* in a TNBC xenograft model. C57 black mice implanted with 2E5 mouse E0071 TNBC cells were treated with vehicle, 1.25e9 pfu ADV/IL-12, or 2.25e9 pfu ADV/IL-12. Representative images of immunohistochemical PD-1 expression in tumor-infiltrating cells in TNBC xenografts are shown (n = 4 per treatment group).

4.2.2 Justification for Dose

Docetaxel Chemotherapy:
The planned dose of docetaxel chemotherapy for this trial is 100 mg/m^2, Q3W.

Pembrolizumab:
The planned dose of pembrolizumab for this trial is 200 mg Q3W. Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies demonstrating flat dose and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg Q2W,
- Clinical data showing meaningful improvement in benefit-risk including OS at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W

Among the 8 randomized dose-comparison studies, a total of 2262 patients were enrolled with melanoma and NSCLC, covering different disease settings (treatment naïve, previously treated, PD-L1 enriched, and all-comers) and different treatment settings (monotherapy and in combination with chemotherapy). Five studies compared 2 mg/kg Q3W versus 10 mg/kg Q2W (KEYNOTE-001 Cohort B2, KEYNOTE-001 Cohort D, KEYNOTE-002, KEYNOTE-010, and KEYNOTE-021), and 3 studies compared 10 mg/kg Q3W versus 10 mg/kg Q2W (KEYNOTE-001 Cohort B3, KEYNOTE-001 Cohort F2, and KEYNOTE-006). All of these studies demonstrated flat dose and exposure-response relationships across the doses studied representing an approximate 5 to 7.5-fold difference in exposure. The 2 mg/kg (or 200 mg fixed dose) Q3W provided similar responses to the highest doses studied. Subsequently, flat dose−exposure−response relationships were also observed in other tumor types including head and neck cancer, bladder cancer, gastric cancer, and classical Hodgkin lymphoma, confirming 200 mg Q3W as the appropriate dose independent of tumor type. These findings are consistent with the mechanism of action of pembrolizumab, which acts by interaction with immune cells, and not via direct binding to cancer cells.

Additionally, pharmacology data clearly show target saturation at 200 mg Q3W. First, PK data in KEYNOTE-001 evaluating target-mediated drug disposition conclusively demonstrated saturation of PD-1 in systemic circulation at doses much lower than 200 mg Q3W. Second, a PBPK analysis was conducted to predict tumor PD-1 saturation over a wide range of tumor penetration and PD-1 expression. This evaluation concluded that pembrolizumab at 200 mg Q3W achieves full PD-1 saturation in both blood and tumor.

Finally, population PK analysis of pembrolizumab, which characterized the influence of body weight and other patient covariates on exposure, has shown that the fixed-dosing provides similar control of PK variability as weight-based dosing, with considerable overlap in the

distribution of exposures from the 200 mg Q3W fixed dose and 2 mg/kg Q3W dose. Supported by these PK characteristics and given that fixed dose has advantages of reduced dosing complexity and reduced potential of dosing errors, the 200 mg Q3W fixed dose was selected for evaluation across all pembrolizumab protocols.

ADV/IL-12:
The planned dose of ADV/IL-12 for this trial is 3.0×10^{11} vp for 3 doses. This dose and number of treatments are based on a completed Phase I/II study in patients with hormone refractory prostate cancer.[75]

4.2.3 Rationale for Endpoints

4.2.3.1 Efficacy Endpoints
The primary efficacy endpoint will be the pCR rate of neoadjuvant docetaxel and pembrolizumab plus ADV/IL-12 gene therapy in patients with anthracycline-refractory TNBC.

4.2.3.2 Biomarker Research
Core Biopsy: Diagnostic biopsy at the time of initial diagnosis of primary breast cancer will be collected, and if not available, a repeat tissue biopsy will be obtained.

Additionally, tissue samples will also be collected at the time of surgery.

Tissues will be collected and prepared as formalin-fixed, paraffin-embedded blocks, as well as snap-frozen and stored at -80^{0}C.

Correlative Blood Collection:

- Patients will undergo correlative blood collection at baseline and after Cycles 1, 2, 3, 4 of docetaxel chemotherapy and pembrolizumab plus ADV/Il-12 and at EOT (± 2 days).

Biopsy and surgical tissue samples and blood samples will be used to evaluate correlates of response to docetaxel chemotherapy and pembrolizumab plus intratumoral ADV/IL-12 gene therapy, including but not limited to expression of PD-1/PD-L1,; effector and suppressor immunocyte populations; cytokine profiles; and TIME composition.

5.0 METHODOLOGY

5.1 Trial Population
Female patients aged ≥18 years with TNBC refractory to anthracycline and showing any imaging correlates of residual disease will be eligible for participation in the trial.

Written informed consent is required before performing any trial-specific tests or procedures. Signing of the informed consent form can occur outside the 28-day screening period. All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before trial entry. Results of standard of care tests or examinations performed prior to obtaining informed consent and within 28 days prior to trial entry (except where

otherwise specified) may be used for screening assessments rather than repeating such tests. The investigator or qualified designee will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

5.1.1 Inclusion Criteria

Patients are eligible to be included in the trial only if all of the following criteria apply:
1. The patient (or legally acceptable representative if applicable) provides written informed consent for the trial.
2. Female ≥18 years of age on the day of informed consent signing.
3. Histologically confirmed triple negative breast cancer is defined as estrogen receptor (ER), progesterone receptor (PR), and
HER2 negative. ER/PR negativity is defined as <10% IHC staining of any intensity.
HER2 negativity is defined as the following per the 2018 American Society of Clinical Oncology and College of American Pathologists guidelines.
4. Refractory to standard neoadjuvant anthracycline-containing chemotherapy regimen, demonstrated on MRI.
5. Bilateral breast cancers that individually meet eligibility criteria are allowed.
6. Prior immunotherapy treatment allowed.
7. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (Appendix 2).
8. Adequate organ function as defined in Table 1. All screening labs should be performed within 28 days of trial treatment initiation.

Table 1: Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/µL
Platelets	≥100,000/µL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[a]
WBCs	>2,500/µL and <15,000/µL
Lymphocyte count	≥500/µL
Renal	
Creatinine OR Measured or calculated[b] creatinine clearance (CrCl) (Glomerular filtration rate can also be used in place of creatinine or CrCl)	≤1.5 × upper limit of normal (ULN) OR ≥30 mL/min for patient with creatinine levels >1.5 × institutional ULN

Hepatic	
Total bilirubin	≤1.5 × ULN OR direct bilirubin ≤ULN for patients with total bilirubin levels >1.5 × ULN (Patients with known Gilbert's disease who have serum bilirubin level ≤3 × ULN may be enrolled)
AST and ALT	≤2.5 × ULN with normal alkaline phosphatase (ALP) OR ≤1.5 × ULN in conjunction with ALP >2.5 × ULN
Coagulation	
International normalized ratio (INR) OR prothrombin time (PT) Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless patient is receiving anticoagulant therapy as long as PT OR aPTT is within therapeutic range of intended use of anticoagulants
[a]Criteria must be met without erythropoietin dependency and without packed red blood cell transfusion within last 2 weeks. [b]CrCl should be calculated per institutional standard.	

