City of Hope National Medical Center
1500 E. Duarte Road
Duarte, CA 91010

TITLE: A Phase 2 Clinical Trial of the Combination of Pembrolizumab and Selective Androgen Receptor Modulator (SARM) GTx-024 in Patients with Metastatic Androgen Receptor (AR) Positive Triple Negative Breast cancer (TNBC)

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SPONSOR/IND NUMBER: City of Hope/ 132754
DISEASE SITE: Breast
STAGE (if applicable): IV
MODALITY: Immunotherapy and Selective Androgen Receptor Modulator
PHASE/TYPE: Phase 2 with Safety lead-in

PRINCIPAL INVESTIGATOR:

CO-INVESTIGATOR(S):

PARTICIPATING CLINICIANS:

BIO-STATISTICIAN:

RESEARCH STAFF::

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Clinical Trial Protocol

A Phase 2 Clinical Trial of the Combination of Pembrolizumab and Selective Androgen Receptor Modulator (SARM) GTX-024 in Patients with Metastatic Androgen Receptor (AR) Positive Triple Negative Breast cancer (TNBC)

Version Date:	7/3/19
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City of Hope #:	16131
Agents:	Pembrolizumab (MK-3475) GTX-024 (SARM)
IND #:	132754
Sponsor:	City of Hope
Industry Partner:	Merck & Co. GTx Inc.
Funding Support:	Merck & Co.
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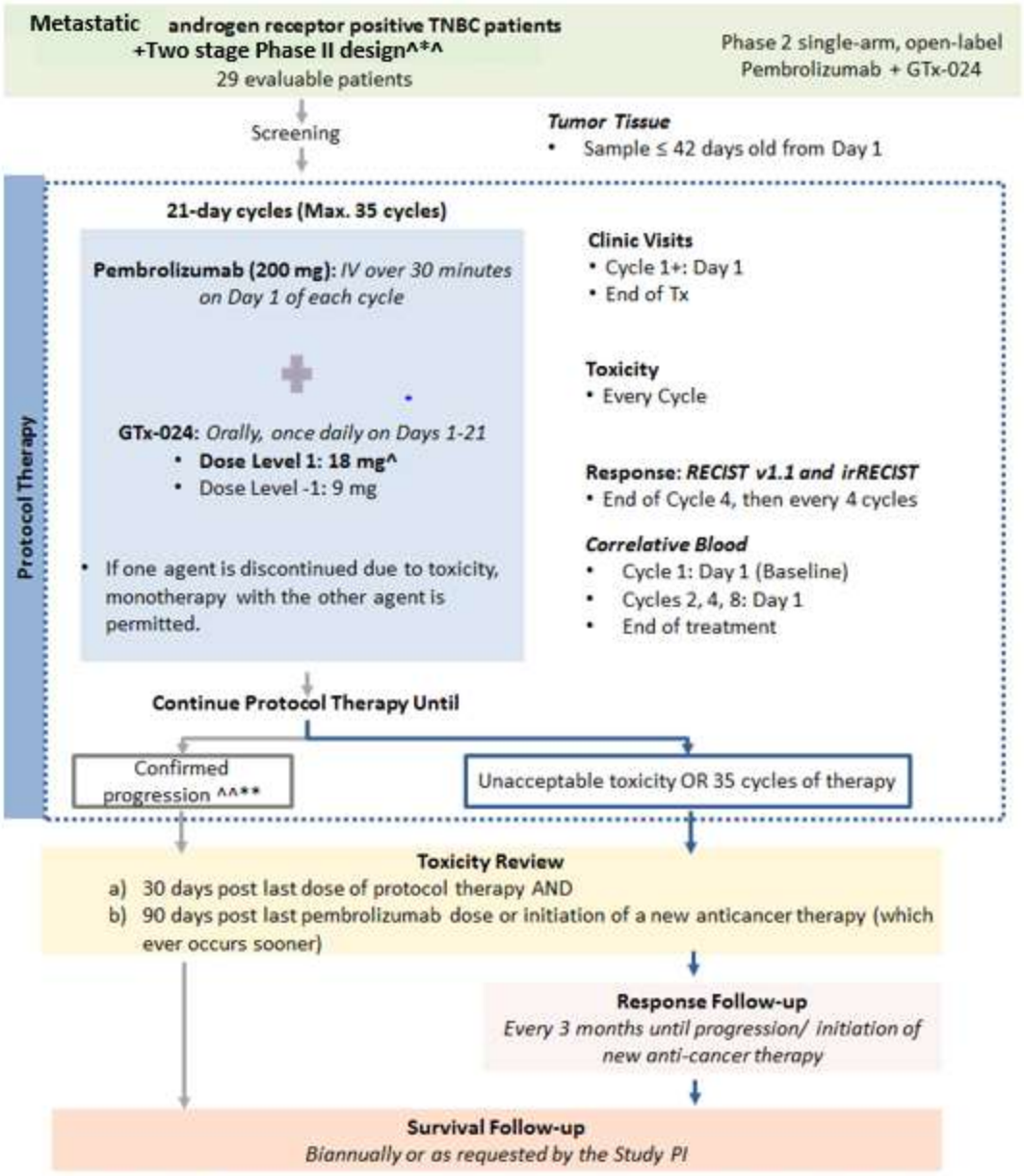
EXPERIMENTAL DESIGN SCHEMA



^ A 3 at-risk design will be utilized for the first 6 patients to assess toxicity for the combination regimen. If Dose Level 1 is not tolerable, then Dose Level -1 will be tested as the starting dose.

^*^ Up to a total of 29 patients pending review of the first stage (see statistical section)

^^**Participants with confirmed radiographic progression per RECIST v1.1 who are clinically stable but do not meet irRECIST criteria for progression will continue to receive protocol therapy following consultation of the Study PI.

PROTOCOL SYNOPSIS

Protocol Title
A Phase 2 Clinical Trial of the Combination of Pembrolizumab and Selective Androgen Receptor Modulator (SARM) GTX-024 in Patients with Metastatic Androgen Receptor (AR) Positive Triple Negative Breast cancer (TNBC)

Study Detail	
Indication(s)	Androgen receptor (AR) positive metastatic triple negative breast cancer (TNBC)
Phase	2
Number of participants	29 evaluable
Estimated Accrual Duration	18 months
Estimated Duration of the Study	Approximately 36 months
Participating Sites	• City of Hope (Duarte), CA •
Study Agents	Pembrolizumab - (Merck & Co.) GTX-024 - (GTx Inc.)
Brief Protocol Title for the Lay Public	Pembrolizumab and GTx-024 in AR positive (+) for Metastatic Triple Negative Breast Cancer.
Sponsor	City of Hope
Industry Partners	Merck & Co. GTx Inc.

Rationale for this Study

Triple negative breast cancer (TNBC) accounts for 15-20% of all breast cancers. Due to the lack of specific markers for effective targeted therapy, chemotherapy is the only therapeutic option for TNBC in the metastatic setting. Thus, novel targeted therapies are desperately needed for this devastating disease. One of the potential therapeutic targets is the androgen receptor (AR). In TNBC, up to 30% of cancers may express AR. In mRNA-based sub-classification of TNBC, luminal AR subtype accounts for approximately 10% of TNBC. Efficacy of AR targeted therapy such as enzalutamide was observed in patients with AR + TNBC.

As a nonsteroidal selective androgen receptor modulator (SARM), GTx-024 demonstrates selective anabolic activity, weak anti-progestin activity and cannot be converted to estrogenic metabolites. The ability of GTx-024 to act as an AR agonist and a weak progesterone receptor antagonist may provide multiple mechanisms to inhibit breast cancer cell growth and by decreasing bone turnover, prevent bone metastases and skeletal related events in women with metastatic breast cancer. Currently there are two phase II clinical trials evaluating the clinical benefit of GTx-024 and the importance of the AR as a therapeutic target in women with ER positive metastatic breast cancer who have responded to previous hormonal therapy and in women with AR +, triple negative breast cancer (AR+ TNBC).

Pembrolizumab is a highly selective humanized monoclonal antibody of the IgG4/kappa isotype that is believed to block the interaction between PD-2 and its ligands, PD-L1 and PD-L2. Pembrolizumab demonstrated sustained tumor regression with a response rate of approximately 24% in melanoma. Single agent pembrolizumab has a reported response rate in a cohort of 27 selected PD-L1+ TNBC patients of 19% [46], with the 5 responses were seen in patients with 8, 6, 6, 3, 1 lines of prior therapy for metastatic disease. ~47% had 3+ lines in that study. In a subsequent study presented after the initiation of this study, a response rate of 5% was observed in 170 mTNBC patients , 44% with 3+ lines and no previously untreated patients. The 5% RR was consistent between PD-L1 positive and negative patients [67]. The combined RR for the two studies is approximately 7%, and the upper confidence interval for the larger study is 9%, so we have modified the discouraging response rate to reflect the more recent and larger study and selected 9% for our updated discouraging response rate of the combination (see statistical considerations).

Immunotherapy and AR targeted therapy representpromising therapeutic strategies for TNBC. Despite the encouraging finding of recent phase II trials of AR targeted therapy such as enzalutamide, single agent AR targeted therapy is not adequate to induce durable clinical benefit. The complementary modes of action and low potential of overlapping toxicity profiles of these two modalities make the combination therapy of AR targeted agent and anti-PD-1 therapy potentially promising. This study is designed to test the efficacy of combining SARM GTx-024 with pembrolizumab in patients with metastatic AR+ TNBC.

Study Design

Following a successful lead-in, we originally proposed an open-label Simon's MiniMax two-stage Phase 2 study for metastatic Androgen Receptor (AR) positive Triple Negative Breast Cancer (TNBC). Eligible participants will receive pembrolizumab in combination with GTx-024. This has been modified as noted in the statistical considerations due to the need to incorporate the more recent literature on the single agent Pembrolizumab response rate.

A 3 at risk design will be utilized for the first 6 participants to assess toxicity for the combination therapy with pembrolizumab and GTx-024. The starting dose will be Dose Level 1. If there are DLTs at Dose Level 1, attributed to protocol therapy then Dose level -1 will be tested as the starting dose.

	Maximum cycles = 35 cycles		
	Dose Levels	**Pembrolizumab** (IV infusion over 30 minutes; on Day 1 of each 21-day cycle)	**GTx-024** (Orally, once daily on Days 1-21 of each 21-day cycle)
	Dose Level -1	200 mg	9 mg
Initial starting dose level	**Dose Level 1**	**200 mg**	**18 mg**

Subsequent participants enrolled on to the study will be treated at the tolerable dose defined during the safety lead-in.

Participants will receive up to 35 cycles of treatment. If one agent is discontinued due to toxicity, then the participant may continue to receive single agent protocol therapy.

Objectives

Primary Objective

- To evaluate the safety/tolerability of the combination regimen
- To determine the response rate (CR or PR via RECIST 1.1) of the combination of pembrolizumab with GTx-024 in patients with advanced AR + TNBC

Secondary Objectives

- To evaluate clinical outcomes by RECIST 1.1 including clinical benefit rate (CBR) at 24 weeks, progression free-survival (PFS), duration of response (DOR), event free survival (EFS), time-to-treatment failure (TTF); and overall survival (OS);
- To evaluate the role of immune-related response criteria (irRECIST);
- To evaluate the association of AR by IHC and clinical response;

Exploratory Objectives

- To evaluate the association of an AR gene expression signature and clinical response:
- To evaluate genomic and phenotypic status of breast tumor
- To evaluate the effect of the combination therapy on peripheral blood circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA)
- To evaluate the effect of combination therapy tumor-derived exosomes (TEX) and TEX associated immune biomarkers
- Immune Correlatives:
  - To evaluate pre-treatment programmed death ligand 1 (PD-L1) and tumor infiltrating lymphocytes (TILs) as a predictor of response to combination therapy;

- To evaluate specific TIL subsets (e.g. CD4,CD8, Treg distribution) and other immunological correlatives (e.g. TCR repertoire analysis) as possible predictors of response;
- To evaluate change in TILs as a result of the combination therapy;
- To evaluate peripheral blood, immune biomarkers

Endpoints

Primary endpoints

- Toxicity will be assessed using CTCAE v 4.0
- DLT **is defined** as any of the following that occur during the **first cycle** that are attributed as possibly, probably, or definitely related to protocol therapy for the safety lead-in:
  - Delay > 14 days in initiating Cycle 2
  - Planned dose of GTx-024 is < 75% during Cycle 1
  - Death not clearly due to underlying disease or extraneous causes

  Hematologic
  - Grade ≥ 3 thrombocytopenia with bleeding requiring transfusion
  - Grade 4 thrombocytopenia
  - Grade 4 neutropenia that persists more than 5 days
  - Grade 3 or 4 febrile neutropenia
    - Grade 3 febrile neutropenia: defined as single temperature of >38.3°C (101°F) or a sustained temperature of ≥38°C (100.4°F) for more than one hour

  Non-Hematologic
  - Any ≥ Grade 3 non-hematologic toxicity with the **following exceptions**:
    - Grade 3 rash that resolves to ≤ Grade 1 within 14 days with appropriate supportive therapy
    - Grade 3 myalgia, fatigue, or constipation that resolves to ≤ Grade 1 within 72 hours
    - Grade 3 electrolytes abnormality that lasts < 72 hours that is not clinically complicated, and resolves spontaneously or responds to conventional medical interventions
    - Grade 3 nausea, vomiting, or diarrhea that lasts < 72 hours with adequate antiemetic and other supportive care.
    - Grade 3 hypersensitivity and injection site reactions
    - *For patients with liver metastases who began treatment with Grade ≤ 2 hepatic transaminase*: transaminase level ≥ 10x ULN lasting ≤ 7 days
  - Hy's law: concurrent ALT/AST > 3.0 x ULN AND total bilirubin > 2.0 x ULN without initial findings of cholestasis and in the absence of any alternative cause
- Response will be assessed using RECIST v 1.1

Secondary endpoints

- The following efficacy endpoints will be assessed using RECIST v 1.1

Endpoint	Patients	Definition
Progression-free survival (PFS)	All patients	Time to disease progression/ relapse or death as a result of any cause
Clinical benefit rate (CBR)	All patients	Lack of progression at 24 weeks
Duration of response (DOR)	In CR or PR patients	Time to progression or death
Overall survival (OS)	All patients	Time to death as a result of any cause
Time to treatment failure (TTF)	All patients	Time to treatment termination for any reason (progression, toxicity, death, patient preference)
Event-free survival (EFS)	All patients	Failure of treatment or death as a result of any cause

- Response and clinical benefit will be assessed using irRECIST

Statistical Considerations

A Simon's MiniMax two-stage Phase 2 design was the original design based on the initial reported response rate of 19% with single agent pembrolizumab [46]. However, this was based on only 27 patients. Subsequently, a study of 170 patients observed a response rate of only 5%.[67] While the original protocol design stated a 19% response rate (the single agent response rate in the first reported study) as the discouraging rate for the combination and required >2/15 responses in the first stage to continue, these rules must be changed to reflect the data from the larger and more recent study that showed a response rate of only 5% for single agent Pembrolizumab (with that same response rate applying to both PD-L1 positive and negative tumors). The later study had an upper 95% confidence limit of 9% RR, and the combined data from the two studies would result in a response rate of approximately 7%. As a result, we have determined that 9% would be a more appropriate discouraging response rate given the more recent and larger report on single agent Pembrolizumab and we consider a promising response rate a 20% increment, or a 29% response rate. With the updated information, we will continue to accrue if at least 2/15 patients response (representing a RR of at least 13.3%). We will maintain the total sample-size at 29 patients. Considering the impact of mid-design changes on hypothesis testing, and the variability of outcomes from the two relevant historical studies, the updated design is based on having at least 2 out of the first 15 patients with a response, and then adding an additional 14 patients to better estimate the response rate of the combination. With 2+/15 with a response to continue, this is better than the upper 95% CI for the response rate reported in the larger and more recent study, and satisfies the rule for continuing based on the SWOG planned versus attained design of Dahlberg (Green SJ and Dahlberg S (1992). Planned Versus Attained Design in Phase II Clinical Trials. *Statistics in Medicine* 11:853-862) for >1/15 responses based on a type I error of 5% and power of 85% for distinguishing between a 9% and a 29% response rate. With 29 total patients the SE of the response rate will be 9% or less.

All eligible patients who start treatment will be considered in the calculation of the response rate.

While both drugs are well-tolerated with non-overlapping toxicities, the initial 6 patients will be followed in a safety lead-in to observe if a dose adjustment is appropriate. If in the first 6 patients we observe 2 or more patients with dose limiting toxicities (DLTs) in the first cycle, this will mandate a reduction in the starting dose for all subsequent patients. If dose level -1 has 2 patients with DLTs in the first 6 patients, the study will hold accrual.

During the safety lead-in, we will permit only 3 patients to be at risk for first cycle toxicities at any one time.

With approximately 29 patients, we expect to have to obtain at least 12 patients with pre- and post- treatment biopsy material adequate for evaluation for AR pathway activity (AR by IHC and gene expression array) and immune profiling. The correlative studies will be used to potentially refine patient selection for future studies, and understand the role of AR pathway activities and immune changes on the activity of the combination of pembrolizumab plus GTx-024. These correlative studies are considered exploratory in the context of this limited Phase II study; however, 12 samples provide 80% power to detect an effective size of 0.8 (80% of the standard deviation), with a one-sided type I error of 5%.

Safety/tolerability

The safety and tolerability of the combination of pembrolizumab and SARM GTx-024 will be evaluated via a safety lead-in. In addition, the CBR will be evaluated at 32 weeks (by RECIST 1.1), the PFS will be evaluated, and the duration of response (time from documentation of tumor response to disease progression or death) will be evaluated.

Abbreviated Eligibility Criteria

Inclusion Criteria

- Adult patients with AR+ TNBC
- Measurable disease per RECIST v 1.1

- ANC ≥ 1,500/mm^3
- Platelets ≥ 100,000/mm^3
- Total serum bilirubin ≤ 1.5 x ULN
- Albumin ≥ 2.5 mg/dL
- AST and ALT ≤ 2.5 x ULN OR ≤ 5.0 x ULN if liver metastases present
- Creatinine clearance of ≥ 60 mL/min or serum creatinine ≤ 1.5 x ULN

Exclusion Criteria

- Any of the following prior therapies
  - Anti-PD-1, PD Ligand-1 (PD-L1), PD Ligand-2 (PD-L2) agent or an antibody targeting other immuno-regulatory receptors or mechanisms
  - AR targeted agents (including GTx-024, enzalutamide or other AR targeted therapies).
- Radiotherapy within 14 days prior to first dose of study treatment
- Investigational agent within 21 days of study entry
- Hormone replacement therapies within 14 days prior to the first dose of study treatment (estrogens, megesterol acetate)
- Live-virus vaccination within 30 days of planned treatment start.
- Systemic cytotoxic chemotherapy, antineoplastic biologic therapy, or major surgery within 3 weeks of the first dose of trial medication.
- Currently taking or have previously taken testosterone or testosterone-like agents (methyltestosterone, oxandrolone, oxymetholone, danazol, fluoxymesterone, dehydroepiandrosterone, androstenedione) other androgenic compounds or anti-androgens
- Chronic systemic steroid therapy or on any other form of immunosuppressive medication.
- History of pneumonitis (non-infectious) that requires steroids or current pneumonitis

Investigational Product Dosage and Administration

Pembrolizumab 200mg iv every 3 weeks iv and GTx-024 18 mg oral daily. The dose of GTx-024 may be reduced to 9 mg orally daily.

Clinical Observations and Tests to be Performed

- Medical history and physical exam
- Safety assessments (CBCs with differential, comprehensive chemistry panel, and thyroid function)
- CT/ MRI scans
- Correlative tumor tissue and blood sample

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ABBREVIATIONS

AE	Adverse Event
ANC	Absolute Neutrophil Count
AR	Androgen Receptor
CBR	Clinical Benefit Rate
CFR	Code of Federal Regulations
COH	City of Hope
CR	Complete Response
CRA	Clinical Research Associate
CRF	Case Report Form
CTC	Circulating tumor cells
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	Circulating tumor DNA
CTEP	Cancer Therapy Evaluation Program
CTMS	Clinical Trial Management System
DCC	Data Coordinating Center
DLT	Dose Limiting Toxicity
DOR	Duration of Response
DSMC	Data Safety Monitoring Committee
EFS	Event-Free Survival
EOT	End of Treatment
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator Brochure
ICF	Informed Consent Form
IDS	Investigational Drug Services
IHC	Immunohistochemistry
IND	Investigational New Drug
IRB	Institutional Review Board
irRECIST	Immune related Response Evaluation Criteria in Solid Tumors
iv	Intravenous
MTD	Maximum Tolerated Dose
OS	Overall Survival
PD	Progressive Disease
PFS	Progression Free Survival
PI	Principal Investigator
PMT	Protocol Monitoring Team
PR	Partial Response
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious Adverse Event
SARM	Selective Androgen Receptor Modulator
SD	Stable Disease
TNBC	Triple Negative Breast Cancer
TTF	Time-to-Treatment Failure
WHO	World Health Organization

1.0 OBJECTIVES

1.1 Primary Objective

- To evaluate the safety/tolerability of the combination regimen
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1.2 Secondary Objectives

- To evaluate clinical outcomes by RECIST 1.1 including clinical benefit rate (CBR) at 24 weeks, progression free-survival (PFS), duration of response (DOR), event free survival (EFS), time-to-treatment failure (TTF); and overall survival (OS);
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  - To evaluate change in TILs as a result of the combination therapy;
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2.0 BACKGROUND

2.1 Introduction/Rationale for Development

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on MK-3475 (pembrolizumab) and GTx-024.

2.1.1 Triple negative breast cancer (TNBC)

TNBC accounts for 15–20% of all breast cancer and is characterized by a lack of expression of estrogen receptor (ER), progesterone receptor (PR), or HER2. TNBC occurs more frequently in younger patients (< 50 years of age) and generally shows a more aggressive behavior[1]. Median survival for patients with advanced TNBC is 12 months, which is much shorter than the survival for patients with other advanced breast cancer subtypes. Due to the lack of specific markers for effective targeted therapy, chemotherapy is the only therapeutic option for TNBC in the adjuvant or metastatic setting. Thus, novel targeted therapies are desperately needed for this devastating disease.

2.1.2 AR positive breast cancer

AR is the most abundantly expressed steroid receptor in breast cancer with up to 95% of ER+ breast cancers expressing AR. In TNBC, 12-55% of cancers express AR depending on the study [2, 3]. Across all subtypes of BC, AR expression is associated with a better overall survival and disease-free survival irrespective of co-expression of ERα in breast cancer [2, 4]. Emerging evidence has shown AR may serve as a therapeutic target for this subset of TNBC [4-6]. A recent phase II trial studying single agent enzalutamide in AR+ TNBC has demonstrated encouraging clinical benefit rate of 35% at 16 weeks follow up, with a median PFS of 14.7 weeks [7]. A novel gene expression array may predict sensitivity to enzalutamide treatment[3].

2.1.3 Selective Androgen Receptor Modulator (SARM)

GTx-024 is an oral nonsteroidal selective androgen receptor modulator (SARM) that is being developed for clinical use because of its selectivity for anabolic activity with minimal androgenic activity (i.e., tissue selectivity). GTx binds to the AR with similar affinity as testosterone. However, GTx-024 is a nonsteroidal ligand that does not bind or activate ER or progesterone receptor and, unlike testosterone and other steroidal androgens, cannot be aromatized to estrogenic metabolites. The underlying hypothesis regarding the selectivity of GTx-024 is that this nonsteroidal molecule induces slight conformational changes in the AR upon binding. The altered conformational change in AR changes the interaction of AR with specific coactivator and corepressor proteins that exist in different tissues, thereby resulting in a different mix of genes being turned on and off and conferring more selective anabolic activities. Differences in intracellular signaling pathways (i.e., non-genomic effects) and/or interactions with steroid biosynthetic enzymes (e.g., 5 alpha(α)-reductase) between GTx-024 and the steroids may also contribute to differences in selectivity. GTx-024 is an anabolic agonist in muscle and bone while acting neutral or antagonistic in androgenic tissues like the skin. Preclinical data coupled with the early clinical success of androgens in breast cancer, support the clinical evaluation of GTx-024 and SARMs as novel targeted therapies to treat AR-positive breast cancer. There are two phase II clinical trials currently being conducted to test the efficacy of GTx-024 in AR+ advanced breast cancer (TNBC: NCT02368691; ER+HER2-BC: NCT02463032).

2.1.4 *Rationale of GTx-024 dosing selection*

GTx-024 has been evaluated in 21 completed and ongoing clinical studies enrolling over 1,500 total subjects. GTx-024 has been generally well-tolerated, including single doses up to 100 mg and multiple doses up to 30 mg once daily for up to 14 days. In longer studies, GTx-024 has also been generally well tolerated, including 1, 3, and 9 mg daily doses for up to 184 days (GTx IB). Previous clinical studies demonstrated that daily doses up to 30 mg of GTx-024 were well tolerated in healthy male volunteers. Both 10 mg and 30 mg daily doses were for up to 14 days. Elevated alanine transaminase (ALT) (any elevation outside upper limit of normal [ULN]) was the most common adverse event (AE) experienced. None of the subjects in the 10 mg dose group were discontinued from the study due to ALT elevations. In the 30 mg dose group, six subjects experienced ALT increases above two times the ULN.

GTx-024 3 mg given daily was evaluated in two completed Phase 3 trials, in over 600 subjects, for the prevention and treatment of muscle wasting (cachexia) in subjects with advanced non-small cell lung cancer receiving chemotherapy. GTx-024 3 mg increased lean body mass in both studies and was safe and well tolerated when dosed for up to 168 days. Subjects in the GTx-024 and placebo groups experienced similar AEs and these AEs were consistent with the background chemotherapy regimen.

Although GTx-024 3 mg was chosen for its anabolic activity in muscle for the completed Phase 3 study, a dose of 9 mg once daily was selected for hormonal therapy in the ongoing Phase 2 trial in ER+ and AR+ metastatic breast cancer in order to achieve a higher exposure that is both safe and more likely to be efficacious in women with advanced breast cancer. Seven out of twenty-two subjects with advanced, heavily pretreated (hormonal therapy, radiation, and chemotherapy) breast cancer demonstrated clinical benefit (CB) (stable disease [SD]) at 6 months. In one subject with SD (by Response Evaluation Criteria in Solid Tumors [RECIST], Version 1.12), tumor regression of 27% was demonstrated. Consistent with the previous studies, GTx-024 remained safe and well tolerated.

In TNBC, doses of 15–20 mg per day should provide saturation of the AR potentially providing better efficacy as opposed to a lower dose with partial occupancy of the AR and absence of any progesterone receptor inhibitory effect. In TNBC patients, the 18 mg dose is preferred over a lower dose due to the aggressive phenotype of the disease and poor prognosis. Based on preclinical data, the 18 mg dose is more likely to saturate the AR and may lead to better clinical outcomes than a lower dose without receptor saturation or progesterone receptor inhibition. Based on the safety data collected to date in both preclinical and clinical settings, 18 mg dose will be safe and generally well tolerated. Currently, an ongoing Phase II study is evaluating the efficacy of GTx-024 at a dose of 18mg po daily in patients with AR+ TNBC (NCT02368691). However, in the event that a subject has a Grade 3 or greater toxicity, the 18 mg dose may be reduced to 9 mg until the AE resolves or for the remainder of treatment based on the Investigator's discretion. The 9 mg dose has been previously studied in postmenopausal women with metastatic breast cancer and was safe and well tolerated.

2.1.5 PD-1 checkpoint inhibition and cancer treatment

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [8]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [9-20]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors [17, 21-26]. One of the major causes of immune tolerance is expression of inhibitory T cell co-receptors such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death1 (PD-1). Strategies reversing immune tolerance to promote anti-tumor immunity have been proven successful. These include anti-

CTLA-4 monoclonal antibody ipilimumab, which improves overall survival in melanoma [27, 28]. An alternative strategy is blocking PD-1.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control [29]. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structures of murine PD-1 alone [30] and in complex with its ligands were first resolved [31, 32]and more recently the NMR-based structure of the human PD-1 extracellular region and analyses of its interactions with its ligands were also reported [33]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding, and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade [34]. The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins [35].

PD-1 has been shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells [36]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells [37] as well as subsets of macrophages[38] and dendritic cells [39]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types [40], including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor.

PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments [40]. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor [41]. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL) [42]. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention [43].

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2[44]. Pembrolizumab demonstrated sustained tumor regression with a response rate of approximately 24% in melanoma, with mainly grade 1 or 2 toxicities in patients regardless of previous ipilimumab use[45]. Single agent activity of pembrolizumab is currently been studied for HER2 positive breast cancer (NCT01848834). Single agent pembrolizumab has a reported response rate in a cohort of 27 selected metastatic PD-L1+ TNBC patients of 19% (ref 46), with the 5 responses seen in patients with 8, 6, 6, 3, 1 lines of prior therapy for metastatic disease. ~47% had 3+ lines in that study. In a subsequent study presented after the initiation of this protocol, a response rate of 5% was observed in 170 mTNBC patients , 44% with 3+ lines and no previously untreated patients. The 5% RR was

consistent between PD-L1 positive and negative patients (ref YYY). The combined RR for the two studies is approximately 7%, and the upper confidence interval for the larger study is 9%, so we have modified the discouraging response rate to reflect the more recent and larger study and selected 9% for our updated discouraging response rate of the combination (see statistical considerations).

2.1.5.1 Rationale for including subjects with PD-L1 negative tumors

The KN 012 TNBC proof-of-concept data was obtained in subjects with PD-L1 positive tumors (PD-L1 staining in ≥1% tumor cells or in stroma) [46]. No data was originally available on the performance of pembrolizumab in breast cancer patients with PD-L1 negative tumors (i.e., PD-L1 staining in <1% tumor cells and no stromal staining), but KEYNOTE-86 Arm A suggests a 5% response independent of PD-L1 status in metastatic TNBC. Combined with thelimited data using PD-L1 expression as a biomarker predicting response to pembrolizumab, and patients with PD-L1 negative tumors may benefit from pembrolizumab, PD-L1 expression will not be used for patient selection. Instead, analysis of PD-L1 expression will be included as a correlative study.

2.1.5.2 Rationale for Dose Selection of Pembrolizumab

The dose of pembrolizumab planned to be studied in this trial is 200 mg Q3W. The dose recently approved in the United States for treatment of melanoma subjects is 2 mg/kg Q3W. Although the dose of pembrolizumab studied in KN 012, which established efficacy and safety in mTNBC, was 10 mg/kg Q2W, recent studies in other tumor types have indicated that 10 mg/kg Q2W and 200 mg Q3W are likely to be similar with regard to efficacy and tolerability.

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors [47]. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights.

MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher

dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

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

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage. The pembrolizumab dose of 200mg Q3W has formed a foundation for ongoing combination therapeutic protocols.

2.1.6 Rationale for studying immunotherapy in combination with AR targeted therapy in AR+ TNBC

Immunotherapy and AR targeted therapy represent the most promising therapeutic strategies for TNBC. Despite the encouraging finding of recent Phase 2 trials of AR targeted therapy such as enzalutamide, single agent AR targeted therapy is not adequate to induce durable clinical benefit. The complementary modes of action and low potential of overlapping toxicity profiles of these two modalities make the combination therapy of AR targeted agent and anti-PD-1 therapy potentially promising.

2.2 Preclinical Studies

2.2.1 Preclinical Data for Pembrolizumab

Refer to the Investigator's Brochure for detailed preclinical data.

2.2.1.1 PD-1 immune checkpoint inhibition

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T cells and ultimately leads to tumor rejection, either as a monotherapy or in combination with other treatment modalities[41, 48, 49]. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated antitumor responses in models of squamous cell carcinoma, pancreatic carcinoma, melanoma, acute myeloid leukemia and colorectal carcinoma [49-53]. In such studies, tumor infiltration by CD8+ T cells and increased IFN-γ, granzyme B and perforin expression were observed, indicating that the mechanism underlying the antitumor activity of PD-1 checkpoint inhibition involved local infiltration and activation of effector T cell function in vivo[51]. Experiments have confirmed the in vivo efficacy of anti-mouse PD-1 antibody as a monotherapy, as well as in combination with chemotherapy, in syngeneic mouse tumor models (see pembrolizumab IB).

2.2.2 Preclinical Data for GTx-024 in breast cancer

The effects of GTx-024 on in vitro proliferation of breast cancer cell lines were examined.MDA-MB-231 breast cancer cells and HCC-38 breast cancer cells stably transfected with the AR were treated with dihydrotestosterone (DHT), GTx-024, or the inactive R-isomer of GTx-024 in the presence and absence of bicalutamide, a known AR antagonist. DHT and GTx-024 inhibited the in vitro proliferation of the breast cancer cell lines with potencies ranging from approximately 1 to 100 nM. These effects were inhibited

by co-treatment with bicalutamide and were not induced by the R-isomer of GTx-024, demonstrating dependence on the AR and the stereochemical configuration of the ligand.

The ability of GTx-024 to stimulate AR action and inhibit progesterone receptor action may provide a dual mechanism to inhibit breast cancer cell growth. MDA-MB-231-AR cells were implanted subcutaneously in nude mice and were treated orally (PO) with vehicle or 30 mg/kg/day GTx-027, a close structural analog of GTx-024[54]. Tumor growth was reduced significantly, with greater than 75% tumor growth inhibition observed, compared with vehicle-treated tumors. The intratumoral expression of genes and pathways that promote breast cancer development through its actions on the AR were also suppressed. Similar tumor suppressive activity was observed in patient-derived xenograft models of TNBC (unpublished data). These data, coupled with the early clinical success of androgens in breast cancer, support the clinical evaluation of GTx-024 and SARMs as a novel targeted therapy to treat AR-positive breast cancer.

2.3 Human Studies

2.3.1 Clinical Data for Pembrolizumab

Refer to the Investigator's Brochure for detailed clinical data.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab was recently approved in the US for the treatment of advanced, unresectable or metastatic malignant melanoma, and for use in melanoma subjects with disease progression after prior treatment with (a) ipilimumab or (b) a BRAF inhibitor, in the case of BRAF V600-mutant disease[55]. It is the first anti-PD-1 therapy to receive regulatory approval in the US, and is currently under regulatory review in the EU. Ongoing clinical trials of pembrolizumab are being conducted in advanced melanoma, non-small cell lung cancer, and a number of other advanced solid tumor indications and hematologic malignancies. For study details please refer to the IB.

Preliminary data from a study of pembrolizumab in PD-L1-enriched selected patients with triple negative stage IV breast cancer suggest 18.3% potentially lasting clinical activity [46], while a larger subsequent study showed a response rate of 5% in mTNBC, with the same response rate for PD-L1-positive and negative patients.[67] Currently there are no completed studies completed evaluating the efficacy of pembrolizumab in ER+ breast cancer. Most common adverse reactions (reported in ≥20% of patients) with: melanoma included fatigue, pruritus, rash, constipation, diarrhea, nausea, and decreased appetite; NSCLC included fatigue, decreased appetite, dyspnea and cough. In The P001 and P002 melanoma and lung subjects, the most commonly reported drug related adverse event across the pembrolizumab dosing regimens are fatigue (27.9%), pruritus (19.0%), rash (14.0%), diarrhoea (12.3%), arthralgia (11.5%), and nausea (10.2%) (Pembrolizumab IB).

2.3.1.1 Pembrolizumab Immune-Medicated Side Effects

The most frequent potential immune-mediated adverse reactions reported in patients treated with pembrolizumab were hypo/hyperthyroidism, pneumonitis, colitis, hepatitis, nephritis, and hypophysitis (Pembrolizumab IB). Refer to Table 6 for dose modification for this immune-mediated side effects.

Immune-Mediated Pneumonitis: Pneumonitis occurred in 32 (2.0%) of 1567 patients receiving KEYTRUDA in melanoma trials, including Grade 1 (0.8%), Grade 2 (0.8%), and Grade 3 (0.4%) pneumonitis. The median time to development of pneumonitis was 4.3 months (range: 2 days to 19.3 months). The median duration was 2.6 months (range: 2 days to 15.1 months). Twelve (38%) of the 32 patients received corticosteroids, with 9 of the 12 receiving high-dose systemic corticosteroids for a

median duration of 8 days (range: 1 day to 1.1 months) followed by a corticosteroid taper. Pneumonitis led to discontinuation of pembrolizumab in 9 (0.6%) patients. Pneumonitis completely resolved in 21 (66%) of the 32 patients.