9. Cardiac ejection fraction ≥45%.
10. A female patient is eligible to participate if she is not pregnant (Appendix 3), not breastfeeding, and at least one of the following conditions applies:
a) Not a woman of childbearing potential (WOCBP) as defined in Appendix 3
OR
b) A WOCBP who agrees to follow the contraceptive guidance in Appendix 3 during the treatment period and for at least 120 days after the last dose of trial treatment. WOCBP must have a negative serum pregnancy test (β-human chorionic gonadotropin [β-HCG]) within 72 hours prior to trial treatment administration.
11. Willing to provide biopsy tissue as required by the trial.
12. Willing and able to comply with the protocol for the duration of the trial including undergoing treatment and scheduled visits and examinations.

5.1.2 Exclusion Criteria

Patients are excluded from the trial if any of the following criteria apply:

1. History of New York Heart Association class III or greater cardiac disease (Appendix 4).
2. History of myocardial infarction, stroke, ventricular arrhythmia, or greater than first-degree conduction defect within the past 12 months.
3. History of congenital QT prolongation.
4. Absolute corrected QT interval of >480 msec in the presence of potassium >4.0 mEq/L and magnesium >1.8 mg/dL.
5. Is currently participating in or has participated in a study of an investigational agent or has used an investigational device within 4 weeks prior to trial treatment administration.

6. NOTE: Patients who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.
7. Concurrent use of any complementary or alternative medicines.
8. Concurrent use of inhibitors or inducers of cytochrome P450 (CYP)3A4 and CYP2D6 (Appendix 5)
9. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (dose exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior to trial treatment administration.
10. Known history of active tuberculosis (Bacillus Tuberculosis).
11. Known or suspected hypersensitivity to any component or excipient of the proposed regimen (docetaxel chemotherapy, gene vector, pembrolizumab).
12. Patients must have recovered from all AEs due to previous therapies to ≤ Grade 1 or baseline. Patients with ≤ Grade 2 neuropathy may be eligible. If patient received major surgery, she must have recovered adequately from the toxicity and/or complications from the intervention prior to starting the trial treatment. Known additional malignancy that is progressing or requires active treatment. NOTE: Patients with basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or cervical cancer in situ that have undergone potentially curative therapy are not excluded.
13. Active autoimmune disease that has required systemic treatment in the past 2 years (i.e., with use of disease-modifying agents, corticosteroids, or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
14. History of (noninfectious) pneumonitis that required steroids or current pneumonitis.
15. Active infection requiring systemic therapy.
16. History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator.
17. Known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
18. Pregnant or breastfeeding, or expecting to conceive children within the projected duration of the trial, starting with the prescreening or screening visit through 120 days after the last dose of trial treatment.
19. Known history of human immunodeficiency virus (HIV).
20. Known history of hepatitis B (defined as hepatitis B surface antigen reactive) or known active hepatitis C virus (HCV; defined as HCV RNA [qualitative] is detected) infection.
21. Received a live vaccine within 30 days prior to trial treatment administration. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.

5.1.3 Lifestyle Restrictions

5.1.3. Contraception

Doxorubicin, docetaxel, cyclophosphamide, and pembrolizumab can cause fetal harm when administered to a pregnant woman. Refer to Appendix 3 for approved methods of contraception.

5.1.4 Pregnancy

If a patient inadvertently becomes pregnant while on the trial treatment, the patient will be immediately discontinued from the trial treatment. The site will contact the patient at least monthly and document the patient's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The investigator or qualified designee will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor and Merck.

5.1.5 Use in Nursing Women

Doxorubicin has been detected in the milk of at least 1 lactating patient. Cyclophosphamide is excreted in breast milk. It is unknown whether docetaxel and pembrolizumab are excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, patients who are breastfeeding are not eligible for enrollment.

5.2 Trial Treatment

The treatment to be used in this trial is outlined below in Table 2.

Table 2: Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period
Docetaxel Chemotherapy + Pembrolizumab + ADV/IL-12				
Docetaxel	100 mg/m^2	Q3W ± 3 days	IV infusion	4 cycles, Day 1 of each 3-week cycle.
Pembrolizumab	200 mg	Q3W ± 3 days	IV infusion	4 cycles, Day 1 of each 3-week cycle
ADV/IL-12	5×10^{11} vp	3 injections	Intratumoral injection	Within 3 days prior to Cycle 2, 3 and 4 of docetaxel

				chemotherapy and pembrolizumab
IV = intravenous; Q3W = every 3 weeks; vp = virus particles.				

Patients will receive 4 cycles of docetaxel chemotherapy and pembrolizumab in combination with ADV/IL-12.

- Docetaxel chemotherapy: Docetaxel (100 mg/m^2 IV infusion over 1 hour), will be administered on Day 1 Q3W (± 3 days).

- Pembrolizumab (200 mg IV infusion over 30 minutes) will be administered on Day 1 Q3W (± 3 days).

- ADV/IL-12 will be administered after Cycles 1, 2 and 3 of the 4 cycles of docetaxel chemotherapy and pembrolizumab, within 3 days prior to cycles 2, 3 and 4. ADV/IL-12 at 5×10^{11} vp in 2-mL volume (vector buffer) will be administered intratumorally using a 21-gauge needle placed under ultrasound guidance. In cases where the tumor cannot be accessed by ultrasound, a computed tomography (CT)-guided injection will be performed. Local anesthesia will be used and patients will be monitored for several hours after the procedure. Mild sedation may also be administered.

Vector preparation will take place in the GMP facility of The Center for Cell and Gene Therapy (CAGT). The vial containing the ADV/IL-12 vector will be pulled from the freezer, quickly thawed, placed in a biological safety cabinet and diluted to 5×10^{11} vp in a 2-mL volume. The diluted vector will be drawn into a syringe, wrapped and transported on ice in a closed container to Houston Methodist Hospital's Interventional Radiology and administered within 90 minutes of thawing. If not administered within this time frame, syringes with unused vector will be discarded in a biohazard waste disposal container or returned to CAGT for disposal under CAGT's internal Standard Operating Procedures (SOPs).

All patients will undergo surgery after neoadjuvant treatment completion (within 30 days of the final dose of trial treatment).

After definitive surgery, patients will receive adjuvant pembrolizumab every 3 weeks for up to nine cycles. Further adjuvant therapy will be according to physicians' choice. Patients will be monitored for at least 90 days after treatment discontinuation of ADV/IL-12 for late immune-related toxicities.

5.2.1 Timing of Dose Administration

The trial treatment will be administered on an outpatient basis.

Patients will receive 4 cycles of docetaxel chemotherapy and pembrolizumab in combination with ADV/IL-12.

- Docetaxel chemotherapy and pembrolizumab will be administered Q3W (± 3 days) for 4 cycles. Docetaxel chemotherapy and pembrolizumab will be administered on Day 1 of each cycle. Pembrolizumab infusion timing should be as close to 30 minutes as possible. However, given the variability of infusion pumps, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min). The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

- ADV/IL-12 will be administered intratumorally after Cycle 1, 2 and 3 of docetaxel chemotherapy and pembrolizumab, within 3 days prior to Cycle 2, 3 and 4.

5.2.2 Dose Modification

Docetaxel Chemotherapy:

Dose modifications for docetaxel chemotherapy are shown in Table 3.

Table 3: Dose Modifications for Docetaxel Chemotherapy

Hematological Toxicity	
ANC/µL	
500−1000	Delay treatment until recovery
<500	Delay treatment until recovery and consider reducing docetaxel by 25% for subsequent cycles
Febrile neutropenia	Delay treatment until recovery and consider reducing docetaxel by 25% for subsequent cycles
Platelets/µL	
75,000 to <100,000	Treatment may continue if patient is clinically well
50,000 to <75,000	Delay treatment until recovery
<50,000	Delay treatment until recovery and consider reducing docetaxel by 25% for subsequent cycles
Hepatic Impairment	
Minimal	Reduce docetaxel by 25%
Mild	Reduce docetaxel by 50%
Moderate	Omit docetaxel
Severe	Omit docetaxel

Peripheral Neuropathy	
Grade 2	Reduce docetaxel by 25%; if persistent, reduce docetaxel by 50%
Grade 3 or 4	Omit docetaxel
Mucositis and Stomatitis	
Grade 2	Delay treatment until toxicity has resolved to Grade 1 or less and reduce the dose for subsequent cycles as follows: 1st occurrence: No dose reduction 2nd occurrence: Reduce docetaxel by 25% 3rd occurrence: Reduce docetaxel by 50% 4th occurrence: Withhold chemotherapy
Grade 3 or 4	Delay treatment until toxicity has resolved to Grade 1 or less and reduce the dose for subsequent cycles as follows: 1st occurrence: Reduce docetaxel by 50% 2nd occurrence: Withhold chemotherapy
Diarrhea	
Grade 2	Delay treatment until toxicity has resolved to Grade 1 or less and reduce the dose for subsequent cycles as follows: 1st occurrence: No dose reduction 2nd occurrence: Reduce docetaxel by 25% 3rd occurrence: Reduce docetaxel by 50% 4th occurrence: Withhold chemotherapy
Grade 3 or 4	Delay treatment until toxicity has resolved to Grade 1 or less and reduce the dose for subsequent cycles as follows: 1st occurrence: Reduce docetaxel by 50% 2nd occurrence: Withhold chemotherapy

ANC = absolute neutrophil count; CrCL = creatinine clearance.

Pembrolizumab:

Dose Modification and toxicity management for irAEs associated with pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These irAEs may occur shortly after the first dose or several months after the last treatment dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids, and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, and skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 4.

Table 4: Dose Modification and Toxicity Management Guidelines for irAEs Associated with Pembrolizumab

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after the AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if the AE does not resolve within 3 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.

IrAEs	**Toxicity grade or conditions (CTCAE v5.0)**	**Action taken with 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 patients for signs and symptoms of pneumonitis • Evaluate patients 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 patients for signs and symptoms of enterocolitis (i.e., diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (i.e., peritoneal signs and ileus) • Patients with ≥ Grade 2 diarrhea suspecting colitis should consider gastrointestinal consultation and performing endoscopy to rule out colitis • Patients with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid
	Grade 4	Permanently discontinue		

				intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
AST/ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer 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 returns to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1−2 mg/kg prednisone or equivalent) followed by taper	
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 patients with T1DM • Administer antihyperglycemic for patients with hyperglycemia	• Monitor patients 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 nonselective beta-blockers (e.g., 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 (e.g., levothyroxine or liothyronine) 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 in 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 causes
	Grade 3 or 4	Permanently discontinue		

All other irAEs	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 but are not limited to: Guillain–Barré Syndrome, encephalitis.		
	Grade 4, or recurrent Grade 3	Permanently discontinue		
1. Decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. **NOTE:** For patients 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). AE = adverse event; ALT = alanine transaminase; AST = aspartate transaminase; irAE = immune-related adverse event; IV = intravenous; T1DM = type 1 diabetes mellitus.				

Dose modification and toxicity management of infusion reactions related to pembrolizumab

Pembrolizumab may cause severe or life-threatening infusion reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines for pembrolizumab-associated infusion reaction are provided in Table 5.

Table 5: Pembrolizumab Infusion Reaction Dose Modification and Treatment Guidelines

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 patient is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hours	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hour to 50 mL/hour). Otherwise dosing will be held until symptoms resolve and the patient should be premedicated for the next scheduled dose. **Patients who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment.**	Patient may be premedicated 1.5 hours (± 30 minutes) prior to infusion with: Diphenhydramine 50 mg p.o. (or equivalent dose of antihistamine). Acetaminophen 500−1000 mg p.o. (or equivalent dose of analgesic).
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: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Patient is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the CTCAE v5.0 in Appendix 1. IV = intravenous; NSAID = nonsteroidal anti-inflammatory drug; p.o. = orally.		

ADV/IL-12:
Significant toxicity has not been observed with the intratumoral administration of ADV/IL-12.[74,75] ADV/IL-12 dose will be de-escalated to 3.0×10^{11} vp if unacceptable toxicity occurs with the planned dose. If toxicity occurs with this dose modification, the dose will be further de-escalated to 1.0×10^{11} vp.

Any toxicity observed during the course of the trial can be managed by dose interruption or dose reduction. Dose interruptions and dose reductions should be reported on the appropriate Dosage Administration case report form (CRF). The maximum time allowed for toxicity-related treatment interruption is 3 weeks from the intended dosing day. If the interruption is >3 weeks, the patient must be discontinued from the trial treatment. When a patient withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of withdrawal. Any AEs that are present at the time of withdrawal should be followed as described in Section 7.1.4.1.1.

5.3 Concomitant Medications/Vaccinations (allowed & prohibited)
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 the trial treatment or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the patient's primary physician. However, the decision to continue the patient on trial treatment requires the mutual agreement of the investigator, the Sponsor, and the patient.

5.3.1 Acceptable Concomitant Medications
All treatments that the investigator considers necessary for a patient’s welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medications will be recorded on the CRF including all prescription, over-the-counter, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included in the CRF.

Patients with medication-controlled hypertension will continue to take their current blood pressure medication.