Immune-Mediated Colitis: Colitis occurred in 31 (2.0%) of 1567 patients receiving pembrolizumab in melanoma trials, including Grade 2 (0.5%), Grade 3 (1.1%), and Grade 4 (0.1%) colitis. The median time to onset of colitis was 3.4 months (range: 10 days to 9.7 months). The median duration of colitis was 1.4 months (range: 1 day to 7.2 months). Twenty-one (68%) of the 31 patients received corticosteroids, all of whom required highdose systemic corticosteroids for a median duration of 6 days (range: 1 day to 5.3 months) followed by a 5 corticosteroid taper. Colitis led to discontinuation of pembrolizumab in 14 (0.9%) patients. Colitis resolved in 27 (87%) of the 31 patients. Colitis occurred in 4 (0.7%) of 550 patients with NSCLC, including Grade 2 (0.2%) or Grade 3 (0.4%) colitis in patients receiving pembrolizumab.

Immune-Mediated Hepatitis: Hepatitis occurred in 16 (1.0%) of 1567 patients with melanoma receiving pembrolizumab, including Grade 2 (0.1%), Grade 3 (0.7%), and Grade 4 (0.1%) hepatitis. The time to onset was 26 days (range: 8 days to 21.4 months). The median duration was 1.2 months (range: 8 days to 4.7 months). Eleven (69%) of the 16 patients received corticosteroids, with 10 of the 11 receiving high-dose systemic corticosteroids for a median duration of 5 days (range: 1 to 14 days) followed by a corticosteroid taper. Hepatitis led to discontinuation of pembrolizumab in 6 (0.4%) patients. Hepatitis resolved in 14 (88%) of the 16 patients.

Immune-Mediated Endocrinopathies: Hypophysitis occurred in 13 (0.8%) of 1567 patients with melanoma receiving pembrolizumab including Grade 2 (0.3%), Grade 3 (0.3%), and Grade 4 (0.1%) hypophysitis. The time to onset was 3.3 months (range: 1 day to 7.2 months). The median duration was 2.7 months (range: 12 days to 12.7 months). Twelve (92%) of the 13 patients received corticosteroids, with 4 of the 12 patients receiving high-dose systemic corticosteroids. Hypophysitis led to discontinuation of KEYTRUDA in 4 (0.3%) patients. Hypophysitis resolved in 7 (54%) of the 13 patients.

Immune-Mediated Thyroid Disorders:

- **Hyperthyroidism** occurred in 51 (3.3%) of 1567 patients with melanoma receiving pembrolizumab, including Grade 2 (0.6%) and Grade 3 (0.1%) hyperthyroidism. The median time to onset was 1.4 months (range: 1 day to 21.9 months). The median duration was 1.7 months (range: 1 day to 12.8 months). Hyperthyroidism resolved in 36 (71%) of the 51 patients.
- **Hypothyroidism** occurred in 127 (8.1%) of 1567 patients with melanoma receiving pembrolizumab, including Grade 3 (0.1%) hypothyroidism. The median time to onset of hypothyroidism was 3.3 months (range: 5 days to 18.9 months). The median duration was 5.4 months (range: 6 days to 24.3 months). Hypothyroidism resolved in 24 (19%) of the 127 patients.

2.3.2 Clinical Data for GTx-024

The clinical experience with GTx-024 comes from 21 Phase I, II and III clinical trials. The effect of food on the pharmacokinetics of GTx-024 3 mg is not clinically relevant. Therefore, GTx-024 may be administered without regard to food intake or the time of a meal.

GTx-024 has generally been well tolerated in clinical trials conducted to date. Certain AEs associated with GTx-024 may occur. The most commonly reported side effects for GTx-024 in the previous 21 clinical studies include: headache; back pain; diarrhea; pain in arm or leg; upset stomach; constipation; fatigue; dizziness; increases in liver enzymes; flu-like symptoms; anemia ; reduction in high density lipoproteins; visual disturbances. Dyspnea was observed more frequently in subjects that received 3 mg

GTx-024 (~13%) as compared to placebo (~7%) in two Phase III trials in patients with stage III and IV NSCLC receiving chemotherapy consisting of a platinum doublet chemotherapy with a taxane or non-taxane.

GTx-024 treatment has been generally well-tolerated, including 1 mg, 3 mg and 9 mg daily doses. The important potential risks identified for GTx-024 are:

- Dose-dependent, transient, asymptomatic increase in alanine aminotransferase (ALT) was observed in some of the Phase 1 and 2 studies. These increases were modest at doses up to 3 mg/day. Most of the subjects studied to date had ALT levels that remained within normal limits. ALT levels returned to normal with continued exposure to GTx-024 in most cases and, when dosing was not continued, levels returned to normal. In the Phase 3 clinical studies (N=651) in subjects with NSCLC receiving chemotherapy, 6 subjects receiving GTx-024 had an ALT lab values ≥ 5 times the ULN. None of these events were considered serious by the investigators, led to discontinuation of study drug or withdrawal of the subject from the study. No significant increases in total bilirubin, gamma-glutamyl transferase (GGT), alkaline phosphatase, or lactate dehydrogenase have been observed in patients with elevated ALT levels in the Phase 3 studies. There were no other consistent, clinically relevant, dose-related effects of GTx-024 on ECGs or vital sign measurements (blood pressure/heart rate).
- Dose-dependent reduction in high density lipoprotein (HDL) of 27-31% were observed at a 3 mg dose in healthy male volunteers and postmenopausal females. This change is consistent with the effects of other orally administered anabolic agents. The clinical significance of these changes is unknown. There were no other consistent, clinically relevant, dose-related effects of GTx-024 on ECGs or vital sign measurements (blood pressure/heart rate). No reductions in HDL were reported during the Phase 3 clinical studies in patients with NSCLC (N=326 receiving GTx-024 3 mg/day for up to 147 days).
- Dyspnea was observed more frequently in subjects that received GTx-024 3 mg/day (42 subjects, ~13%, 54 events reported) as compared to placebo (23 subjects, ~7%, 33 events reported) in two Phase 3 trials in patients with Stage III and IV NSCLC receiving chemotherapy consisting of a platinum doublet chemotherapy with a taxane (N=321) or non-taxane (N=330). Twelve of these events were serious, and were equally distributed among subjects receiving placebo and GTx-024 3 mg/day. There was only one other serious event of dyspnea for a subject administered GTx-024 1 mg in a prior study. Dyspnea was observed in 3 subjects in the Phase 2 breast cancer study (GTx-024 9 mg/day), but none of these events were serious.

In cancer trials, treatment-related AEs occurred at a similar frequency in each treatment group (Table 2.3.1). The most commonly occurring treatment-related events were those associated with the gastrointestinal system (nausea, vomiting, and diarrhea). These events occurred in <3% of subjects (GTx-024 IB).

Table 2.3.1 AEs from GTx-024 vs. Placebo cancer trials attributed to drug treatment arm with a frequency of ≥0.5%

	GTx-024 (N=432)	Placebo (N=378)	All subjects (N=810)
Treatment-related AEs	**n (%)**	**n (%)**	**n (%)**
Any treatment related adverse event	*67 (15.5)*	*46 (12.2)*	*113 (14.0)*
Nausea	9 (2.1)	8 (2.1)	17 (2.1)
Vomiting	8 (1.9)	4 (1.1)	12 (1.5)

Treatment-related AEs	GTx-024 (N=432) n (%)	Placebo (N=378) n (%)	All subjects (N=810) n (%)
Diarrhoea	5 (1.2)	9 (2.4)	14 (1.7)
Headache	5 (1.2)	3 (0.8)	8 (1.0)
Alanine aminotransferase increased	4 (0.9)	2 (0.5)	6 (0.7)
Constipation	4 (0.9)	2 (0.5)	6 (0.7)
Fatigue	4 (0.9)	4 (1.1)	8 (1.0)
Abdominal pain	3 (0.7)	1 (0.3)	4 (0.5)
Anaemia	3 (0.7)	0 (0)	3 (0.4)
Dyspnoea	3 (0.7)	0 (0)	3 (0.4)
Hyperglycaemia	3 (0.7)	0 (0)	3 (0.4)
Polyneuropathy	3 (0.7)	1 (0.3)	4 (0.5)
Weight Increased	3 (0.7)	0 (0)	3 (0.4)
Alopecia	2 (0.5)	1 (0.3)	3 (0.4)
Aspartate aminotransferase increased	2 (0.5)	1 (0.3)	3 (0.4)
Deafness	2 (0.5)	0 (0)	2 (0.2)
Decreased appetite	2 (0.5)	0 (0)	2 (0.2)
Dehydration	2 (0.5)	0 (0)	2 (0.2)
Dermatitis allergic	2 (0.5)	0 (0)	2 (0.2)
Dizziness	2 (0.5)	0 (0)	2 (0.2)
Gastrooesophageal reflux disease	2 (0.5)	0 (0)	2 (0.2)
Insomnia	2 (0.5)	1 (0.3)	2 (0.2)
Leukopenia	2 (0.5)	0 (0)	2 (0.2)
Mucosal inflammation	2 (0.5)	0 (0)	2 (0.2)
Muscular weakness	2 (0.5)	0 (0)	2 (0.2)
Rash	2 (0.5)	0 (0)	2 (0.2)
Vision blurred	2 (0.5)	0 (0)	2 (0.2)

2.4 Overview of Proposed Study

This is an open-label, Phase 2, single institutional trial of pembrolizumab in combination with GTx-024 for patients with histologically proven diagnosis of metastatic AR+ TNBC who have measurable disease (per RECIST 1.1 Criteria). Patients will receive pembrolizumab at a dose of 200 mg iv once every 21 days in combination with GTx-024 at a dose of 18 mg orally with a safety lead-in. The first 6 participants will enroll at a 3 at risk safety lead-in to ensure the safety/ tolerability of the combination regimen.

Patients may be therapy naïve or may have failed any number of prior therapies in the metastatic setting. Paraffin-embedded tumor tissue must be available for assessment of biomarkers and patient must be able to provide tissue for PD-L1 biomarker analysis from a newly obtained formalin fixed tissue or recent biopsy of a tumor lesion not previously irradiated. Patients may not have prior treatment with any other anti-programmed cell death protein-1 (anti-PD-1), or PD ligand-1 (PD-L1) or PD ligand-2 (PD-L2) agent or an antibody targeting other immune-regulatory receptors or mechanisms. Patients may also not have previous treatment with AR targeted agents (including but not limited GTx-024, bicalutamide or enzalutamide).

Since each agent may have its own anti-cancer properties if one agent is discontinued due to toxicity, then the participant may continue to receive single agent protocol therapy.

Study treatment will continue until disease progression, unacceptable adverse event(s) (AEs), concurrent illness that prevents further administration of study treatment, investigator's decision to withdraw the subject from study treatment or withdrawal.

Since pembrolizumab is an immunotherapy agent, participants with confirmed radiographic progression per RECIST v1.1 who are clinically stable but do not meet irRECIST criteria for progression will continue to receive protocol therapy following consultation of the Study PI.

Participants who discontinue treatment will be assessed for safety 30 days post-last dose of protocol therapy through to 90 days post last dose of protocol therapy or until initiation of a new anti-cancer therapy (whichever occurs sooner).

Follow up response assessment for participants who end treatment and yet to progress will be every 3 months until disease progression (verified by restaging imaging) or initiation of a new anti-cancer therapy. Participants who progress or initiated a new anticancer therapy will be followed biannually or as requested by the Study Principal Investigator (PI).

2.4.1 *Correlatives and biomarker analysis*

2.4.1.1 Tumor tissue correlatives

Previous research data suggested that CD8 T cell infiltration and PD-L1 expression within tumors may predict efficacy for pembrolizumab, but definitive biomarkers are not yet available [56-60]. To identify biomarkers to predict and/or follow efficacy for pembrolizumab in combination with GTx-024 in patients with metastatic TNBC, we propose the following correlative studies to analyze the immune and stromal cells within metastatic tumors, as well as immune cells in peripheral blood, before and after therapy:

Biopsies of tumor tissue(s) prior to study initiation and at the end of study will be obtained. Key questions we will address include:

- Immune and stromal cell characteristics before treatment that correlate with clinical response [56]. This includes PD-L1 expression levels.
- Changes in immune and stromal cells after therapy that correlate with clinical response [57].

The following methods will be utilized to address the above questions:

- **12-color FACS** analysis to enumerate/phenotype immune cell subsets and functional readouts, including cytokine production and signaling (phosflow).
- **TCR repertoire analysis via deep sequencing**. Expansion of the T cell repertoire after therapy will be evidence for an immunological response and may indicate epitope spread.
- **Immunohistology** using a novel quantitative, spatial image analysis system (Vectra, Perkin Elmer) that will enable us to analyze immune, stromal, and cancer cells in metastatic tumors via 8-color histology.
- **Gene expression analysis of stromal cells via RNA-Seq or microarrays**: Messenger RNA (mRNA) expression profiling in tumor specimens and peripheral blood will be completed to assess expression either by RNAseq or microarrays, to define a gene set critical for clinical response to pembrolizumab. The hypothesis to be tested is that pembrolizumab induces responses in tumors that reflect an inflammatory/immune cell-rich phenotype based on gene expression. Expression of individual genes related to the immune system may also be evaluated, such as immune signatures and critical cytokines.
- **Morphological / IHC TIL analysis of tumor biopsies**: Tumor infiltrating lymphocytes have been shown to provide prognostic and potentially predictive value, particularly in TNBC and HER2-overexpressing breast cancer. Hematoxylin and eosin (H&E)-stained breast tumor sections can be evaluated for TILs, according to a recently published standardized methodology [61]. We will evaluate: (1) pre-treatment TILS as a predictor of response to combination therapy; (2) specific

TIL subsets (e.g. CD4, CD8, Treg distribution) and other immunological correlatives (e.g. TCR repertoire analysis) as possible predictors of response; (3) change in TILs as a result of the combination therapy.

Sequencing technologies, such as whole genome sequencing or single cell RNA sequencing, allow global assessment of DNA mutations and gene expression profiles, which can provide molecular phenotyping that identifies distinct characteristics of tumors, including the ultimate outcome of disease. We will use single cell sequencing technology to characterize a given tumor for both its mutational profile and phenotypic status using fresh tumor tissue.

2.4.1.2 Peripheral blood immune biomarkers (Serial cytokine measurements)

Blood samples will be obtained to track the temporal dynamics of the host immune response.

The 30 cytokine panel includes: epidermal growth factor (EGF), eotaxin, basic fibroblast growth factor (FGF-basic), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), interferon alpha (IFN-α); IFN-gamma (γ), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist (IL-1RA), IL-2; interleukin-2 receptor (IL-2R), IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40/p70, IL-13, IL-15, IL-17, IFN-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), monokine induced by IFN-γ (MIG), monocyte inflammatory protein-1 alpha (MIP-1α), monocyte inflammatory protein-1 beta (MIP-1β), regulated upon activation, normal T cell expressed and secreted cytokine (RANTES), tumor necrosis factor (TNF)-α, and VEGF.

2.4.1.3 Androgen Receptor Signaling

Tumor specimen will be processed for mRNA extraction and AR signaling pathway will be analyzed. Association of AR signaling pathway and response to the combination therapy will be studied.

2.4.1.4 Peripheral blood circulating tumor DNA (ctDNA)

Circulating DNA fragments carrying tumor-specific sequence alterations (ctDNA) are found in the cell-free fraction of blood, representing a variable and generally small fraction of the total circulating DNA. Advances in sequencing technologies have enabled the rapid identification of somatic genomic alterations in individual tumors, and these can be used to design personalized assays for the monitoring of ctDNA. Studies have shown the feasibility of using ctDNA to monitor tumor dynamics in a limited number of patients with various solid cancers, but few cases of breast cancer have been analyzed [62].

We will acquire serial peripheral blood and will examine the peripheral blood ctDNA and the effect of the combination of GTx-024 pembrolizumab on the ctDNA profiling.

2.4.1.5 Tumor-derived exosomes

Exosomes are small membrane-bound vesicles that present abundantly in peripheral blood. Tumor-derived exosomes (TEX) are thought to carry immunosuppressive molecules and factors which can interfere with immune cell functions [63]. Through delivering suppressive cargos consisting of proteins similar to those in parent tumor cells to immune cells, TEX may influence the antitumor activities of immune cells. Hence, TEX may potential to serve as noninvasive biomarkers of tumor progression. In this study, we will evaluate TEX's and TEX-associated immune biomarkers in association with patient's response to AR targeted therapy and Pembrolizumab combination.

2.5 Study Endpoints

A primary endpoint is to evaluate the safety and tolerability of the combination therapy. Safety analysis will be carried out based on toxicities assessed by CTCAE version 4.0. Adverse events will be analyzed

including but not limited to all AEs, SAEs, fatal AEs, and laboratory changes. Immune-related adverse events (irAEs) will also be collected.

The other primary endpoint is to determine the response rate (CR or PR via RECIST 1.1) of the combination of pembrolizumab with GTx-024 in patients with AR positive advanced TNBC.

Other secondary end points includes: clinical benefit rate (CBR) at 24 weeks; duration of response (DOR), PFS, EFS, TTF, OS to test the efficacy of therapy of the novel drug combination assessed by RECIST version 1.1. Any event (toxicity-related or progression) will be counted as “treatment failure”. Kaplan-Meier estimates will be generated for PFS, failure-free survival and OS.

Furthermore, secondary endpoint include assessing efficacy using irRECIST, an adaptation of RECIST version 1.1 to account for the unique tumor response characteristics to treatment with new immunotherapeutic agents, including pembrolizumab.

3.0 ELIGIBILITY CRITERIA

Patient MRN (COH Only):	Patient Initials (F, M, L):
Institution:	

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1 Inclusion Criteria

Informed Consent and Willingness to Participate

__1. Documented informed consent

__2. Willing to provide a sample from a recently obtained (within 42 days prior to initiation of Day 1) biopsy of a tumor lesion (See Section 9.1 for details).