All concomitant medications received within 28 days before the start of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered 30 days after the last dose of trial treatment should be recorded for SAEs and events of clinical interest (ECIs) as defined in Section 7.2.

5.3.2 Prohibited Concomitant Medications/Therapies

Patients are prohibited from receiving the following for the duration of the trial:

- Radiation therapy or targeted/biological therapy.

- Immunotherapy not specified in this protocol.
- Investigational agents other than ADV/IL-12, and pembrolizumab.
- Live vaccines within 30 days prior to the start of trial treatment administration 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, Bacillus Calmette−Guérin, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an ECI of suspected immunologic etiology.

Patients 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. Patients may receive other medications that the investigator deems to be medically necessary.

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial.

There are no prohibited therapies during the post-treatment follow-up phase.

5.3.3 Rescue Medications & Supportive Care
Patients should receive appropriate supportive care measures as deemed necessary by the treating investigator.

Docetaxel Chemotherapy:
Supportive care, such as premedication with oral corticosteroids (e.g., dexamethasone) to reduce the incidence and severity of fluid retention as well as the severity of hypersensitivity reactions, prophylactic granulocyte-colony-stimulating factor to mitigate the risk of hematological toxicities, and antiemetics, will be administered in accordance with institutional guidelines.

Pembrolizumab:
Patients should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 5.2.2 (Table 5). Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

NOTE: If after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance. Refer to Table 5 in Section 5.2.2 for guidelines regarding dose modification and supportive care.

5.4 Patient Withdrawal/Discontinuation Criteria

Patients may discontinue trial treatment at any time for any reason or be dropped from the trial treatment at the discretion of the investigator should any untoward effect occur. In addition, a patient may be discontinued from trial treatment by the investigator or the Sponsor if the trial treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding procedures to be performed at trial treatment discontinuation are provided in Section 7.1.3 – Other Procedures.

A patient must be discontinued from trial treatment but continue to be monitored in the trial for any of the following reasons:

- The patient or patient's legally acceptable representative requests to discontinue trial treatment
- Confirmed radiographic disease progression
- Any progression or recurrence of any malignancy, or any occurrence of another malignancy that requires active treatment
- Dose interruption that exceeds 3 weeks
- Unacceptable adverse experiences as described in Section 5.2.2
- The patient has a medical condition or personal circumstance that, in the opinion of the investigator and/or Sponsor, places the patient at unnecessary risk from continued administration of trial treatment
- Confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- Recurrent Grade 2 pneumonitis
- All chemotherapy orders will be halted for patients with known and active Covid-19 infection, on clinical trial and for routine clinical care, as best practiceLost to follow-up
- Death
- Administrative reasons

5.5 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to patients
4. Plans to modify or discontinue the development of the study drugs

In the event of Merck decision to no longer supply study drug (pembrolizumab), ample notification will be provided so that appropriate adjustments to patient treatment can be made.

6.0 TRIAL FLOW CHART

6.1 Trial Flow Chart

	Screening[a]	Baseline[b]	Docetaxel Chemotherapy + Pembrolizumab + ADV/IL-12				EOT
Cycle			**1**	**2**	**3**	**4**	
Scheduling Window (Days):	**-28 to -7**	**-7 to -1**	**± 5**	**± 5**	**± 5**	**± 5**	
Informed Consent	X						
Inclusion/Exclusion Criteria	X						
Demographics	X						
Medical History	X						
Physical Exam[c]	X	X	X	X	X	X	X
ECOG Performance Status	X	X	X	X	X	X	X
12-Lead ECG[d]	X						
MUGA Scan or ECHO[e]	X						
Serum Pregnancy Test (β-HCG)[f]	X	X					
PT/INR and aPTT[g]	X						
Hematology[h]	X	X	X	X	X	X	X
Clinical Chemistry[i]	X	X	X	X	X	X	X
T3, FT4, and TSH[j]		X					
Urinalysis[k]		X					
Breast MRI[l]		X					X
Breast Ultrasound[m]		X					X
Docetaxel Chemotherapy[n]			X	X	X	X	
Pembrolizumab[n]			X	X	X	X	
ADV/IL-12[n]				X	X	X	
Biopsy[o]		X					
Correlative Blood Collection[p]		X	X	X	X	X	X
AEs and SAEs[q]			X	X	X	X	X
Surgical Sample Collection[r]							X

AE = adverse event; ALP = alkaline phosphatase; ALT = alanine transaminase; aPTT = activated partial thromboplastin time; AST = aspartate transaminase; β-hCG = beta-human chorionic gonadotropin; BUN = blood urea nitrogen; CBC = complete blood count; ECG = electrocardiogram; ECHO = echocardiogram; ECOG = Eastern Cooperative Oncology Group; EOT = end of treatment; FT4 = free thyroxine; INR = international normalized ratio; IV = intravenous; LDH = lactate dehydrogenase; MRI = magnetic resonance imaging; MUGA = multigated acquisition; PT = prothrombin time; Q3W = every 3 weeks; SAE = serious adverse event; T3 = triiodothyronine; TSH = thyroid-stimulating hormone; vp = virus particles; WBC = white blood cell; WOCBP = women of childbearing potential.

A window of ± 5 days is allowed for trial visits and assessments/procedures (except as otherwise specified).

a. Within 28 days prior to Cycle 1, Day 1 of docetaxel chemotherapy and pembrolizumab.
b. Within 7 days prior to Cycle 1, Day 1 of docetaxel chemotherapy and pembrolizumab. Only screening procedures not performed within 7 days prior to Cycle 1, Day 1 of docetaxel chemotherapy and pembrolizumab are required at baseline.
c. Physical exam will be performed at screening/baseline, before each cycle of docetaxel chemotherapy, pembrolizumab and ADV/IL-12 and at EOT. Physical exam will include vital sign (blood pressure, oral or temporal temperature, pulse, and respiratory rate) and weight measurements. Height will be measured at screening only.
d. A 12-lead ECG will be performed at screening and when clinically indicated.
e. MUGA scan or ECHO will be performed at screening and when clinically indicated. The same method (MUGA scan or ECHO) must be used throughout the duration of the trial.
f. For WOCBP, serum β-hCG pregnancy testing is to be performed at screening and within 72 hours prior to Cycle 1, Day 1 of docetaxel chemotherapy and pembrolizumab. Serum β-hCG pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise expected.
g. Coagulation parameters (INR/PT/aPTT) will be tested at screening.
h. CBC with platelet count and differential WBC count will be performed at screening/baseline, before each cycle of docetaxel chemotherapy, pembrolizumab and ADV/IL-12, and when clinically indicated.
i. Clinical chemistry panel (glucose, albumin, sodium, potassium, carbon dioxide, calcium, chloride, BUN, creatinine, total protein, total bilirubin, ALP, ALT, and AST) and evaluation of magnesium, uric acid, and LDH will be performed at screening/baseline, before each cycle of docetaxel chemotherapy, pembrolizumab and ADV/IL-12, and when clinically indicated.
j. TSH, FT4, and total T3 testing will be performed at baseline and when clinically indicated.
k. Urinalysis (blood, glucose, protein, specific gravity) will be performed at baseline and when clinically indicated.
l. Breast MRI will be performed at baseline and at EOT. NOTE: Breast MRI performed as standard of care will be used for baseline assessment if performed within 90 days prior to Cycle 1, Day 1 of docetaxel chemotherapy and pembrolizumab.
m. Breast ultrasound will be performed at baseline and at EOT.
n. Docetaxel chemotherapy (100 mg/m^2 IV), and pembrolizumab (200 mg IV) will be administered on Day 1 Q3W (± 5 days) for 4 cycles. ADV/IL-12 at 5×10^{11} vp will be administered intratumorally at 3 time points: after Cycle 1 and within 3 days prior to Cycle 2; after Cycle 2 and within 3 days prior to Cycle 3; after Cycle 3 and within 3 days prior to Cycle 4 of docetaxel chemotherapy and pembrolizumab.
o. Diagnostic biopsy will be collected at baseline. If not available, a repeat biopsy will be conducted.

p. Correlative blood collection will be performed at baseline, after Cycles 1, 2, 3 and 4 (± 2 days) of docetaxel chemotherapy, pembrolizumab and ADV/IL-12 and at EOT. Blood will be collected into standard vacutainer tubes (3 green top tubes).
q. AEs and SAEs will be captured from the time of informed consent signing up to and including 30 and 90 days after the last treatment dose, respectively.
r. After definitive surgery, patients will receive adjuvant pembrolizumab every 3 weeks for up to nine cycles.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart − Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to patient safety. In some cases, such evaluation/testing may require that additional informed consent be obtained from the patient.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The investigator or qualified designee must obtain documented consent from each potential patient prior to their participation in the trial.

Consent must be documented by the patient's dated signature or by the patient's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the patient before participation in the trial.

The initial informed consent form and any subsequent revised written informed consent form provided to the patient must receive institutional review board (IRB) approval in advance of use. The patient or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the patient's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the patient's dated signature or by the patient's legally acceptable representative's dated signature.

The informed consent will adhere to IRB requirements, applicable laws and regulations, and Sponsor requirements.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the patient qualifies for the trial.

7.1.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the investigator. Details regarding the disease for which the patient has enrolled in this trial will be recorded separately and not listed as medical history.

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications
The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the patient within 28 days before starting the trial. Treatment for the disease for which the patient has enrolled in this trial will be recorded separately and not listed as a prior medication.

7.1.1.4.2 Concomitant Medications
The investigator or qualified designee will record medication, if any, taken by the patient during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details
The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.1.5.2 Prior Treatment Details
The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation, and surgeries.

7.1.1.5.3 Subsequent Anticancer Therapy Status
The investigator or qualified designee will review all new antineoplastic therapy initiated after the last dose of trial treatment. If a patient initiates a new anticancer therapy within 30 days after the last dose of trial treatment, the 30 day safety follow-up visit must occur before the first dose of the new therapy.

7.1.2 Clinical Procedures/Assessments
A visit window of ± 5 days is allowed for trial visits and assessments/procedures (except as otherwise specified).

7.1.2.1 Adverse Event Monitoring
The investigator or qualified designee will assess each patient to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. AEs will be graded and recorded throughout the trial and during the follow-up period according to the NCI CTCAE v5.0. Toxicities will be characterized in terms of seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to Section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Physical Exam
Physical exam will be performed at screening/baseline, before every cycle of docetaxel chemotherapy, pembrolizumab and ADV/IL-12 and at end of treatment (EOT). Weight will be measured at each physical exam. Height will be measured at screening only.

7.1.2.3 Vital Signs
Vital signs (blood pressure, oral or temporal temperature, pulse, and respiratory rate) will be measured at each physical exam.

7.1.2.4 ECOG Performance Status
ECOG performance status will be assessed at screening/baseline, before every cycle of docetaxel chemotherapy, pembrolizumab and ADV/IL-12, and at EOT.

7.1.2.5 Electrocardiogram/Multigated Acquisition Scan/Echocardiogram
12-lead electrocardiogram (ECG) will be performed at screening, and when clinically indicated.

Multigated acquisition (MUGA) scan or echocardiogram (ECHO) will be performed at screening and when clinically indicated. The same method (MUGA scan or ECHO) must be used throughout the duration of the trial.

7.1.2.6 Breast MRI
Breast MRI will be performed at baseline and at EOT. NOTE: Breast MRI performed is performed as the standard of care and can be used for baseline assessment if performed within 90 days prior to Cycle 1, Day 1 of docetaxel chemotherapy and pembrolizumab.

Disease status will be assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1.

7.1.2.6.1 RECIST 1.1
The RECIST 1.1 will be used to assess treatment response. All measurable lesions (up to 5 measurable lesions [2/organ]) representative of all involved organs should be identified as target lesions and recorded and measured at baseline. A sum of the longest diameter for all target lesions will be calculated and reported as the baseline sum longest diameter.

- CR

Disappearance of all target lesions.

- PR

At least a 30% decrease in the sum of the longest diameter of target lesions, using the baseline sum of the longest diameter as a reference.

- SD

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for progressive disease (PD), using the smallest sum longest diameter recorded since treatment start as a reference.

- PD

At least a ≥20% increase in the sum of the longest diameter of the target lesions, using as a reference the smallest sum longest diameter recorded since treatment start, or the appearance of one or more new lesions. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.

- Clinical PD

Patients who in the opinion of the treating principal investigator have clinical evidence of PD may be classified as having PD.

7.1.2.7 Tumor Tissue and Blood Collection for Correlative Studies

Biopsy

Diagnostic biopsy at the time of initial diagnosis of primary breast cancer will be collected, and if not available, a repeat tissue biopsy will be obtained to confirm TNBC.

Additionally, tissue samples will also be collected at the time of surgery.

Tissues will be collected and prepared as formalin-fixed, paraffin-embedded blocks, as well as snap-frozen and stored at -80^{0}C.

Correlative Blood Collection

- Patients will undergo correlative blood collection at baseline, after every cycles (± 2 days) of the 2 cycles of docetaxel chemotherapy, pembrolizumab and ADV/IL-12 and at EOT.

Biopsy and surgical tissue samples and blood samples will be used to evaluate correlates of response to docetaxel chemotherapy and pembrolizumab plus intratumoral ADV/IL-12 gene therapy, including but not limited to expression of PD-1/PD-L1, effector and suppressor immunocyte populations; cytokine profiles; and TIME composition.