- If recently-obtained samples are unavailable an archived metastatic specimen not previously irradiated may be submitted upon agreement from the study PI.

Age Criteria, Performance Status and Life Expectancy

__3. Age: ≥ 18 years

__4. ECOG performance status of ≤ 1 (see Appendix A)

__5. Life expectancy of > 3 months

Nature of Illness and Treatment History

__6. Metastatic triple negative breast cancer (TNBC)

__7. Measurable disease per RECIST v1.1 criteria: At least 1 lesion of >10 mm in long axis diameter for non-lymph nodes or >15 mm in short axis diameter for lymph nodes that is serially measurable according to RECIST 1.1 using computerized tomography, magnetic resonance imaging, or panoramic and close-up color photography (see Appendix D).

__8. Histologically proven diagnosis of TNBC per current ASCO/CAP guideline.

- ER negative (ER expression ≤ 10% positive tumor nuclei), PR negative (PR expression ≤ 10% positive tumor nuclei) and HER2 negative breast cancer by IHC and/or FISH.

__9. Androgen Receptor positive (AR+)

- Defined as ≥50% nuclear AR staining by immunohistochemistry (IHC) in either the primary or metastatic lesion
- NOTE: Research testing of AR status is available at COH Pathology. Refer to Section 10.0 for details.

__10. Resolution of grade 2 and above toxicities of most recent therapy except for stable sensory neuropathy (≤ Grade 2) and alopecia.

Patient MRN:	Patient Initials (F, M, L):

Contraception

__11. *Female (childbearing potential)*: use an adequate method of birth control (except hormonal contraception) or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication. Refer to Appendix B.

- Childbearing potential defined as not being surgically sterilized or have not been free from menses for > 1 year.

__12. *Male*: use an adequate method of contraception with the first dose of study therapy through 120 days after the last dose of study therapy. Refer to Appendix B

- Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

Laboratory Criteria (to be performed within 14 days prior to Day 1 of protocol therapy)

__13. ANC ≥ 1500/mm^3	ANC:	Date:
__14. Platelets ≥ 100,000/mm^3	Plts:	Date:
__15. Hemoglobin ≥ 9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)	Hgb:	Date:
__16. Serum total bilirubin ≤ 1.5 x ULN **OR** Direct bilirubin ≤ ULN if total bilirubin levels > 1.5 x ULN	ULN: Bil: Direct Bil:	Date:
__17. Albumin ≥ 2.5 mg/dL	Albumin:	Date:
__18. AST and ALT ≤ 2.5 x ULN **OR** ≤ 5.0 x ULN if liver metastases present	ULN: ALT: ULN: AST:	Date: Date:
__19. Serum creatinine ≤ 1.5 x ULN **OR** Creatinine clearance* ≥ 60 mL/min if creatinine levels > 1.5 x ULN * If measured or calculated per institutional standard. GFR can also be used in place of creatinine or CrCl $\text{CrCl (mL/min)} = \frac{(140\text{-age}) \times \text{actual body weight (kg)}}{72 \times \text{serum creatinine (mg/dL)}}$ (x 0.85 for females) **Or** $\text{CrCl (mL/min)} = \frac{(140\text{-age}) \times \text{actual body weight (kg)}}{0.8136 \times \text{serum creatinine (umol/L)}}$ (x 0.85 for females)	ULN: Serum Cr: Cr Cl:	Date:
__20. *Female of childbearing potential only*: Negative urine or serum pregnancy test. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required	Urine test: Serum test:	Date:

Patient MRN:	Patient Initials (F, M, L):

3.2 Exclusion Criteria

Prior Therapy and Concomitant Therapy

__1. Anti-programmed cell death protein-1 (anti-PD-1), PD Ligand-1 (PD-L1) PD Ligand-2 (PD-L2) agent, an antibody targeting other immuno-regulatory receptors or mechanisms.

__2. Radiotherapy within 14 days prior to Day 1 of protocol therapy

__3. AR targeted agents (including GTx-024, enzalutamide or other AR targeted therapies).

__4. Investigational agent within 21 days prior to Day 1 of protocol therapy

__5. Hormone replacement therapies (estrogens, megesterol acetate) within 14 days prior to Day 1 of protocol therapy

__6. Live-virus vaccination within 30 days prior to Day 1 of protocol therapy.

__7. Systemic cytotoxic chemotherapy, antineoplastic biologic therapy, or major surgery within 21 days of the first dose of trial medication.

__8. Testosterone or testosterone-like agents (methyltestosterone, oxandrolone, oxymetholone, danazol, fluoxymesterone, dehydroepiandrosterone, androstenedione) other androgenic compounds or anti-androgens within 30 days prior to Day 1 of protocol therapy.

__9. Chronic systemic steroid therapy or on any other form of immunosuppressive medication.

Other Illnesses and Conditions

__10. Unstable or untreated brain/leptomeningeal metastasis.

__11. Clinically active diverticulitis, intra-abdominal abscess, gastrointestinal (GI) obstruction, or abdominal carcinomatosis (known risks factors for bowel perforation).

__12. Active central nervous system metastases and/or carcinomatous meningitis.

__13. Severe hypersensitivity reaction to treatment with another monoclonal antibody

__14. Active autoimmune disease that has required systemic treatment in the past 2 years (replacement therapies for hormone deficiencies are allowed).

__15. Known history of HIV, Hepatitis B or Hepatitis C

__16. History of pneumonitis (non-infectious) that required steroids or current pneumonitis

__17. Diagnosed with or treated for cancer within the previous two years, other than breast cancer or non-melanoma carcinoma of the skin

__18. Unable to swallow capsules

__19. Currently on bisphosphonate or denosumab with elevated serum calcium levels corrected for albumin/ionized calcium levels outside of institutional normal limits

__20. Female: Pregnant or lactating

Patient MRN:	Patient Initials (F, M, L):

__21. Concomitant medical condition that precludes adequate study treatment compliance or assessment, or increases subject risk, in the opinion of the Investigator, such as but not limited to:

- Myocardial infarction or arterial thromboembolic events within 6 months prior to baseline or severe or unstable angina, New York Heart Association (NYHA) Class III or IV disease, or a QTCB (corrected according to Bazett's formula) interval > 470 msec; serious uncontrolled cardiac arrhythmia grade II or higher according to NYHA; uncontrolled hypertension (systolic > 150 and/or diastolic > 100 mm Hg)
- Acute and chronic active infectious disorders and non-malignant medical illnesses that are uncontrolled or whose control may be jeopardized by the complications of this study therapy
- Impairment of gastrointestinal function or gastrointestinal disease that may significantly alter the absorption of study drugs (e.g., ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome)

Noncompliance

__22. Prospective participants who, in the opinion of the investigator, may not be able to comply with all study procedures (including compliance issues related to feasibility/logistics).

Eligibility Confirmed* by (Choose as applicable):	**Print Name**	**Signature**	**Date**
☐ **Site PI**			
☐ **Authorized study MD**			
☐ **Study Nurse**			
☐ **Study CRA/ CRC**			
☐ **Other:_____**			
*Eligibility should be confirmed per institutional policies.			

4.0 PARTICIPANT ENROLLMENT

NOTE: Sites must meet activation requirements prior to enrolling participants.

4.1 Pre-Enrollment Informed Consent and Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility) may be used for baseline values or to evaluate suitability for treatment, even if the studies were done before informed consent was obtained. The informed consent process is to be fully documented (see Section 16.4), and the prospective participant must receive a copy of the signed informed consent document. Screening procedures are listed Study Calendar, Section 10.0.

4.2 Registration Requirements/Process

4.2.1 COH DCC Availability and Contact Information

Eligible subjects will be registered on the study centrally by the Data Coordinating Center (DCC) at City of Hope. DCC staff are available between the hours of 8.00 am and 5.00 pm PST, Monday through Friday (except holidays). DCC contact information is as follows:

- Phone: (626) 256-4673 ext. 83968
- E-mail: DCC@coh.org

4.2.2 Slot verification and reservation

Designated study staff should call the DCC to verify current slot availability, and to reserve a slot for a specific prospective subject. Slots can only be held for a limited time. Refer to Section 5.3 for Safety Lead-in and Phase 2 treatment plan details.

Eligible subjects must be registered prior to start of protocol therapy. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a subject does not receive protocol therapy following registration, the subject's registration on the study may be canceled after discussion with the PI. The Data Coordinating Center should be notified of cancellations as soon as possible.

4.2.3 Registration Process

To register a participant the subsequent procedure is to be followed:

1. The study team at City of Hope or the participating site should contact the DCC via telephone or email to provide notification regarding the pending registration and communicate desired timeline of the registration, especially if it must be completed promptly to meet the registration window.
2. The data manager/coordinator/research nurse should then email copies to DCC@coh.org (participating site – please use #secure# in subject line) of the following documents:
    - Registration Cover Sheet (Appendix X)
    - Completed Eligibility Criteria List (printed from Section 3.0 of the protocol)
    - Source documentation to support eligibility criteria**
    - Signed Informed Consent document (participating sites – please send as per your institutional guidelines)

- Signed subject's Bill of Rights (COH only)
- Signed HIPAA authorization form (if separate from the informed consent document)
  ** For COH participants, provide copies of source documentation only if not readily available as a finalized record in the COH Electronic Medical Record (EMR). For participating sites, please include all source documentation and de-identify as per your institutional policy.

3. After having received all transferred documentation, the DCC will complete the review the documents to verify eligibility, working with the study team as needed to resolve any missing required source elements. A participant failing to meet all protocol eligibility requirements will not be registered.
4. Once eligibility has been confirmed, DCC staff will register the participant by: assigning a subject accession number, register the subject on study centrally into a COH clinical trials management system (e.g. MIDAS), and enter the subject into the eCRF system, Medidata RAVE.
5. Once registration has been completed, DCC staff will send a Confirmation of Registration Form within 24 hours, including the participant study number to:
   - The site study team: Principal Investigator, treating physician, protocol statistician, protocol nurse, CRC and COH IDS Pharmacy.
   - the COH sponsor team designees

4.3 **Screen Failures and Registered participants who do not Begin Protocol Therapy**

The DCC is to be notified of all participants who sign consent but do not meet eligibility criteria or do not initiate protocol therapy. For non-COH sites, the reason for screen failure will be documented in the registration coversheet (see Appendix X) and submitted to the DCC.

5.0 TREATMENT PROGRAM

5.1 Treatment Overview

This is an open-label Simon's MiniMax two-stage Phase 2 trial for advanced Androgen Receptor (AR) + TNBC. The first 6 patients will be monitored as part of a safety lead-in in a 3 at risk design to determine safety and tolerability of the combination regimen- pembrolizumab and GTx-024.

Participants will be administered protocol therapy in the outpatient setting in each 21-day cycle (see Section 5.3 and Section 5.4). Participants will receive up to 35 cycles of treatment. If one agent is discontinued due to toxicity, then the participant may continue to receive single agent protocol therapy.

Participants who end protocol therapy (Section 5.6) will undergo follow-up (Section 5.7). Windows for all assessments and treatments are detailed in Section 10.0.

5.2 Treatment Cycle Definition

Each cycle is defined as the administration of protocol therapy.

In the absence of a delay of initiating a new cycle due to toxicity, each treatment cycle lasts 21 days, with a window of ± 3 days for participant convenience.

Day 1 of each treatment cycle is defined by the administration of pembrolizumab. If pembrolizumab is permanently discontinued and GTx-024 is being continued, then each cycle will be 21 ± 3 days.

5.3 Treatment Plan

5.3.1 Safety Lead-in Cohort

Table 5.3 describes the combination regimen. A 3 at risk design will be utilized for the first 6 participants to assess toxicity for the regimen. Dose limiting toxicities (DLT) are defined in Section 12.1 and will be evaluated during Cycle 1.

Table 5.3 Dosing regimen and schedule

	Dose Level	**Pembrolizumab** (IV infusion over 30 minutes; on Day 1 of each 21-day cycle)	**GTx-024** (Orally, once daily on Days 1-21 of each 21-day cycle)
	Dose Level -1	200 mg	9 mg
Initial starting dose level	**Dose Level 1**	**200 mg**	**18 mg**

5.3.2 Phase 2 Cohort

Subsequent participants enrolled on to the study will be treated at the tolerable dose defined during the safety lead-in. Refer to Section 12.0 for details.

5.4 Agent Administration

5.4.1 Pembrolizumab

Pembrolizumab (200 mg) will be administered as a 30 minute IV infusion on Day 1 of each 21-day cycle. Every effort should be made to target infusion timing to 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 pembrolizumab dose is fixed at 200 mg. There are no dose reductions. Management and dose delays associated with pembrolizumab AEs are outlined in Section 6.2.

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

On Day 1 of Cycle 1, pembrolizumab will be given first.

5.4.2 GTx-024

GTx-024 should be taken daily (e.g. Days 1-21 of each 21-day cycle). Participant may be provided sufficient supply to include the 3 day cycle duration window (21 ± 3 days).

On Day 1 of Cycle 1, GTx-024 should be taken after pembrolizumab.

GTx-024 should be taken with water at approximately the same time each day with or without food.

GTx-024 capsules should be swallowed whole.

If a participant misses a dose and > 12 hours have passed since the scheduled dose time, the missed dose will be skipped and will not be made up. Doses that are vomited will not be made up.

Participants will be given a patient diary to document each dose of GTX-024 that is taken or missed (Appendix C).

Management and dose modification associated with GTx-024 adverse events are outlined in Section 6.2.

5.5 Assessments and Special Monitoring

Refer to Section 10.0 summarizes the trial procedures to be performed. **Note:** Protocol therapy should be administered on Day 1 of each cycle after all procedures/safety assessments have been completed.

It may be necessary to perform study procedures at unscheduled time points if deemed clinically necessary by the investigator.

5.5.1 Special Monitoring

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of pembrolizumab

Diarrhea/colitis

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

Hyperthyroidism or hypothyroidism

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

Management of infusion reactions

- Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.
- Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.

5.5.2 Bone metastases and disease evaluation

Bone scans will also be utilized to assess osseous metastases for participants with a known history of bone metastases or if clinically indicated during screening (see Section 10.0).

A bone scan at follow up is required only if the participant develop new or worsening symptoms or if the investigator believes the participant has attained a Complete Response.

Additionally, plain X-ray evaluation will be obtained for symptomatic sites with negative bone scan evaluations.

5.5.3 Brain metastases and disease evaluation

Brain imaging should be performed in patients with known brain metastases at baseline and during the study. Imaging should occur as stated in Section 10.0.

5.6 Duration of Therapy and Criteria for Removal from Protocol Therapy

Participants will receive protocol therapy until one of the below criteria are met:

- Confirmed disease progression
  - *Note*: Participants with confirmed radiographic progression per RECIST v1.1 who are clinically stable but do not meet irRECIST criteria for progression will continue to receive protocol therapy following consultation of the Study PI.
- Completed 35 cycles of treatment (~24 months)
- Participant is deemed intolerant to protocol therapy because of toxicity, despite dose modification/ delay
  - *Note*: If one agent is discontinued due to toxicity, then the participant may continue to receive single agent protocol therapy
- General or specific changes in the patient's condition which render the patient unacceptable for further treatment in the judgment of the investigator
- Withdrawal of consent for further protocol therapy (See Section 16.5)

Once participants meet criteria for removal from protocol therapy, the participant should then proceed to End of Protocol Therapy assessments.

Documentation of the reason for discontinuing protocol therapy and the date effective should be made in the source documentation and appropriate CRF. The COH DCC should be promptly notified of the change in participant status.

5.7 Follow Up

Following completion of protocol therapy, all participants will enter follow-up after ending protocol treatment. This is comprised of:

- **Follow-up for safety**, 30 days post-last dose of protocol therapy through to (a) 90 days post-last dose of protocol therapy or (b) until initiation of a new anticancer therapy (whichever occurs sooner)
  - *Exception:* If a participant received 4 cycles of GTx-024 monotherapy (~ 90 ± 7 days) beyond pembrolizumab discontinuation, then then the participant will only be followed for 30 days post last dose of protocol therapy.
  - Note the period for safety follow-up will be extended until stabilization or resolution for all reportable AEs (per the agreement of the Study PI) and accompanying follow-up safety report.
- **Follow-up for response** for those who have yet to have disease progression.
- **Follow-up for survival** for those who progressed or initiated a new anti-cancer therapy.
  - Follow-up for survival may be obtained by reviewing the medical record, contacting the participant or review of public records and the Social Security Index Registry.

Assessment time points and windows are detailed in Section 10.0.

5.8 Duration of Study Participation

The reason(s) for discontinuing study participation should be documented and may include:

- Completion of study activities (treatment and follow-up)
- Withdrawal of consent (See Section 16.5)
- Participant is lost to follow-up. All attempts to contact the participant must be documented.
- At the discretion of the investigator for safety, behavioral, study termination or administrative reasons

The reason for study completion and associated date must be documented in the source documentation and the study-specific case report form (CRF). The participant's status is to be modified in the COH clinical trial management system once the participant completes the study. The COH DCC should be promptly notified of the change in participant status.

5.9 Supportive Care, Prohibited Treatments and Concomitant Medications

Participants must be instructed not to take any additional medications (including over-the-counter products) during the trial without prior consultation with the investigator..

5.9.1 **Prohibited** concomitant medication

The following are **prohibited** until from Day 1 of protocol therapy until end of protocol therapy (last day of study agent or decision to end study agent(s) whichever occurs later):

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Other investigational agents other than pembrolizumab and GTx-024
- Live vaccines
- Testosterone or testosterone-like agents (methyltestosterone, oxandrolone, oxymetholone, danazol, fluoxymesterone, dehydroepiandrosterone, androstenedione) other androgenic compounds or anti-androgens

- Hormone replacement therapy: estrogens and/or megesterol acetate
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the PI.

Participants who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the study.