7.1.2.8 Laboratory Procedures/Assessments

For WOCBP, serum β-hCG pregnancy testing will be performed at screening and within 72 hours prior to trial treatment initiation. Serum β-hCG pregnancy testing is to be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise expected. Coagulation parameters (INR/PT/aPTT) will be tested at screening. Thyroid-stimulating hormone, free thyroxine, and total triiodothyronine testing will be performed at baseline and when clinically indicated. Urinalysis (blood, glucose, protein, specific gravity) will be performed at baseline and when clinically indicated. Complete blood count with platelet count and differential WBC count, clinical chemistry panel (glucose, albumin, sodium, potassium, carbon dioxide, calcium, chloride, blood urea nitrogen, creatinine, total protein, total bilirubin, ALP, ALT, and AST), and evaluation of magnesium, uric acid, and lactate dehydrogenase will

be performed at screening/baseline, before every cycle of docetaxel chemotherapy, pembrolizumab and ADV/IL-12 and at EOT, as well as when clinically indicated.

Laboratory tests for hematology, chemistry, urinalysis, and other are specified in Table 6.

Table 6: Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hemoglobin	Albumin	Blood	Serum β-hCG†
Platelet count	ALP	Glucose	PT (INR)
WBC count (total and differential)	ALT	Protein	aPTT
ANC	AST	Specific gravity	Total T3
	LDH	(*Microscopic exam if abnormal results are noted*)	Free T4
	Carbon dioxide		TSH
	Creatinine		
	Uric acid		
	Calcium		
	Chloride		
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total bilirubin		
	Direct bilirubin *(If total bilirubin is elevated above the ULN)*		
	Total protein		
	BUN		

†Perform on WOCBP only.

ANC = absolute neutrophil count; ALP = alkaline phosphatase; ALT = alanine transaminase; aPTT = activated partial thromboplastin time; AST = aspartate transaminase; β-hCG = beta-human chorionic gonadotropin; BUN = blood urea nitrogen; INR = international normalized ratio; LDH = lactate dehydrogenase; PT = prothrombin time; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone; ULN = upper limit of normal; WBC = white blood cell; WOCBP = women of childbearing potential.

7.1.3 Other Procedures

7.1.3.1 Withdrawal/Discontinuation

When a patient discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any AEs which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 − Assessing and Recording Adverse Events.

7.1.4 Visit Requirements

Visit requirements are outlined in Section 6.0 − Trial Flow Chart. Specific procedure-related details are provided above in Section 7.0 − Trial Procedures.

7.1.4.1 Post-Treatment Visits

7.1.4.1.1 Safety Follow-Up Visit

The safety follow-up visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anticancer treatment, whichever comes first. All AEs that occur prior to the safety follow-up visit should be recorded. Patients with an AE of > Grade 1 will be followed until the resolution of the AE to Grade 0–1 or the beginning of a new antineoplastic therapy, whichever occurs first. SAEs that occur within 90 days of EOT or before initiation of a new anticancer treatment should also be followed and recorded.

7.2 Assessing and Recording Adverse Events

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (e.g., abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the trial treatment is also an AE. Progression of the cancer under study is not considered an AE.

All AEs, SAEs, and other reportable safety events that occur after the consent form is signed but before trial treatment initiation must be reported by the investigator if the patient is receiving placebo run-in or other run-in treatment, if the event causes the patient 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, or a procedure.

- All AEs from the time of trial treatment initiation through 30 days following cessation of trial treatment must be reported by the investigator.

- All AEs meeting serious criteria from the time of trial treatment initiation through 90 days following cessation of trial treatment or 30 days following cessation of trial treatment if the patient initiates new anticancer therapy, whichever is earlier, must be reported by the investigator.

- All pregnancies and exposure during breastfeeding from the time of trial treatment initiation through 120 days following cessation of trial treatment or 30 days following cessation of trial treatment if the patient initiates new anticancer therapy must be reported by the investigator.

- Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately by the investigator if the event is considered to be related to the trial treatment.

Investigators are not obligated to actively seek AE or SAE or other reportable safety events in former study patients. However, if the investigator learns of any SAE, including a death, at any time after a patient has been discharged from the trial, and he/she considers the event to be

reasonably related to the trial treatment or trial participation, the investigator must promptly notify Merck.

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of pembrolizumab overdose. In the event of overdose, the patient should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an AE(s) is associated with ("results from") pembrolizumab overdose, the AE(s) is reported as a SAE, even if no other seriousness criteria are met.

If a dose of pembrolizumab 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 ECI, using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an AE must be reported within 24 hours to the Sponsor (CTOMgmt@houstonmethodist.org and/or RATM@houstonmethodist.org) and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215-661-6229).

7.2.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and infant exposure during breastfeeding are not considered AEs, it is the responsibility of the investigator to report any pregnancy or lactation in a patient (spontaneously reported to them) that occurs during the trial.

Pregnancies and infant exposures during breastfeeding that occur after the consent form is signed but before trial treatment initiation must be reported by the investigator if they cause the patient 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 infant exposures during breastfeeding that occur from the time of trial treatment initiation through 120 days following cessation of trial treatment or 30 days following cessation of trial treatment if the patient 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 the infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor (CTOMgmt@houstonmethodist.org and/or RATM@houstonmethodist.org) and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215-661-6229).

7.2.3 Immediate Reporting of Adverse Events to the Sponsor and to Merck

7.2.3.1 Serious Adverse Events

A SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Results in persistent or significant disability/incapacity
- Results in or prolongs an existing inpatient hospitalization, unless hospitalization is for:
    - routine treatment or monitoring of the studied indication and not associated with any deterioration in condition
    - elective or preplanned treatment for a preexisting condition that is unrelated to the indication under study and has not worsened since the start of trial treatment
    - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
    - social reasons and respite care in the absence of any deterioration in the patient's general condition
- Is a congenital anomaly/birth defect
- Is another important medical event
- **NOTE:** In addition to the above criteria, AEs meeting either of the below criteria, although not serious per International Conference on Harmonisation (ICH) definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.
    - Is a new cancer (that is not a condition of the trial)
    - Is associated with an overdose

Refer to Table 7 for additional details regarding each of the above criteria.

For the time period beginning when the consent form is signed until trial treatment initiation, any SAE or follow-up to a SAE including death due to any cause that occurs to any patient must be reported within 24 hours to the Sponsor (CTOMgmt@houstonmethodist.org and/or RATM@houstonmethodist.org) and within 2 working days to Merck Global Safety if it causes the patient 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.

From the time period beginning at trial treatment initiation through 90 days following cessation of trial treatment or 30 days following cessation of trial treatment if the patient initiates new anticancer therapy, whichever is earlier, any SAE or follow-up to a SAE, including death due to any cause whether or not related to the trial treatment, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Additionally, any SAE considered by an investigator who is a qualified physician to be related to the trial treatment that is brought to the attention of the investigator at any time following consent through the end of the safety follow-up period specified in the paragraph above or at

any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and Merck Global Safety.