5.9.2 Use with **Caution**

The following medications (see Table 5.11) should be used with caution when co-administering GTx-024:

- **Potent CYP3A4 inducers** may decrease the rate and extent of exposure of GTx-024 and its glucuronide metabolite
- **UDP inhibitors** may increase GTx-024 and glucuronide exposure
- **Substrates of BCRP** may increase exposure of the BCRP substrate

Table 5.11 Potential Interactions with GTx-024

Strong CYP3A4 Inducers	Strong CYP3A4 Inducers	BCRP Substrates	UGT Inhibitors
Barbiturates	Phenobarbital	Ofloxacin	Gemfibrozil
Carbamazepine	Primidone	Paratusin	Ketoconazole
Dexamethasone	Rifabutin	Pitavastatin	
Fosphenytoin	Rifapentine	Rosuvastatin	
Modafinil	Rifampin	Sulfasalazine	
Nafcillin	Troglitazone		
Oxcarbazepine	Herbals:		
Phenytoin	Ginko Biloba		
Pioglitazone	St John's Wort		

5.9.3 Other Considerations

Refer to Appendix B for contraception related guidance.

5.9.4 Supportive Care Guidelines

With the exception of therapies listed in Section 5.9.1, participants should receive appropriate supportive care measures as deemed necessary by the treating investigator.

Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. 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**.

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

5.9.4.1 Pneumonitis

- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

Grade 2

- Administer systemic corticosteroids.
- When symptoms improve to ≤ Grade 1, steroid taper should be started and continued over no less than 4 weeks.

Grade 3 or 4

- Immediately treat with IV steroids.
- Administer additional anti-inflammatory measures, as needed.

5.9.4.2 Diarrhea/colitis

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

Grade 2 diarrhea/colitis

- Administer oral corticosteroids.
- When symptoms improve to ≤ Grade 1, steroid taper should be started and continued over no less than 4 weeks.

Grade 3 or 4 diarrhea/colitis

- IV steroids followed by high dose oral steroids
- When symptoms improve to ≤ Grade 1, steroid taper should be started and continued over no less than 4 weeks.

5.9.4.3 Type 1 diabetes mellitus OR ≥ Grade 3 hyperglycemia***

- *if new onset, including diabetic ketoacidosis [DKA])
- **if associated with ketosis (ketonuria) or metabolic acidosis (DKA)

*For **T1DM** or **Grade 3-4** hyperglycemia*

- Insulin replacement therapy is recommended.
- Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

5.9.4.4 Hypophysitis

Grade 2 (i.e. symptomatic)

- Treat with corticosteroids.
- When symptoms improve to ≤ Grade 1, steroid taper should be started and continued over no less than 4 weeks.
- Replacement of appropriate hormones may be required as the steroid dose is tapered.

Grade 3 or 4 Treat with an initial dose of IV corticosteroids followed by oral corticosteroids

- When symptoms improve to ≤ Grade 1, steroid taper should be started and continued over no less than 4 weeks.
- Replacement of appropriate hormones may be required as the steroid dose is tapered.

5.9.4.5 Hyperthyroidism or hypothyroidism

*Grade 2 **Hyper**thyroidism*

- Non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.

*Grade 3 or 4 **Hyper**thyroidism*

- Treat with an initial dose of IV corticosteroid followed by oral corticosteroids.
- When symptoms improve to ≤ Grade 1, steroid taper should be started and continued over no less than 4 weeks.
- Replacement of appropriate hormones may be required as the steroid dose is tapered

*Grades 2-4 **Hypo**thyroidism*

- Thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.

5.9.4.6 Hepatic

Grade 2

- Monitor liver function tests more frequently until returned to baseline values (consider weekly).
- Treat with IV or oral corticosteroids

Grade 3 or 4

- Treat with intravenous corticosteroids for 24 to 48 hours.
- When symptoms improve to ≤ Grade 1, steroid taper should be started and continued over no less than 4 weeks

5.9.4.7 Renal failure or nephritis

Grade 2

- Treat with corticosteroids

Grade 3 or 4

- Treat with systemic corticosteroids.
- When symptoms improve to ≤ Grade 1, steroid taper should be started and continued over no less than 4 weeks

5.9.4.8 Management of infusion reactions

- Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Grade 1: Mild reaction; infusion interruption not indicated; intervention not indicated

- Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.

Grade 2: Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs

- Stop Infusion and monitor symptoms.
- Additional appropriate medical therapy may include but is not limited to:
  - IV fluids
  - Antihistamines
  - NSAIDS
  - Acetaminophen
  - Narcotics
- Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.
- If symptoms **resolve within one hour** of stopping drug infusion:
  i. May restart infusion at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr).
- If symptoms **DO NOT resolve within one hour** of stopping drug infusion:
  i. Hold the dose until symptoms resolve
  ii. Premedicate for the next scheduled dose
- **At subsequent dosing** may premedicate 1.5h (± 30 minutes) prior to infusion of pembrolizumab with:
  - Diphenhydramine: 50 mg orally (or equivalent dose of antihistamine).
  - Acetaminophen: 500-1000 mg orally (or equivalent dose of antipyretic).
- **Participants who develop Grade 2 toxicity despite adequate premedication should be removed from protocol therapy**.

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)

OR

Grade 4: Life-threatening; pressor or ventilatory support indicated

- Stop Infusion
- Additional appropriate medical therapy may include but is not limited to:
  - IV fluids
  - Antihistamines
  - NSAIDS
  - Acetaminophen
  - Narcotics
  - Oxygen
  - Pressors
  - Corticosteroids
  - Epinephrine
- Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.

- Hospitalization may be indicated.
- Participant should not receive any further treatment with pembrolizumab.

5.9.4.9 Myocarditis

- For suspected immune-mediated myocarditis, ensure adequate evaluation to exclude other etiologies
- Administer corticosteroids as appropriate

6.0 ANTICIPATED TOXICITIES AND DOSE DELAY/ MODIFICATIONS

6.1 Anticipated Toxicities

6.1.1 Pembrolizumab

Per the IB (Edition 16, 2018) the expected toxicities for pembrolizumab are as follows (asterisk signifies ≥ 10%, very common; no asterisk signifies 1-10%, common, † signifies 0.1- 1%, uncommon and ^ signifies 0.01-0.1% rare). For percentage of subjects with adverse event of special interest by toxicity grade, refer to table 32 of IB.

Blood and lymphatic system disorders	Anemia, neutropenia†, thrombocytopenia†, leukopenia†, lymphopenia†, eosinophilia†, hemolytic anemia†, immune thrombocytopenic purpura†
Cardio-Vascular	Myocarditis†, hypertension †
Endocrine	Hyperthyroidism, hypothyroidism, hypophysitis †, secondary adrenal insufficiency †, thyroiditis†
Eye	Uveitis †, dry eye †
Gastrointestinal	Diarrhea*, nausea*, abdominal pain, vomiting, colitis, constipation, dry mouth, pancreatitis†, small intestinal perforation†
General Disorders and Administration Site	Fatigue*, asthenia, edema, pyrexia, influenza-like illness, chills
Hepatobiliary	Hepatitis†
Immune system	Infusion related reactions, severe infusion reactions† sarcoidosis†, solid organ transplant rejection^
Investigations (excluding hematologic)	AST/ALT increased, blood alkaline phosphatase increased, blood creatinine increased, low sodium levels†, low potassium levels†, low calcium levels†, blood bilirubin increased†, amylase increased†, increased calcium†
Metabolism and Nutrition	Decreased appetite, Type 1 diabetes mellitus †
Musculoskeletal and Connective Tissue	Arthralgia, back pain, myositis, musculoskeletal pain, arthritis, pain in extremity, tenosynovitis^
Nervous system	Headache, dizziness, dysgeusia, epilepsy†, lethargy†, Guillian-Barré syndrome †, encephalitis†, peripheral neuropathy†, myasthenic syndrome†,
Psychiatric disorders	Insomnia†
Renal and urinary	Nephritis †
Respiratory, Thoracic and Mediastinal	Cough, pneumonitis, dyspnea, penumonia †
Skin and Subcutaneous Tissue	Pruritus*, rash*, vitiligo, dry skin, erythema, lichenoid keratosis †, psoriasis†, dermatitis †, dermatitis acneiform †, papule†, hair color changes†, eczema†, alopecia†, severe skin reactions* , erythema nodosum ^, Steven-Johnson Syndrome^ and toxic epidermal necrolysis^)

6.1.2 GTx-024

Per the IB (Version 16, 2017) the expected toxicities for GTx-024 are listed below (no asterisk signifies ≥ 10%; and * signifies 1-10%):

Gastrointestinal	Constipation
Investigations	Alanine aminotransferase increased*, blood creatinine increased*
Nervous System	Headache
Respiratory, Thoracic and Mediastinal	Dyspnea
Skin and Subcutaneous Tissue	Rash*

Also reported on GTx-024 trials but with undetermined relationship to GTx-024 is:

Metabolism and nutrition: Hypercalcemia. (This toxicity may be included in the consent, but is considered unexpected to the agent in terms of safety reporting classification).

6.2 Dose Delay/ Modification Guidelines

- Toxicities will be graded using the NCI CTCAE Version 4.0.
- Baseline values are from the last values obtained prior to treatment.
- If one agent is permanently discontinued, then the participant may continue treatment with the other agent treatment.
- Guidelines for dose modification/ delays related to pembrolizumab and GTx-024 are stated in Table 6.2.
- Participants already receiving 9 mg/day of GTx-024 and requiring further dose reduction must discontinue GTx-024.

Table 6.2 Dose Delay/Modification Guidelines for AEs

Toxicity	Agent Most Likely Attributed to	Dose delay/ modification guidelines
Hematological		
Platelets Grade 3-4 *G3: 25,000-50,000/mm³* *G4: <25,000/mm³*	-	• Hold study drugs until toxicity resolves to ≤ Grade 1. • Dose modify/ delay per investigator discretion based on attribution.
ANC Grade 4*:* *<500/mm³*		
Febrile neutropenia Grade 3-4 *G3: ANC< 1000/mm³ with a single temperature of > 38.3 °C or a sustained temperature of ≥ 38 °C for > 1 hour.* *G4: Life-threatening consequences; urgent intervention indicated*		
Gastrointestinal (Diarrhea/Colitis)		*Follow supportive care guidelines in Section 5.9.4.2.*

Toxicity	Agent Most Likely Attributed to	Dose delay/ modification guidelines
Diarrhea Grade 2-3 *G2: stool frequency of 4-6 over baseline per day* *G3: stool frequency of ≥7 over baseline per day*	*Pembrolizumab (ir)* GTx-024	• Hold study drugs until toxicity resolves to ≤ Grade 1 and corticosteroid dose is < 10 mg/day. • Resume GTx-024 at the same dose or lower dose per investigator discretion following consultation with the Study PI. Resume pembrolizumab per protocol • Discontinue study regimen if toxicity does not resolve to ≤ Grade 1 within 12 weeks of last dose or inability to reduce prednisone or equivalent to ≤ 10 mg per day within 12 weeks.
Diarrhea Grade 4 *Life-threatening consequences; urgent intervention indicated*		Permanently discontinue study regimen
Colitis Grade 2-3 *G2: Abdominal pain; mucus or blood in stool* *G 3: Severe or persistent abdominal pain; fever; ileus; peritoneal signs*	*Pembrolizumab (ir)*	• Hold pembrolizumab until toxicity resolves to ≤ Grade 1 and corticosteroid dose is < 10 mg/day. • Discontinue pembrolizumab if toxicity does not resolve to ≤ Grade 1 within 12 weeks of last dose or inability to reduce prednisone or equivalent to ≤ 10 mg per day within 12 weeks.
Colitis Grade 4 *Life-threatening consequences; urgent intervention indicated*		Permanently discontinue pembrolizumab.
Hepatic (AST, ALT, or Increased Bilirubin)		*Follow supportive care guidelines in Section 5.9.4.6*
Grade 2 *AST or ALT Grade2: >3.0 -5.0 x ULN* *Bilirubin Grade 2: >1.5 -3.0 x ULN*	*Pembrolizumab (ir)* *GTx-024*	• Hold study drugs until toxicity resolves to ≤ Grade 1 and corticosteroid dose is < 10 mg/day. • Resume GTx-024 at the same dose or lower dose per investigator discretion. Resume pembrolizumab per protocol. • Discontinue study regimen if toxicity does not resolve to ≤ Grade 1 within 12 weeks of last dose.
Grade 3-4 AST or ALT G3: >5.0 -20.0 x *ULN* *Bilirubin G3: >3.0 -10.0 x ULN* *AST or ALT G4: >20.0 x ULN* *Bilirubin G4: >10.0 x ULN*		• Permanently discontinue study regimen. • For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by ≥ 50% relative to baseline and lasts for at least 14 days then patients should be discontinued from protocol therapy.

Toxicity	Agent Most Likely Attributed to	Dose delay/ modification guidelines
Metabolism and Nutrition		
Type 1 Diabetes Mellitus (if new onset) or Grade 3-4 hyperglycemia *Hyperglycemia Grade 3: >250-500 mg/dL; > 13.9-27.8 mmol/L; hospitalization indicated* *Hyperglycemia Grade 4: > 500 mg/dL; > 27.8 mmol/L; life-threatening consequences*	*Pembrolizumab (ir)*	• Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure. • *Follow supportive care guidelines in Section 5.9.4.3* • Resume pembrolizumab when patients are clinically and metabolically stable
Hypercalcemia Grade 2-4 *G2: Corrected serum calcium of > 11.5-12.5 mg/dL; >2.9-3.1 mmol/L; ionized calcium > 1.5-1.6 mmol/L; symptomatic* *G3: Corrected serum calcium of > 12.5-13.5 mg/dL; >3.1-3.4 mmol/L; ionized calcium > 1.6-1.8 mmol/L; hospitalization indicated* *G4: Corrected serum calcium of > 13.5 mg/dL; >3.4 mmol/L; ionized calcium > 1.8 mmol/L; life-threatening consequences*	*GTx-024*	• Hold GTx-024 until toxicity resolves to ≤ Grade 1. • Resume GTx-024 at the same dose or lower dose per investigator discretion.
Hypophysitis		*Follow supportive care guidelines in Section 5.9.4.4*
Hypophysitis-Grade 2-4 (i.e. Symptomatic)	*Pembrolizumab (ir)*	• Hold pembrolizumab until toxicity resolves to ≤ Grade 1 and corticosteroid dose is < 10 mg/day. • Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted. • Discontinue pembrolizumab if toxicity does not resolve to ≤ Grade 1 within 12 weeks of last dose or inability to reduce prednisone or equivalent to ≤ 10 mg per day within 12 weeks.
Hyperthyroidism		*Follow supportive care guidelines in Section 5.9.4.5*
Hyperthyroidism-Grade 3	*Pembrolizumab (ir)*	• Hold pembrolizumab until toxicity resolves to ≤ Grade 1 and corticosteroid dose is < 10 mg/day. • Discontinue pembrolizumab if toxicity does not resolve to ≤ Grade 1 within 12 weeks of last dose or inability to reduce prednisone or equivalent to ≤ 10 mg per day within 12 weeks.
Hyperthyroidism-Grade 4		Permanently discontinue pembrolizumab
Hypothyroidism		*Follow supportive care guidelines in Section 5.9.4.5*
Hypothyroidism- Any Grade	*Pembrolizumab (ir)*	• Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted.
Infusion Reaction		*Follow supportive care guidelines in Section 5.9.4.8*

Toxicity	Agent Most Likely Attributed to	Dose delay/ modification guidelines
Infusion Reaction-Grade 2	*Pembrolizumab (ir)*	• Hold pembrolizumab until toxicity resolves to ≤ Grade 1. • If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). • Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. • Permanently discontinue pembrolizumab if toxicity develops despite adequate premedication.
Infusion Reaction-Grade 3-4		Permanently discontinue pembrolizumab.
Pneumonitis		*Follow supportive care guidelines in Section 5.9.4.1*
Pneumonitis-Grade 2	*Pembrolizumab (ir)*	• Hold pembrolizumab until toxicity resolves to ≤ Grade 1 and corticosteroid dose is < 10 mg/day. • Discontinue pembrolizumab if toxicity does not resolve to ≤ Grade 1 within 12 weeks of last dose or inability to reduce prednisone or equivalent to ≤ 10 mg per day within 12 weeks. • **Permanently discontinue protocol therapy if Grade 2 pneumonitis recurs**.
Pneumonitis-Grade 3-4		Permanently discontinue pembrolizumab
Renal Failure or Nephritis		*Follow supportive care guidelines in Section 5.9.4.7*
Grade 2 *Serum Creatinine>1.5-3.0X baseline; >1.5X-3.0 X ULN*	*Pembrolizumab (ir)*	• Hold pembrolizumab until toxicity resolves to ≤ Grade 1 and corticosteroid dose is < 10 mg/day. • Discontinue pembrolizumab if toxicity does not resolve to ≤ Grade 1 within 12 weeks of last dose or inability to reduce prednisone or equivalent to ≤ 10 mg per day within 12 weeks.
Grade 3-4 *Grade 3: Serum Creatinine > 3.0 X baseline; >3.0-6.0 X ULN* *Grade 4: Serum Creatinine > 6.0 X ULN*		Permanently discontinue pembrolizumab

Toxicity	Agent Most Likely Attributed to	Dose delay/ modification guidelines
Cardiac		*Follow supportive care guidelines in Section 5.9.4.9*
Myocarditis Grade 2 *Symptoms with mild to moderate activity or exertion*	*Pembrolizumab (ir)*	• Hold pembrolizumab until toxicity resolves to ≤ Grade 1 and if applicable, corticosteroid dose is < 10 mg/day. • Discontinue pembrolizumab if toxicity does not resolve to ≤ Grade 1 within 12 weeks of last dose or if applicable, inability to reduce prednisone or equivalent to ≤ 10 mg per day within 12 weeks
Myocarditis ≥ Grade 3 *G3: Severe with symptoms at rest or with minimal activity or exertion; intervention indicated* *G4: Life-threatening consequences; urgent intervention indicated (e.g., continuous IV therapy or mechanical hemodynamic support)*		Permanently discontinue pembrolizumab
Skin and subcutaneous		
Steven-Johnson Syndrome ≥ Grade 3 *G3: Skin sloughing covering <10% BSA with associated signs (e.g., erythema, purpura, epidermal detachment and mucous membrane detachment)* *G4: Skin sloughing covering 10 - 30% BSA with associated signs (e.g., erythema, purpura, epidermal detachment and mucous membrane detachment)*	*Pembrolizumab (ir)*	• Hold pembrolizumab. • Refer patient to specialized care for assessment and treatment. • If Steven-Johnson Syndrome is confirmed, permanently discontinue pembrolizumab.
Toxic epidermal necrolysis ≥ Grade 4 *G4: Skin sloughing covering >=30% BSA with associated symptoms (e.g., erythema, purpura, or epidermal detachment)*	*Pembrolizumab (ir)*	• Hold pembrolizumab and confirm toxic epidermal necrolysis. • Refer patient to specialized care for assessment and treatment. • If toxic epidermal necrolysis is confirmed, permanently discontinue pembrolizumab.

Toxicity	Agent Most Likely Attributed to	Dose delay/ modification guidelines
Other unspecified toxicities related to <u>GTx-024 only</u>		
≥ Grade 3	*GTx-024*	• Dose reduce (option 1) OR hold drug for up to 7 days (option 2) per investigator discretion *Option 1: Dose reduction* • Re-challenge with 18 mg or maintain at 9 mg per investigator discretion once the AE resolves to ≤ Grade 1 *Option 2: Hold drug for 7 days* • After 7 days rechallenge at the same or lower dose of GTX-024 OR discontinue GTx-024
Other unspecified toxicities related to <u>Pembrolizumab only</u>		
Intolerable or persistent Grade 2	*Pembrolizumab*	• Hold pembrolizumab at physician discretion. • Permanently discontinue pembrolizumab for persistent Grade 2 adverse reactions for which treatment with pembrolizumab has been held, that do not recover to ≤ Grade 1 within 12 weeks of the last dose.
Grade 3 or Severe		• ***Non-irAE*:** Hold pembrolizumab until toxicity resolves to ≤ Grade 1. *OR* • ***irAE:*** Hold pembrolizumab until toxicity resolves to ≤ Grade 1 and corticosteroid dose is < 10 mg/day. • Discontinue pembrolizumab if toxicity does not resolve to ≤ Grade 1 within 12 weeks of last dose or inability to reduce prednisone or equivalent to ≤ 10 mg per day within 12 weeks. • **Permanently discontinue for any severe or Grade 3 drug-related AE that recurs, or any life- threatening event.**
Grade 4		Permanently discontinue pembrolizumab
Other unspecified toxicities related to <u>both agents</u>		
Intolerable or persistent Grade 2	*Pembrolizumab* GTx-024	• Hold study drugs at physician discretion. • Permanently discontinue study regimen for persistent Grade 2 adverse reactions for which treatment with study agents has been held, that do not recover to ≤ Grade 1 within 12 weeks of the last dose.
Grade 3 or Severe		• ***Non-irAE*:** Hold study drugs until toxicity resolves to ≤ Grade 1. OR • ***irAE:*** Hold pembrolizumab until toxicity resolves to ≤ Grade 1 and corticosteroid dose is < 10 mg/day. • Resume GTx-024 at the same dose or lower dose per investigator discretion. Resume pembrolizumab per

Toxicity	Agent Most Likely Attributed to	Dose delay/ modification guidelines
		protocol. • Discontinue study regimen if toxicity does not resolve to ≤ Grade 1 within 12 weeks of last dose or inability to reduce prednisone or equivalent to ≤ 10 mg per day within 12 weeks. • **Permanently discontinue for any severe or Grade 3 drug-related AE that recurs, or any life- threatening event.**
Grade 4		Permanently discontinue study regimen
Other unspecified toxicities –UNRELATED to Protocol Therapy		
Any Grade	UNRELATED	• Maintain treatment with study agent(s). • Interruption of protocol therapy is permitted if the investigator consults with the Study PI to determine that this is in the best interest of the participant.

7.0 DATA & SAFETY MONITORING PLAN, ADVERSE EVENT AND UNANTICIPATED PROBLEM REPORTING

7.1 COH Data and Safety Monitoring

Definition of Risk Level

This is a Risk Level 4 study as defined in the City of Hope Institutional Data and Safety Monitoring Plan. This determination was made because the study involves a COH IND.

Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) is responsible for monitoring the data and safety of this study. The PMT consists of the Principal Investigator (PI), Biostatistician, Research Protocol Nurse, and Clinical Research Coordinator.

The PMT is required to submit periodic status reports (i.e., the PMT Report) according to the frequency prescribed in the City of Hope Institutional Data and Safety Monitoring Plan. Important decisions made during PMT meetings (i.e., dose escalation, de-escalation, etc.) only need to be noted in the PMT Report submitted to the Data and Safety Monitoring Committee (DSMC).

Adverse Events and Serious Adverse Events

The PI will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events.

Adverse Event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after protocol specific intervention, whether or not it is considered to be related to the protocol intervention.

Reporting Non-serious Adverse Events – Adverse events will be collected after the patient is given the study treatment or any study related procedures. Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the PMT Report.

Serious Adverse Event (SAE) [per 21 CFR 312.32] - defined as *any expected* or *unexpected adverse event*s that result in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Reporting Serious Adverse Events - begins after study treatment or any study related procedures. All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to the approved City of Hope's Institutional policy. Serious Adverse Events that require expedited reporting will be submitted electronically using iRIS.

Adverse Event Name and Severity

The PI will determine the adverse event name and severity (grade) by using the CTCAE version 4.0.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event, OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Unexpected Adverse Event [21 CFR 312.32 (a)] – An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Adverse Event Attribution

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure.

Definite - The AE is clearly related to the investigational agent or study procedure and unrelated to any other cause.

Probable - The AE is likely related to the investigational agent or study procedure and unlikely related to other cause(s).

Possible -The AE may be related to the investigational agent or study procedure and may be related to another cause(s).

Unlikely -The AE is doubtfully related to the investigational agent or study procedure and likely related to another cause(s).

Unrelated -The AE is clearly not related to the investigational agent or study procedure and is attributable to another cause(s).

Expedited AE Reporting Guidelines

Each AE will be assessed to determine if it meets the criteria for expedited reporting. Adverse event reporting is to occur according to the site's specific IRB guidelines, and as outlined in this Section.

Expedited Adverse Event Reporting to Local IRB

Sites are to report to their local IRB per their site's specific institutional and IRB guidelines. As soon as possible, non-COH sites will provide to the DCC copies of the IRB submission and corresponding IRB response.

Expedited Adverse Event Reporting to DCC/ Study PI

- Adverse events that meet the specified guidelines below are to be reported to the DCC and Study PI within the timelines and per the procedures in the sections that follow.

- Report the following per Table 7.8.2 to the DCC /Study PI **within 24 hours** of being aware that the event met reporting criteria:

Expedited Reporting Guidelines

Time point	What to report expeditiously
From the signing of the consent to study completion	All unanticipated problems
From time of signing of the consent to Day 1 of protocol therapy	All SAEs related to protocol procedures
From Day 1 of protocol therapy through (i) 30 days post-last dose visit **AND** (ii) 100 days post last dose visit or until initiation of a new anticancer therapy.	• All SAEs regardless of relationship to protocol therapy, study procedure, underlying disease or concomitant treatment. • AEs that meet the definition of an unanticipated problem • Potential drug induced liver injury • Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug • Secondary malignancy • Pregnancy and lactation • Overdose • Grade 3/4 infusion reactions • Discontinuation of protocol therapy due to unusual or unusually severe AE considered related to study agents.
After the 100 days post last dose visit (i.e. follow-up)	• All SAEs that are considered possibly, probably, or definitely related to therapy. • Pregnancy and lactation (up to 7 months post last dose of nivolumab)
Note: All reportable events will require follow up until stabilization or resolution per the agreement of the Study PI.	

7.1.1 Expedited reporting to the DCC/Study PI – Non COH Sites

1. Document/describe AE/UP on each of the following:
   a. MedWatch 3500A or local IRB submission document*
      i. MedWatch 3500A is downloadable form at http://www.fda.gov/medwatch/getforms.htm
      ii. *The local IRB submission document may be used if the document template is approved by the DCC
   b. Expedited Reporting Coversheet
      i. The Expedited Reporting Coversheet is found in Appendix G. A modifiable Microsoft Word document is also available from the DCC. An electronic signature on the document will be accepted.
2. Scan and email above documents to DCC@coh.org with the subject title as "Yuan Pembro GTX-024 SAE".
   a. All expedited reports received at this account are forwarded immediately to the Study PI, and to DCC personnel.

b. While not required, if available and applicable, please also include the local IRB submission for this event in the submission.

3. If an email receipt from DCC personnel is not received within one working day, please call 626-256-4673 x 83968 and/or email DCC@coh.org.

COH Held IND

Serious Adverse Events meeting the requirements for expedited reporting to the Food and Drug Administration (FDA), as defined in 21 CFR 312.32, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting.

The criteria that require reporting using the Medwatch 3500A are:

- Any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]
- Any adverse experience associated with use of the drug that is both serious and unexpected must be submitted no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]
- Any follow-up information to a study report shall be reported as soon as the relevant information becomes available. [21 CFR 312.32(d)(3)]

The COH PI or designee will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the approved City of Hope's Institutional policy.

Deviations and Unanticipated Problems

Deviation - A deviation is a divergence from a specific element of a protocol that occurred without prior IRB approval. Investigators may deviate from the protocol to eliminate immediate hazard(s) for the protection, safety, and well-being of the study subjects without prior IRB approval. For any such deviation, the COH PI will notify the COH DSMC and COH IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy.

Single Subject Exception (SSE)

An SSE is a planned deviation, meaning that it involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific patient. It is a deviation that is anticipated and receives prior approval by the PI and the IRB. The SSE must be submitted as a "Single Subject Exception Amendment Request" via iRIS in accordance with COH IRB guidelines and the Clinical Research Protocol Deviation policy. An IRB approved SSE does not need to be submitted as a deviation to the DSMC.

Unanticipated Problem (UP) – Any incident, experience, or outcome that **meets all three** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**

2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

Any UP that occurs during study conduct will be reported to the COH DSMC and COH IRB in accordance with the City of Hope's Institutional policy using iRIS.

COH Held IND

The Office of IND Development and Regulatory Affairs (OIDRA) will assist the COH PI in reporting the event to the Food and Drug Administration (FDA).

7.2 Events of Special Interest Requiring Expedited Reporting

7.2.1 Definition of an Overdose

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 overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an AE(s) is associated with ("results from") the overdose of a pembrolizumab, 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 "accidental or intentional overdose without adverse effect."

7.2.2 Pregnancy and Lactation

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a participant (spontaneously reported to them) that occurs during the trial; See Table 7.3.

Efforts must be made by the investigator to follow the outcome of pregnancy outcome per institutional policies.

Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events).

If the pregnancy continues to term, the outcome (health of infant) must also be reported; weight and length of child, Apgar scores (with an explanation of score <10), and congenital abnormalities. If the new-born is healthy additional follow-up is not necessary.

7.2.3 Abnormal liver function tests

Liver function tests that meet the following criteria as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing must be reported expeditiously to Merck and/or GTx.

7.2.3.1 Merck:

- An elevated AST or ALT ≥ 3X ULN **in combination with** total bilirubin ≥ 2X ULN **and** an alkaline phosphatase lab value that is < 2X ULN.

7.2.3.2 GTx:

- An elevated AST or ALT ≥ 3X ULN **in combination with** total bilirubin ≥ 2X ULN
- An elevated AST or ALT ≥ 3X ULN **in combination with** clinical jaundice
- Note: for participants with liver metastasis and elevated AST or ALT > 3X ULN at baselines, the values will not be reported as an SAE if ≤ 5X ULN.

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.

7.3 Reporting to Merck and GTx

The City of Hope PI or designee will forward reports to Merck and GTx in a timely fashion per below stated guidelines.

7.3.1 Reporting to Merck

1. The following events will be reported using a MedWatch form in an expedited manner to Merck Global Safety **within 24 hours** of being aware of the event (via OIDRA) (Table 7.3).
2. SAE reports and any other relevant safety information will be forwarded to the Merck Global Safety facsimile number 215 993-1220 (Attn: Worldwide Product Safety).
3. Copies of all FDA reports cross-referencing the IND will also be submitted to Merck (Attn: Worldwide Product Safety; FAX 215 993-1220) at time of FDA submission (via OIDRA).
4. Report to Merck aggregate safety information every 3 months at time of COH PMT report.
5. Participating sites will report these events directly to the COH PI and the DCC who in turn will report to Merck.

7.3.2 Reporting to GTx

1. The following events will be reported using a MedWatch form in an expedited manner to GTx Inc. **within 24 hours** of being aware of the event (via OIDRA) (Table 7.3).
2. SAE reports and any other relevant safety information will be forwarded via facsimile or email (FAX: 001 866 966 2970 or email: sae@cmedresearch.com).
3. Copies of all FDA reports cross-referencing the IND will also be submitted to GTx Inc. (FAX: 001 866 966 2970 or email: sae@cmedresearch.com) at time of FDA submission (via OIDRA).
4. Report to GTx Inc. aggregate safety information every 3 months at time of COH PMT report.
5. Participating sites will report these events directly to the COH PI and the DCC who in turn will report to GTx.

Table 7.3 Expedited reporting to Merck and GTx

Time point	What to report to Merck and GTx
Screening up to Day 1 of protocol therapy	• Pregnancy and lactation

Time point	What to report to Merck and GTx
	• Any reason for not starting Day 1 of protocol therapy
For the time period beginning at treatment through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier **All reportable events will require follow up until stabilization or resolution per the agreement of the Study PI.**	• All SAEs regardless of relationship to protocol therapy, study procedure, underlying disease or concomitant treatment. • Death due to any cause other than progression of the cancer under study • All AEs that meet the definition of a UP • Overdose (Section 7.2.1) of either agent • Pregnancies and lactation (Section 7.2.2) • Abnormal liver function tests (Section 7.2.3)
For participants yet to initiate anti-cancer therapy: From Day 1 of therapy up to 120 days post-last pembrolizumab dose	• Pregnancies and lactation (Section 7.2.2)
Post Safety follow-up to removal from study	• All SAEs that are considered possibly, probably, or definitely related to pembrolizumab.

8.0 AGENT INFORMATION

8.1 Pembrolizumab

Please refer to the IB for a detailed description. Pembrolizumab is FDA approved for metastatic melanoma, metastatic NSCLC whose tumors express PD-L1 and for patients with recurrent or metastatic HNSCC with disease progression on or after platinum-containing chemotherapy.

8.1.1 Other Names

KEYTRUDA®, MK-3475.

8.1.2 Description and Molecular Weight

Type	IgG4 kappa monoclonal
Source:	Humanized (from mouse)
Target:	PD-1 receptor
Molecular weight:	147 kDa

8.1.3 Mechanism of Action

Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T cell proliferation and cytokine production. Upregulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. Pembrolizumab is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.

8.1.4 Pharmacokinetics

Half-life elimination: 26 days (per package insert).

8.1.5 Human Toxicity

See Section 6.1.1.

8.1.6 Formulation

Pembrolizumab is provided in 50 mg lyophilized powder for reconstitution, or a 100 mg/4 mL solution, in single-use vials.

8.1.7 Storage and Stability

8.1.7.1 Non-diluted product

The 50 mg lyophilized product and 100 mg/4 mL solution should be stored between 2 °C -8°C.

8.1.7.2 Reconstituted and diluted solutions

Pembrolizumab contains no preservative, hence reconstituted, diluted solutions should not be stored at room temperature for more than 4 hours after preparation. This includes storage of reconstituted vials, storage of infusion solutions in the IV bag and the duration of infusion.

Under refrigeration (2°C - 8°C) diluted solutions should not be stored for more than 24 hours from the time of preparation. If refrigerated, allow the diluted solution to come to room temperature prior to administration. **Do not freeze**.

8.1.8 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the agent.

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

8.1.9 Preparation

Add 2.3 mL of Sterile Water for Injection, USP by injecting the water along the walls of the vial (not directly on the lyophilized powder), resulting in a concentration 25 mg/ml. Swirl the vial slowly. Allow up to 5 minutes for the bubbles to clear. Do not shake the vial. Visually inspect the reconstituted solution for particulate matter and discoloration prior to administration. Reconstituted pembrolizumab is a clear to slightly opalescent, colorless to slightly yellow solution. Discard reconstituted vial if extraneous particulate matter other than translucent to white proteinaceous particles is observed. Alternatively, Pembrolizumab solution provided at 100mg/4mL (25mg/mL) will be used.

Withdraw the required volume from the vial(s) of pembrolizumab and transfer into an intravenous (IV) bag containing 0.9% Sodium Chloride Injection, USP. Mix diluted solution by gentle inversion.

8.1.10 Administration

The final concentration of the diluted solution should be 1 mg/mL - 10 mg/mL and administered IV over 30 minutes, through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter.

See also Section 5.4.1.

8.1.11 Supplier

The agent will be supplied free of charge by Merck.

8.1.12 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using a drug accountability log.

8.1.13 Destruction and Return

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to participants, and the amount remaining at the conclusion of the trial.

Any unused agent at the end of the study, expired agent, and damaged agent will be destroyed according to applicable federal, state, local and institutional guidelines and procedures. Destruction will be documented in a drug accountability log.

Prior to the destruction of Pembrolizumab, the DCC should be notified and an acknowledgement to proceed from the DCC should be received.

8.2 GTx-024

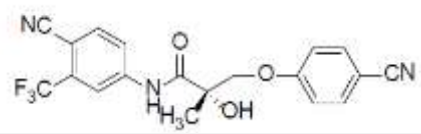
Please refer to the IB for a detailed description. GTx-024 has not been approved for any FDA indication.

8.2.1 Other Names

Ostarine®, Enobosarm, MK-2866, S-22

8.2.2 Description and Molecular Weight

Structural formula:



Empirical formula:	$C_{19}H_{14}F_3N_3O_3$
Molecular weight:	389.33 g/mol
Chemical Name:	(S)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2-hydroxy-2-methylpropanamide

8.2.3 Mechanism of Action

GTx-024 is an oral nonsteroidal selective androgen receptor modulator (SARM). It is hypothesized that GTx-024 may alter the binding of coactivators and repressors to the androgen receptor in a tissue specific manner. Clinically, GTx-024 has been demonstrated to have potent muscle and bone anabolic activity with minimal androgenic effects.

8.2.4 Pharmacokinetics

Refer to the IB.

8.2.5 Human Toxicity

See Section 6.1.2.