All patients with SAEs must be followed up for outcome.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-661-6229

SAE reports should also be sent to the Houston Methodist Research Institute Office of Regulatory Affairs & Translational Management at:

RATM@houstonmethodist.org
fax line: 713-793-7001

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by the Food and Drug Administration (FDA) and other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally, investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to the FDA.

7.2.3.2 Events of Clinical Interest

Selected nonserious and serious AEs are also known as ECIs and must be reported within 24 hours to the Sponsor (CTOMgmt@houstonmethodist.org and/or RATM@houstonmethodist.org) and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215-661-6229).

From the time period beginning when the consent form is signed until trial treatment initiation, any ECI or follow-up to an ECI that occurs to any patient must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the patient 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.

From the time period beginning at trial treatment initiation through 90 days following cessation of trial treatment or 30 days following cessation of trial treatment if the patient initiates new anticancer therapy, whichever is earlier, any ECI or follow-up to an ECI, whether or not related to the trial treatment, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

ECIs for this trial include:

1. an overdose of pembrolizumab, as defined in Section 7.2.1 − Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and To Merck, that is not associated with clinical symptoms or abnormal laboratory results.
2. an elevated AST or ALT lab value that is ≥3 × ULN and an elevated total bilirubin lab value that is ≥2 × ULN and, at the same time, an ALP lab value that is <2 × ULN, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

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

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all AEs according to the NCI CTCAE v5.0. Any AE that changes CTCAE grade over the course of a given episode will have each change of grade recorded on the Adverse Event CRF.

All AEs regardless of CTCAE grade must also be evaluated for seriousness.

Table 7: Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all AEs as to:

v5.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).**
	Grade 3	**Severe or medically significant but not immediately life threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life-threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A SAE is any untoward medical occurrence that at any dose:	
	†**Results in death**; or	
	†**Is life threatening; or** places the patient, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an AE that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (Hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. Note: Hospitalization for an elective procedure to treat a preexisting condition that has not worsened is not a SAE. A preexisting condition is a clinical condition that is diagnosed prior to the use of the trial treatment and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of patient taking the product regardless of time to diagnosis); or	
	Is a new cancer (that is not a condition of the trial) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and within 2 working days to Merck to meet certain local requirements); or	
	Is an overdose (whether accidental or intentional). Any AE associated with an overdose is considered a SAE for collection purposes. An overdose that is not associated with an AE is considered a nonserious ECI and must be reported within 24 hours to the Sponsor and within 2 working days to Merck.	

	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a SAE when, based upon appropriate medical judgment, the event may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the AE. If less than 1 day, indicate the appropriate length of time and units.	
Action taken	Did the AE cause the trial treatment to be discontinued?	
Relationship to the trial treatment	Did the trial treatment cause the AE? The determination of the likelihood that the trial treatment caused the AE will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory timeframe. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the trial treatment and the AE based upon the available information. **The following components are to be used to assess the relationship between the trial treatment and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the trial treatment caused the AE:	
	Exposure	Is there evidence that the patient was actually exposed to the trial treatment such as: reliable history, acceptable compliance assessment, expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the trial treatment? Is the time of onset of the AE compatible with a drug-induced effect?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors?

Relationship to Merck Product (continued)	**The following components are to be used to assess the relationship between Merck product (pembrolizumab) and the AE: (continued)**	
	Dechallenge	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponsor's product; or (3) the trial is a single-dose drug trial; or (4) Sponsor's product(s) is/are only used one time.)
	Rechallenge	Was the patient re-exposed to the Merck product in this trial? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial; or (3) Sponsor's product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN AE THAT WAS SERIOUS AND THAT MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE PATIENT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the CRF by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of Merck product relationship).**	
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.	
No, there is not a reasonable possibility of Merck product relationship	Patient did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR the AE is more likely explained by another cause than the Merck product. (Also entered for a patient with overdose without an associated AE.)	

7.2.5 Sponsor Responsibility for Reporting Adverse Events

All AEs will be reported to regulatory authorities, the IRB, the FDA (via the Houston Methodist Research Institute Office of Regulatory Affairs & Translational Management), and investigators in accordance with all applicable laws and regulations.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan Summary

The safety/toxicity profile of the treatment will be assessed through summaries of AEs, SAEs, AEs leading to treatment discontinuation, and treatment-related death. The safety analysis will report the frequency of all AEs and laboratory abnormalities as well as the frequency of dose interruptions and toxicity-related treatment discontinuation. Toxicity rates will be presented using the worst NCI CTCAE grade per patient. All analyses will be conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The power calculation presented was accomplished using nQuery Advisor 7.0 (Statistical Solutions Ltd., Cork, Ireland).

Assuming a nominal pCR rate of no more than 5% for the standard of care and a single stage design, a sample of 30 anthracycline-resistant TNBC patients achieves 82.1% power to detect an improvement from 5% to 22% using a one-sided Fisher's exact test at a significance level of 0.05. An initial cohort of 6 patients will be followed through the first cycle. If no more than 2 of 6 patients experience grade 3/4 adverse event (AE) that does not resolve within 3 days, then the study will be fully opened with toxicity monitored after the first 6 patients based on a beta-binomial model, assuming a priori that the probability of grade 3/4 AE, p, is distributed beta(1, 1). Study accrual will be suspended and the safety profile of the therapy will be reviewed if $P_r(p > .25 \mid \text{data}) > 0.90$. Study stopping criterion will include any patient experiencing a grade 5 adverse event (death related to AE). Any death that occurs within 30 days of receiving the study products and any death that is possibly related to the administration of the study products will trigger study suspension to allow for a comprehensive safety review before enrolling additional subjects. This set of stopping rules yields the following stopping bounds where the numerator represents the number of events needed to suspend accrual and the denominator represents the number treated at that point in the study: 3/6, 4/7, 5/10, 6/13, 7/16, 8/19, 9/23, 10/26, and 11/29. The operating characteristics for this set of safety stopping rules are presented in the Table below. Scenario 2 of the operating characteristics table indicates that the probability of stopping the study early for safety is very low when the therapy has a favorable safety profile; that is, the study has only a 2.1% chance of stopping early when the true grade 3/4 AE rate is 10%. The study has an 98.0% chance of stopping for safety if the true grade 3/4 AE rate is 50% and on average would not last past the initial cohort of patients

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Table 8. Operating characteristics for safety monitoring rule.