8.2.6 Formulation

GTx-024 3.0 mg Softgels will be supplied as opaque, white to off-white, size 5, oval Softgels with “GTx” imprinted in black ink on the outer shell containing 3.0 mg of GTx-024. The liquid Softgel fill is composed of GTx-024 dissolved in polyethylene glycol 400.

8.2.7 Storage and Stability

Recommended storage will be at controlled room temperature 15°C-25°C (59°F-77°F), with excursions permitted to 30°C (86°F), protected from moisture.

8.2.8 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the agent.

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

8.2.9 Administration

See Section 5.4.2.

8.2.10 Supplier

The agent will be supplied free of charge by GTx Inc.

8.2.11 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using a Drug Accountability Record.

8.2.12 Destruction and Return

The investigator is responsible for keeping accurate records of the clinical supplies received from GTx Inc. or designee, the amount dispensed to participants, and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, 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 provided that appropriate records of disposal are kept.

Prior to the destruction of GTx-024, the DCC should be notified and an acknowledgement to proceed from the DCC should be received.

9.0 CORRELATIVE/SPECIAL STUDIES

9.1 Tumor Tissue Studies

An overview of collection, processing, and analysis details are shown in Table 9.1.

Table 9.1 Tumor tissue studies overview

Tissue Type	Timepoint of collection	Materials per timepoint	Material Submitted	Laboratory Performing the Analysis	Type of Laboratory Analysis
Formalin fixed (FFPE) tissue from core needle or surgical specimen or other procedure	Baseline	4 unstained slides	ProbeOn Plus slides	QualTek	PD-L1 expression
	• Baseline • *Optional*: during treatment • End of protocol therapy	5 unstained slides	Slides	Dr. Peter Lee at COH	Changes in immune and stromal cells after therapy
		5 unstained slides	Slides	Holly Yin, COH	Nanostring Expression analysis
		10 unstained slides	Slides	Tempus	Tempus DNA + RNA sequencing
Fresh tissue	• Baseline • *Optional*: during treatment • End of protocol therapy	2-3 cores of 18 gauge needle biopsy specimen, or cancer cells collected from pleural fluid/ascites, or other biopsy /surgical samples.	Fresh tumor in PBS or RPMI media on ice (See Section 9.1.2)	COH Biorepository Core	Tumor heterogeneity and genomic analysis

- *Baseline tissue*:
  - *FFPE (up to 42 days old from Day 1 of protocol therapy)*: A sample should be submitted within 14 days post-registration. If a recently-obtained sample is unavailable an archived metastatic specimen not previously irradiated may be submitted upon agreement from the Study PI.
  - *Fresh tissue (after consent)*: If a standard of care procedure is to be performed, attempts should be made to take extra tissue for research.
- *During treatment from partial responders (optional)*: Submit fixed and if available, fresh tissue for research if the participant is willing.
- *End of protocol therapy*: FFPE and if also available fresh tissue obtained for standard of care purposes to be submitted within 1 month of discontinuing therapy.

9.1.1 Labeling

Label samples with COH protocol #, subject ID and timepoint of collection (e.g. baseline or EOT). A sample manifest will be maintained by the PI or designee.

9.1.2 Distribution to laboratories for analysis

Fresh tissue samples in PBS or RPMI media should be submitted to Karen Miller or designee at the COH Biorepository Core immediately on ice within 2 hours of collection. If possible, at **least 1 day advance notice** should be given to Karen Miller or designee.

FFPE samples and PBMCs: If applicable, the COH Biorepository Core will distribute fixed tissue/ diagnostic tissue to internal and external laboratories for analysis (see Table 9.1). FFPE slides will be stored in locked 4°C refrigerator in Beckman and PBMCs will be stored in locked -80°C freezer in Dr. Tim Synold's lab until batch-shipped to Tempus. Tumor DNA and RNA sequencing will be performed at Tempus. No identifiable information will be sent to Tempus and all unused specimen including DNA/RNA extract, FFPE slides and fresh frozen specimen will be returned to COH investigator. Tempus will not store nor conduct any other research with the unused or leftover tissue. Tempus: 15 FFPE +1 H&E for NGS and -PD (20% tumor -- ratio of tumor nuclei to benign nuclei; -25mm^2), plus normal sample (buffy coat from 2 x 8 ml tubes whole blood).

9.2 Overview of Peripheral Blood Studies

Blood samples will be collected from an indwelling venous catheter or by venipuncture for the below stated analyses (see Table 9.2).

Table 9.2 Peripheral blood studies overview

Timepoint of collection	Section	Volume per Timepoint	Tube Type	Processing/ Receiving Laboratory	Laboratory/ Vendor Performing the Analysis	Type of Laboratory Analysis
• Cycle 1 Day 1 (Baseline) • Cycle 2 Day 1 • Cycle 4 Day 1 • Cycle 8 Day 1 • End of treatment	Section 9.3	30 mL	Heparin green-top	Analytical Pharmacology Core Facility (APCF) at COH Duarte campus Or Designated laboratory at each site	Plasma cytokines: Dr. Tim Synold (APCF) PBMC: Dr. Peter Lee (COH Immuno-oncology Core)	Plasma and PBMC isolation to assess serial cytokines and immune response
		10 mL	Purple-top	COH APCF for initial processing Or Designated laboratory at each site	Dr. Tim Synold	Processing and storage for Flow cytometry and Tempus PMBC DNA sequencing (as a control for tumor DNA sequence)

9.3 Blood for immune correlatives

9.3.1 COH APCF Notification, Blood Collection and Labeling

NOTE: It is **highly recommended** that non-Duarte sites arrange for a courier **prior to the participant's clinic visit** given the tight turnaround time for sample delivery to the COH APCF and COH Biorepository

(Duarte Campus). For non COH sites, please notify the local blood sample processing laboratory for sample delivery.

Notification to COH APCF of Pending Collection	Tube Type	Labeling and Collection Details	Post-collection Instructions
• Notify **at least one** day in advance) • **Send calendar invite via e-mail**: Leslie Smith-Powell (Lsmith-Powell@coh.org) or Dauhlian Chi (dchi@coh.org)	Green-top	1. Label tubes with COH protocol # (For non-COH sites, local protocol #), subject ID, actual collection time in 24-hour format, institution (for CP sites), and timepoint of collection (e.g. D1C1 for Day1 of Cycle 1), and if applicable patient initials. 2. Timepoints of collection are stated in Table 9.2. 3. Blood samples will be collected from an indwelling venous catheter or by venipuncture 4. Invert tubes eight times after collection. 5. **Green-top tube(s): Immediately** place the tubes **on ice**.	• **Efforts should be made to promptly** deliver the blood samples to the COH APCF, Shapiro room 1042 (Duarte Campus) for processing **within 1-2 hours (± 30 minutes).** - **If delivery of samples fall outside of the above specified window, (1)** a note should be made to the study regulatory binder and other applicable documents (and a reason provided) **AND (2)** the reason should also be communicated to the Study PI/ designee. • **Deliver green top tubes on ice or 4°C.** • **For COH Community Practice sites and non-COH sites, courier deliveries** should be made to the below address: Dr. Tim Synold Analytical Pharmacology Core Facility Shapiro 1042 City of Hope National Medical Center 1500 E. Duarte Road Duarte, CA 91010
• Notify **at least one** day in advance) Send calendar invite via e-mail: **Biospecimen coordinator**	Purple-top	1. Label tubes with COH protocol # (For non-COH sites, local protocol #), subject ID, actual collection time in 24-hour format, institution (for CP sites), and timepoint of collection (e.g. D1C1 for Day1 of Cycle 1), and if applicable patient initials. 2. Timepoints of collection are stated in Table 9.2. 3. Blood samples will be collected from an indwelling venous catheter or by venipuncture 4. Invert tubes eight times after collection. 5. **Purple-top tube(s): Immediately** place the tubes **on ice**.	• **Efforts should be made to promptly** deliver the blood samples to the COH Biorepository Core (Duarte Campus) for processing **within 1-2 hours (± 30 minutes).** • **Deliver purple top tubes on ice or 4°C.** • **For COH Community Practice sites and non-COH sites, courier deliveries** should be made to the below address: Shabnam Pathan COH Biorepository Core City of Hope National Medical Center 1500 E. Duarte Road Duarte, CA 91010

Notification to COH APCF of Pending Collection	Tube Type	Labeling and Collection Details	Post-collection Instructions

9.3.2 Processing of samples

Keep blood samples on a rocker set at low speed to mimic circulation and avoid clot formation until processing. Efforts should be made to process the samples **within 4 hours** of collection.

Tube Type and Volume	Processing Details		Downstream assay
Green-top (21 mL)	Plasma	1. For plasma preparation **use 14 mL whole blood** from green-top tubes. 2. Centrifuge for 10 minutes at 1800 x g at 4 °C. 3. The resulting upper plasma layer from each tube will be drawn up sequentially into a sterile 5 mL syringe and pushed through a sterile 0.2/0.8 micron disposable filter. a. **Save** the plasma-depleted portion **for isolation of PBMC (see below)**. 4. The filtered plasma will then be transferred in 500 µL aliquots into multiple appropriately-labeled Starstedt microfuge tubes. 5. To **one aliquot**, add 0.5 mL glycerol/0.02% sodium azide solution to dilute the plasma 50/50 v/v. Keep the diluted plasma sample at -20˚C and do not freeze. 6. All the remaining plasma aliquots will be stored frozen at -80˚C until use.	Cytokine analysis (COH APCF)
	Peripheral blood mononuclear cells (PBMC)	7. Any blood remaining in the two green-top tubes used to prepare plasma above will be diluted 1:1 with Hank's Balanced Salt Solution (or equivalent) and combined with the whole blood from the unused green-top tube in a sterile 50 ml conical centrifuge tube. 8. PBMC will then be isolated by Ficoll-gradient per **COH APCF procedures**. 9. Isolated PBMC will be aliquoted and stored at -80˚C until use.	FACS (COH ImmueOncology Core)
Purple-top (~10 mL)	Plasma and Plasma depleted whole blood cells (PDWB)	1. Centrifuge for 10 minutes at 820 x g at room temperature. 2. Remove the tubes from the centrifuge. Do not disturb the cellular layer. 3. Extract plasma carefully. a. **Do not disturb** the **buffy coat** while pipetting plasma; leave ~3-4mm of plasma behind to ensure the buffy coat is undisturbed. 4. Freeze plasma at -80°C in 1-2 mL aliquots. Do not fill tubes beyond 70% capacity. 5. Mix the remaining PDWB. 6. Freeze PDWB at -80°C in 1-2 mL aliquots.	Sequencing-cell free circulating tumor DNA and germline DNA (COH Dr. Synold's Lab)

9.3.3 Sample maintenance and distribution/shipping to laboratories

A sample manifest will be maintained by the PI or designee. Samples will be maintained at APCF until distribution to internal collaborators/external vendors. Samples will be batch shipped to non-COH vendors.

Procedure for plasma/ buffy coat isolation from EDTA tubes: Collect blood in tube until filled. Mix tube by inverting 3-5 times and transport to Dr. Synold's lab for processing. Samples must be processed within one hour of collection to minimize the possibility of white blood cell lysis. For processing, centrifuge the samples at 820g for 10 min at room temperature and transfer 1 mL aliquots of the plasma to sterile 2 mL microtubes. Freeze plasma aliquots at -80 °C. Remove the Buffy coat layer into a separate pre-labeled tube and store at -80^{0}C. Record corresponding information such as collection time, freezing time, and aliquot number.

Flow Cytometry and single cell RNA sequencing: Peripheral blood mononuclear cells (PBMCs) will be isolated from the blood of patients per manufacturer protocol. For flow cytometry, cells will be stained for immune subtype markers and sorted by fluorescence-activated cell sorting (FACS) or CyTOF. For single cell sequencing, the cells will be loaded in Smarter ICELL8 single cell mRNA chip or the 10x, imaged via microscopy, and single cell libraries will be prepared per manufactures instructions. Next generation sequencing will be performed.

9.4 Bacteriomic profiling

16S Gut Microbiome rRNA Analysis

Gut microbiome has been associated with response to immune checkpoint inhibitors in patients with solid tumors. Fecal samples are relatively easy to collect and non-invasive. They provide an indication of the gut microbiome which may be an indicator of general health, impact drug availability, and indicate the presence of communities associated with inflammation, digestive inefficiencies, and pathogens. Monitoring the gut microbiome may allow us to predict the risk of possible side effects of immune check point inhibitors (colitis and decreased appetite) and therapeutic efficacy.

The exploratory objective to monitor the gut microbiota at baseline, on treatment and end of treatment using fecal samples. The differences in gut microbiota within and between fecal samples will be compared using alpha and beta diversity metrics based on 16S rRNA sequencing.

Stool Specimen Collection

Refer to Appendix F for more specific information on the planned analysis to be performed.

Fecal material will be collected in a Zymo Research DNA/RNA Shield Fecal Collection Tube by patients as instructed. A standard operating procedure (SOP) has been generated for stool collection, as outlined in Appendix F. A **DIET and STOOL FREQUENCY LOG - GENERAL INSTRUCTIONS** is listed in Appendix G. Stool collection kit contents are listed in Appendix H. A copy of this SOP will be provided to the patient and their understanding of the SOP will be documented by the PI.

Samples will be collected pre-treatment (within 7 days of C1D1), C5 Day 1 (+/-7 days), every 4 cycles (+/-7 days), and end of treatment (+/-7 days). Patient will bring samples on the scheduled study visits.

All samples will be collected by participants at home or during study visit. Study team will collect the samples and transport to Biospecimen Coordinator in COH Biorepository.

10.0 STUDY CALENDAR

All procedures may increase in frequency if clinically indicated.

Table 10.0 Study Activity Calendar

Protocol Activity	Screening [a]	Protocol Therapy [b, c]		EOT [f]	Follow-up		
		Cycle 1 D1 [d]	Cycle 2+ D1 [e]		Safety 30 & 90-days post [g]	Response [h]	Survival [i]
Informed Consent [j]	X						
Eligibility Criteria [k]	X						
Registration [l]	X						
Medical History [m]	X						
Physical Exam [n]	X	X	X	X	X	X	
Vital Signs [o]	X	X	X	X	X	X	
ECOG Status (Appx. A)	X	X	X	X	X	X	
Con-med review [p]	X	X	X	X	X		
AE assessment[q]	X[q]	----------X[r]----------					
Research AR testing[s]	X						
Pregnancy [t]	X [u]						
CBC w/diff, plt	X [u]	X	X	X			
Serum chemistry [v]	X [u]	X	X	X			
Urinalysis [w]	X [u]						
Fasting serum lipid profile [x]	X [u]		C3, C6 only	X			
Thyroid function [y]	X [u]		C4 then every 4 cycles				
Immune blood (green-top) [z]		X	C2, C4, C8 only	X			
ctDNA blood [aa]		X	C2, C4, C8 only	X			
Bacteriomic profiling[ll]	X		C5 then every 4 cycles	X			
Tumor tissue	X [bb]	X [cc]		X [dd]			
CT/MRI imaging [ee]	X		X [ff]	X [ee]		X	
Brain imaging [gg]	X		X [ff]	X		X	
Bone scan/ X-ray[hh]	X		X [ff]	X		X	
Response[ii]			X [ff]	X		X	
Pembrolizumab [jj]		Day 1 of each 21-day cycle					
GTx-024 [kk]		Days 1-21 of each 21-day cycle					
Pill diary [ll]		X	X	X			
Survival [i]							X

a. Screening evaluations will be performed within 28 days from start of protocol therapy, except laboratory assessments to be performed within 14 days prior to start of therapy (footnote u).

b. Protocol therapy may last up to 35 cycles, until unacceptable toxicity or confirmed disease progression (see

Section 5.6 for more comprehensive list).

c. In the absence of a delay due to toxicity, each treatment cycle lasts 21 days ± 3 days.

d. Day 1 of Cycle 1 is defined as the day of first administration of pembrolizumab. Screening evaluations performed within 14 days prior to start of protocol therapy may serve as Day 1 baseline evaluations.

e. Activities (except imaging and response assessment- footnote gg) and safety assessment review to be performed within 72 hours prior to initiation of the new cycle.

f. End of Treatment (EOT) assessments to be performed no later than 7 days after decision to end treatment (except tumor imaging- see footnote ff); assessments performed after last dose of study agent and within 7 days after the decision to end treatment may serve as End of Treatment assessments.

g. Safety visits to occur (i) 30 (-2/+7) days post-last dose of protocol therapy **AND** (ii) 90 (± 7) days post-last dose of pembrolizumab or at initiation of a new anticancer therapy (whichever occurs first). Expedited reporting will occur during this period. Safety follow-up may be extended until resolution/ stabilization of reportable AEs. **NOTE:** If a participant received at least 4 cycles of GTx-024 monotherapy beyond pembrolizumab discontinuation, then the 90 post-last dose visit is waived.

h. For participants who end protocol therapy but do not progress, Response Follow-up to occur ~ every 3 months (± 7 days) from the day of last response evaluation until progression or the initiation of a new anticancer therapy.

i. *Survival Follow-up*: For participants who progressed or initiated alternate anticancer therapy. Survival follow-up to occur bi-annually or as requested by the Study PI via medical record review, review of social security registry, or telephone call.

j. Informed consent process to be fully documented (see Section 16.4). Informed consent must occur prior to any research only (non-SOC) screening procedures.

k. Eligibility criteria are detailed in Section 3.0.

l. Registration into the COH clinical trial management system (CTMS) (e.g. MIDAS).

m. Medical history – to include a review of treatment history, any ongoing medical conditions and medical history pertaining to study eligibility and involvement.

n. The investigator or qualified designee will perform a full physical exam during the screening period. Directed physical exam should be performed as clinically indicated.

o. Vital signs: Weight, heart rate, blood pressure, respiration rate, and temperature. Height required only at baseline.

p. *Concomitant medication*: All medications starting from 28 days of prior to start of protocol therapy through to the end of the Safety Follow-up visits should be recorded in the source documents. If concomitant therapy must be changed, including over-the-counter medications or alternative therapies, the reason and name of the agent/therapy should be recorded in the eCRF and source document(s). See Section 5.9 for concomitant medication/therapy restrictions and guidelines.