Scenario	True Probability of Toxicity	Probability of Stopping Early	Median # of Patients (25%, 75%)
1	0.05	0.002	30 (30, 30)
2	0.10	0.021	30 (30, 30)
3	0.20	0.177	30 (30, 30)
4	0.35	0.722	12 (6, 30)
5	0.50	0.980	6 (6, 9)
6	0.60	> 0.999	6 (6, 6)

At the end of the study, the safety profile of the study will be summarized and reported along with the demographics of the study cohort. The pCR rate will be estimated and the associated 95% Wilson score confidence intervals (CI) will be reported as well as comparing the observed pCR rate to the nominal rate of 5%. With 30 patients, if 7 patients experience pCR, the estimate rate will be 23.3% with a CI width of 29.1 percentage points (11.8%, 40.9%) and the one-sided test as compared to the nominal rate of 5% would have a p-value of 0.0006. The Bayesian portion of the safety stopping rule was obtained using Multc99 software developed by the Biostatistics Department of the University of Texas M. D. Anderson Cancer Center. All analyses will be conducted using SAS 9.4 [SAS Institute Inc., Cary, NC, USA].

9.0 LABELING, PACKAGING, STORAGE, AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

The investigator or qualified designee shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and usage of investigational products in accordance with the protocol and any applicable laws and regulations.

Pembrolizumab will be provided by Merck as summarized in Table 9.

Table 9: Product Description

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

IL-12 is a substance that is normally made by certain immune cells in the body. IL-12 stimulates T cells, a special type of immune cell in the blood, to respond to threats to your body like cancer. IL-12 gene therapy works by inserting the gene that makes IL-12 into the cancer

cells. A safe virus known as an adenovirus (ADV) is used as the carrier to insert the IL-12 gene into the cancer cells. Once the adenovirus carrying the IL-12 gene (ADV/IL-12) is inserted into the cancer cells, the cancer cells can make IL-12. IL-12 stimulates T cells to attack and destroy the cancer cells.

The hypothesis of this study is that ADV/IL-12 will dramatically reshape the immune environment around the tumor and thus increase the therapeutic effect of pembrolizumab for the treatment of TNBC. Preliminary, pre-clinical experiments support the effectiveness of this combination therapy.

9.2 Packaging and Labeling Information
Supplies will be labeled in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure
This trial is open label; therefore, the patient, investigator, trial site personnel, Sponsor, and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text.

9.4 Storage and Handling Requirements
Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

9.5 Returns and Reconciliation
The investigator or qualified designee is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the patients, and the amount remaining at the conclusion of the trial.

Upon completion or termination of the trial, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures and that appropriate records of disposal are kept.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Compliance with Trial Registration and Results Posting Requirements
Under the terms of the FDA Modernization Act and FDA Amendments Act, the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow patients to identify potentially appropriate trials for their disease

conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.2 Data Management

CRFs will be designed and utilized to capture all patient data. An electronic database will be designed to store patient CRFs. Data quality control will be performed regularly by the research coordinator/research nurse to ensure timely, accurate, and complete patient data collection. Queries will be generated and resolved prior to the generation of interim and final summary reports.

11.0 ETHICAL CONSIDERATIONS

Compliance with laws and regulations. This trial will be conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki. The trial will comply with U.S. FDA regulations and applicable local, state, and federal laws.

IRB. The protocol and informed consent form will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any patient is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the trial. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented patients need to be re-consented.

Informed Consent. The consent form will be IRB approved and the patient will be asked to read and review the document. The investigator will explain the research trial to the patient and answer any questions that may arise. All patients will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the trial and of their rights as research participants. Patients will have the opportunity to carefully review the written consent form and ask questions prior to signing. Patients should have the opportunity to discuss the trial with their surrogates or think about it prior to agreeing to participate. The patient will sign the informed consent document prior to any procedures being done specifically for the trial. Patients may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the patients for their records. The rights and welfare of the patients will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this trial.

Confidentiality. The Sponsor maintains confidentiality standards by coding each patient enrolled in the trial through assignment of a unique patient identification number. Patient medical information obtained by this trial is confidential and may be disclosed to third parties only as permitted by the informed consent form signed by the patient, unless permitted or required by law. Data generated by this trial must be available for inspection upon request by representatives of the U.S. FDA and other national and local health authorities and the IRB.

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13.0 APPENDICES

Appendix 1: Common Terminology Criteria for Adverse Events v5.0

The descriptions and grading scales found in the revised NCI CTCAE version 4.03 will be utilized for AE reporting.

(https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf)

Appendix 2: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
Oken MM, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-55.	

Appendix 3: Contraceptive Guidance and Pregnancy Testing

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy

  NOTE: Documentation can come from the site personnel's review of the patient's medical records, medical examination, or medical history interview.

- Postmenopausal female
  - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
  - Females on HRT and whose menopausal status is in doubt will be required to use one of the nonhormonal highly effective contraception methods if they wish to continue their HRT during the trial. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before trial enrollment.

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in Table below during the protocol-defined timeframe in Section 5.1.1.

Highly Effective Contraception Methods

Highly Effective Contraceptive Methods That Are User Dependent[a] *Failure rate of <1% per year when used consistently and correctly.*
● Combined (estrogen and progestogen-containing) hormonal contraception[b] ○ Oral ○ Intravaginal ○ Transdermal ○ Injectable
● Progestogen-only hormonal contraception[b] ○ Oral ○ Injectable
Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
● Progestogen-only contraceptive implant[b] ● Intrauterine system ● Intrauterine devise ● Bilateral tubal occlusion
● **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
● **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the trial treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the trial and the preferred and usual lifestyle of the patient.
NOTE:

Use should be consistent with local regulations regarding the use of contraceptive methods for patients of clinical studies. a) Typical use failure rates are lower than perfect-use failure rates (i.e., when used consistently and correctly). b) If locally required, in accordance with Clinical Trial Facilitation Group guidelines, acceptable hormonal contraceptives are limited to those that inhibit ovulation.

WOCBP should only be included after a negative serum β-HCG pregnancy test.

Pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected.

Appendix 4: New York Heart Association Functional Classifications

Class	Description
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on physical activity without discomfort. Symptoms of cardiac insufficiency or of angina syndrome may be present at rest. If any physical activity is undertaken, discomfort is increased.

Appendix 5: Inhibitors and Inducers of CYP3A4 and CYP2D6

- Strong CYP3A inhibitors: itraconazole, telithromycin, clarithromycin, protease inhibitors boosted with ritonavir or cobicistat, indinavir, saquinavir, nelfinavir, boceprevir, telaprevir

- Moderate CYP3A inhibitors: ciprofloxacin, erythromycin, diltiazem, fluconazole, verapamil

- Strong CYP3A inducers: phenobarbital, enzalutamide, phenytoin, rifampicin, rifabutin, rifapentine, carbamazepine, nevirapine, St. John's Wort

- Moderate CYP3A inducers: bosentan, efavirenz, modafinil

- Strong CYP2D6 inhibitors: bupropion, fluoxetine, paroxetine, quinidine, quinine, cinacalcet, ritonavir

- Moderate CYP2D6 inhibitors: terbinafine, sertraline, duloxetine

- Strong CYP2D6 inducers:

- Moderate CYP2D6 inducers: dexamethasone, rifampicin