q. Adverse event (AE) will be assessed using CTCAE v.4.0. SAEs related to study procedures will be recorded and reported from time of informed consent until Day 1 of protocol therapy.

r. AE recording and reporting will continue until the completion of Safety follow up or until resolution or stabilization of any reportable AEs occurring during Safety Follow up period. For events of special interest requiring expedited reporting refer to Section 7.2.

s. Participants who have undergone AR testing may use those results and do not have to have the test repeated. To request research AR testing at COH Pathology contact Dr. Daniel Schmolze (email: dschmolze@coh.org or phone: 626-256-4673 ext. 80727).

t. *Women of child bearing potential only*: Serum or urine pregnancy test.

u. Clinical labs to be performed within 14 days prior to initiating protocol therapy.

v. Serum chemistry to include albumin, alkaline phosphatase, ALT, AST, CO_2 or bicarbonate, calcium, chloride, glucose, phosphorus, potassium, sodium, magnesium, total bilirubin, direct bilirubin (if total bilirubin is elevated

above the ULN), total protein, and blood urea nitrogen (BUN).

w. Urinalysis to include glucose and protein.

x. Fasting serum lipid profile to include low-density lipoprotein, high-density lipoprotein and triglycerides.

y. Thyroid function- T3, free T4 and TSH.

z. *Immune blood* Green-top tubes (30 mL per timepoint) should be placed on ice. See Section 9.2. **NOTE**: APCF notification instructions of a pending collection at least one day before collection should occur per Section 9.3.1.

aa. *ctDNA blood (10 mL per timepoint)*: purple-top tube (10 mL per timepoint) should be placed on ice. See Section 9.2. **NOTE**: COH Biorepository Core notification instructions of a pending collection at least one day before collection should occur per Section 9.3.1.

bb. Fixed tissue (≤ 42 days old from Day 1) and if applicable, extra fresh tissue (Duarte Only) taken from a standard of care procedure to be submitted. If fixed tissue is unavailable, an archived metastatic specimen not previously irradiated may be submitted after consulting the Study PI. See Section 9.1 for details, notification of pending fresh tissue collection from Duarte participants and submission time frames.

cc. *Optional:* Research tumor tissue (fixed and if applicable fresh (Duarte Only)) from partial responders; only if the participant is willing. See Section 9.1 for details, notification of pending fresh tissue collection from Duarte participants and submission time frames.

dd. Fixed tissue and if applicable, extra fresh tissue (Duarte Only) taken from a standard of care procedure to be submitted within 1 month of discontinuing therapy. See Section 9.1 for details, notification of pending fresh tissue collection from Duarte participants and submission time frames.

ee. *CT/MRI*: chest, abdomen, pelvis, any clinically indicated sites of disease, and of bone lesions. The same technique must be performed throughout the trial. **NOTE:** If imaging was obtained within 28 days prior to the date of discontinuation, then tumor imaging at treatment discontinuation is not required.

ff. *Imaging and response*: end of Cycle 4 (- 7 days), then every 4 cycles (- 7 days).

gg. *Brain imaging*: Only if known brain metastases or if clinically indicated.

hh. *Bone scans*: Only if known history of bone metastases or if clinically indicated.

ii. *Response*: Per RECIST v 1.1 (Appendix D) and irRECIST (Appendix E). **NOTE:** Participants with confirmed radiographic progression per RECIST v1.1 who are clinically stable and do not meet irRECIST criteria for progression will continue to receive protocol therapy following consultation of the Study PI. If a biopsy is indicated following progression per RECIST, attempts should be made to take extra tissue for research purposes per Section 9.1.

jj. The dosing plan for the agents is described in Section 5.3; recommended supportive care is described in Section 5.9.4. Refer to Section 6.2 for dose delay/ modification guidelines.

kk. GTx-024 pill diary will be given to the participant and reviewed for adherence (Appendix C).

ll. Stool samples must be collected within 7 days prior to cycle 1 day 1, cycle 5 day 1 (+/- 7 days), every 4 cycles (+/- 7 days), and end of treatment (+/- 7 days).

11.0 ENDPOINT EVALUATION CRITERIA/MEASUREMENT OF EFFECT

11.1 Primary Endpoints

Safety lead-in: The safety and tolerability of the pembrolizumab plus GTx-024 combination. Safety analysis will be carried out based on toxicities assessed by CTCAE, version 4.0 criteria. AEs will be analyzed including but not limited to all AEs, SAEs, fatal AEs, and laboratory changes. Immune-related adverse events (irAE) will be collected (also see Section 12.3). See Section 12.1 for DLT definitions.

Phase 2: The primary endpoint is to assess the response rate (CR or PR via RECIST 1.1) of the combination of pembrolizumab with GTx-024 in patients with AR+ advanced TNBC (see Appendix D).

11.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints for the study are stated in Table 11.2. Since any event (toxicity-related or progression) will be counted as "progression", the feasibility and efficacy endpoints will provide useful information for future trial designs. RECIST 1.1 will be used to assess response, EFS, TTF; Kaplan-Meier estimates will be generated for overall survival (OS). Analysis to assess response and clinical benefit will be carried out using irRECIST (see Appendix E).

Table 11.2 Secondary Efficacy Endpoints

Endpoint	Patients	Definition
Progression-free survival (PFS)	All patients	Time to disease progression/ relapse or death as a result of any cause
Clinical benefit rate (CBR)	All patients	Lack of progression at 24 weeks
Duration of response (DOR)	In CR or PR patients	Time to progression or death
Overall survival (OS)	All patients	Time to death as a result of any cause
Time to treatment failure (TTF)	All patients	Time to treatment termination for any reason (progression, toxicity, death, patient preference)
Event-free survival (EFS)	All patients	Failure of treatment or death as a result of any cause

11.3 Correlative Endpoints

Proposed candidate biomarkers are listed in Table 11.3. These endpoints will be assessed at baseline and at selected timepoints during protocol therapy. Additional endpoints may be evaluated.

Table 11.3 Exploratory Endpoints to Evaluate Candidate Biomarkers by Potential Role

Tissue	Biomarkers	Pharmaco-dynamic	Predictive	Response
Tumor	AR expression profile		X	X
Tumor	Programmed death ligand 1 (PD-L1) and tumor infiltrating lymphocytes (TILs) profile, immune signatures and genomic analysis		X	X
Fresh tumor	Nanostring Expression Tumor heterogeneity and genomic analysis		X	X
Tumor/ blood	Temporal profile of TIL subsets (e.g. CD4,CD8, Treg distribution) and other immunological correlatives (e.g. TCR repertoire analysis)	X	X	X
Tumor/ blood	miRNA/mRNA profiling to evaluate changes in disease state and regulation of key immune checkpoint molecules	X	X	X
Blood	Temporal profile of tumor-derived exosomes	X	X	X

Tissue	Biomarkers	Pharmaco -dynamic	Predictive	Response
Blood	Number of circulating tumor cells (CTCs) and quantitation of circulating tumor DNA (ctDNA)	X	X	X

12.0 STATISTICAL CONSIDERATIONS

12.1 Dose-limiting toxicities

Dose limiting toxicity (DLT) is defined as any of the following that occur during the **first cycle** that are attributed as possibly, probably, or definitely related to protocol therapy for the **safety lead-in**:

- Delay > 14 days in initiating Cycle 2
- Planned dose of GTx-024 is < 75% during Cycle 1
- Death not clearly due to underlying disease or extraneous causes

Hematologic

- Grade ≥ 3 thrombocytopenia with bleeding requiring transfusion
- Grade 4 thrombocytopenia
- Grade 4 neutropenia that persists more than 5 days
- Grade 3 or 4 febrile neutropenia
  - Grade 3 febrile neutropenia: defined as ANC < 1000/ mm^3 with a single temperature of > 38.3 ^oC or a sustained temperature of ≥ 38 ^oC for > 1 hour

Non-Hematologic

- Any ≥ Grade 3 non-hematologic toxicity with the **following exceptions**:
  - Grade 3 rash that resolves to ≤ Grade 1 within 14 days with appropriate supportive therapy
  - Grade 3 myalgia, fatigue, or constipation that resolves to ≤ Grade 1 within 72 hours
  - Grade 3 electrolytes abnormality that lasts < 72 hours that is not clinically complicated, and resolves spontaneously or responds to conventional medical interventions
  - Grade 3 nausea, vomiting, or diarrhea that lasts < 72 hours with adequate antiemetic and other supportive care.
  - Grade 3 hypersensitivity and injection site reactions
  - *For patients with liver metastases who began treatment with Grade ≤ 2 hepatic transaminase*: transaminase level ≥ 10x ULN lasting ≤ 7 days.
- Hy's law: concurrent ALT/AST > 3.0 x ULN AND total bilirubin > 2.0 x ULN without initial findings of cholestasis and in the absence of any alternative cause

12.2 Evaluable Participants

Participants will be evaluable for toxicity from time of their first treatment. Participants who receive at least 1 dose of study drug will be evaluable for response.

In order for participants to be evaluable for DLT during the **safety lead-in** (See Section 5.3) during Cycle 1 participants must:

- Receive ≥ 75% of the planned dose for GTx-024 and undergo all safety monitoring assessments for Cycle 1 **OR**
- Experience a DLT.

Participants who experience a Grade 3/4 pembrolizumab related infusion reaction during Cycle 1 of the safety lead-in will be replaced.

12.3 Study Design

This is a Phase 2 single institutional trial of pembrolizumab in combination with GTx-024 for patients with histologically proven diagnosis of adenocarcinoma of breast with evidence of metastatic AR+ TNBC who have measurable disease (per RECIST 1.1 Criteria). Patients must have confirmed AR+ TNBC. Eligible patients will receive pembrolizumab 200 mg IV every 3 weeks in combination with GTx-024 at a dose of either 18 mg po or 9 mg po daily as determined by the safety lead-in (see Section 5.3).

Study treatment will continue until disease progression, unacceptable adverse event(s) (AEs), concurrent illness that prevents further administration of study treatment, investigator's decision to withdraw the subject from study treatment, consent withdrawal, becoming lost-to-follow-up, death or for administrative reasons requiring cessation of treatment.

After discontinuation of study treatment, each subject will be followed for 30 days for AE monitoring. Reportable adverse events will be collected for 90 days after the end of study treatment or until subject initiates new anticancer therapy (whichever occurs earlier).

Subjects who discontinue study treatment for reasons other than disease progression will have post-treatment follow-up for disease status every 3 months until disease progression or initiation of a non-study anticancer treatment.

All subjects will be followed by biannually (or as requested by the Study PI) via medical record review, review of social security registry, or telephone call for overall survival until consent withdrawal, becoming lost to follow-up, death or end of the study.

12.4 Sample Size Accrual Rate

Sample-size: 29 patients. The estimated accrual rate will be one patient per month for a total of 18 months.

12.5 Statistical Analysis Plan

12.5.1 Primary Objective: Response Rate

A Simon's MiniMax two-stage Phase 2 design was the original design based on the initial reported response rate of 19% with single agent pembrolizumab. However, this was based on only 27 patients. Subsequently, a study of 170 patients observed a response rate of only 5%. [67] While the original protocol design stated a 19% response rate (the single agent response rate in the first reported study) as the discouraging rate for the combination and required >2/15 responses in the first stage to continue, these rules must be changed to reflect the data from the larger and more recent study that showed a response rate of only 5% for single agent Pembrolizumab (with that same response rate applying to both PD-L1 positive and negative tumors). The later study had an upper 95% confidence limit of 9% RR, and the combined data from the two studies would result in a response rate of approximately 7%. As a result, we have determined that 9% would be a more appropriate discouraging response rate given the more recent and larger report on single agent Pembrolizumab and we consider a promising response rate a 20% increment, or a 29% response rate. With the updated information, we will continue to accrue if at least 2/15 patients response (13.3%). We will maintain the total sample-size at 29 patients. Considering the impact of mid-design changes on hypothesis testing, and the variability of outcomes from the two relevant historical studies, the updated design is based on having at least 2 out of the first 15 patients with a response, and then adding an additional 14 patients to better estimate the response rate of the combination, rather than conduct a hypothesis test. With 2+/15 with a response to

continue, this is better than the upper 95% CI for the response rate reported in the larger and more recent study, and satisfies the rule for continuing based on the SWOG planned versus attained design of Dahlberg (Green SJ and Dahlberg S (1992). Planned Versus Attained Design in Phase II Clinical Trials. *Statistics in Medicine* 11:853-862) for >1/15 responses based on a type I error of 5% and power of 85% for distinguishing between a 9% and a 29% response rate. With 29 total patients the SE of the response rate will be 9% or less.

While both drugs are well-tolerated with non-overlapping toxicities, the initial 6 patients will be followed in a safety lead-in to observe if a dose adjustment is appropriate. If in the first 6 patients we observe 2 or more patients with dose limiting toxicities (DLTs) (See Section 6.2) in the first cycle this will mandate a reduction in the starting dose for all subsequent patients. If dose level -1 has 2 patients with DLTs in the first 6, the study will hold accrual.

During the safety lead-in, we will permit only 3 patients to be a risk for first cycle toxicities at any one time.

With approximately 29 patients, we expect to have to obtain at least 12 patients with pre- and post-treatment biopsy material adequate for evaluation for AR pathway activity (AR by IHC and gene expression array) and immune profiling. The correlative studies will be used to potentially refine patient selection for future studies, and understand the role of AR pathway activities and immune changes on the activity of the combination of pembrolizumab plus GTx-024. These correlative studies are considered exploratory in the context of this limited Phase 2 study; however, 12 samples provide 80% power to detect an effective size of 0.8 (80% of the standard deviation), with a one-sided type I error of 5%.

12.5.2 Safety/tolerability

The safety and tolerability of the combination of pembrolizumab and SARM GTx-024 will be evaluated via a safety lead-in. In addition, the CBR will be evaluated at 24 weeks (by RECIST 1.1), the PFS will be evaluated, and the duration of response (time from documentation of tumor response to disease progression or death) will be evaluated.

13.0 DATA HANDLING, DATA MANAGEMENT, RECORD KEEPING

13.1 Source Documents

Source documents are original documents, data, and records (e.g., medical records, pharmacy dispensing records, recorded data from automated instruments, laboratory data) that are relevant to the clinical trial. The Site Investigator or their designee will prepare and maintain adequate and accurate source documents. These documents are designed to record all observations and other pertinent data for each patient enrolled in this clinical trial. Source documents must be adequate to reconstruct all data transcribed onto the case report forms.

13.2 Data Capture Methods and management

Data for this trial will be collected using Medidata RAVE, City of Hope's electronic capture system. Medidata RAVE is a web based, password protected system that is fully compliant with global regulatory requirements, including 21CRF Part 11 compliant.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF).

13.3 Case Report Forms/ Data Submission Schedule

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available.

The investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the investigator. All case report forms must be completed by designated study personnel. The completed case report forms must be reviewed, signed and dated by the Site Investigator or designee in a timely fashion.

All data will be collected using electronic data collection system described in Section 13.2, and will be submitted according to the timelines indicated in Table 13.3.

Table 13.3: Data Submission Schedule

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 calendar days of registration
Baseline Assessment Forms	Within 14 calendar days of registration
Treatment Forms	Within 14 calendar days of treatment administration
Adverse Event Report Forms	**Safety lead-in Cycle 1 only**: within 7 calendar days of AE assessment/notification **All other cycles of the safety lead-in and Phase 2**: Within 14 calendar days of the study visit
Response Assessment Forms	Within 10 calendar days of the response assessment
Other Assessment Forms (concomitant medications etc.)	Within 10 calendar days of the assessment
Off Treatment/Off Study Forms	Within 10 calendar days of completing treatment or being taken off study for any reason
Follow up/ Survival Forms	Within 14 calendar days of the protocol defined follow up visit

Form	Submission Timeline
	date or call

13.4 Regulatory Records

The investigator will maintain regulatory records, including updating records in accordance with Good Clinical Practice guidelines and FDA regulations.

14.0 ADHERENCE TO THE PROTOCOL

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. Protocol deviations may be on the part of the subject, the investigator, or study staff.

All deviations from the protocol must be documented in study subject source documents and promptly reported. The Study PI will report the deviation according to City of Hope's deviation policy for reporting deviations (See Section 7.0).

15.0 STUDY OVERSIGHT, QUALITY ASSURANCE, AND DATA & SAFETY MONITORING

15.1 All Investigator Responsibilities

An investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of drugs under investigation.

All investigators agrees to:

- Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when necessary to protect the safety, rights or welfare of subjects.
- Personally conduct or supervise the study (or investigation).
- Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
- Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
- Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
- Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
- Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
- Promptly report to the IRB and the Sponsor all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
- Seek IRB and Sponsor approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
- Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

Site Lead Investigator Responsibilities

The Site Lead Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations (CFR). The Site Lead Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

It is the responsibility of the Site Lead Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events, deviations, and unanticipated problems.

The Site Lead Investigator will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms at his/her site. For remote or onsite monitoring and auditing, the Site Lead Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Site Lead Investigator and will require his/her final signature to verify the accuracy of the data.

An investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of participants under the investigator's care; and for the control of drugs under investigation.

15.2 Study Principal Investigator Responsibilities

The Study Principal Investigator is responsible for the conduct of the clinical trial, including overseeing that sponsor responsibilities as defined in § 21 CFR 312.

15.3 Protocol Management Team (PMT)

See Section 7.0.

15.4 Monitoring/Auditing

Clinical site monitoring/auditing is conducted to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and regulatory requirements, and that the quality and integrity of study data and data collection methods are maintained. Monitoring/ auditing for this study will be performed by the City of Hope Office of Clinical Trials Auditing and Monitoring (OCTAM).

Documentation of monitoring/auditing activities and findings by OCTAM will be provided to the study team, the PI, and the COH DSMC.

15.5 City of Hope Data and Safety Monitoring Committee

The DSMC is a multidisciplinary committee charged with overseeing the monitoring of safety of participants in clinical trials, and the conduct, progress, validity, and integrity of the data for all clinical trials that are sponsored by City of Hope. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. The committee reviews the progress and safety of all active research protocols that are not monitored by another safety and data monitoring committee or board.

The COH DSMC will review and monitor toxicity and accrual data from this trial. Information that raises any questions about participant safety will be addressed with the PI, statistician and study team.

Refer to Section 7.0 for details.

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

16.1 Ethical Standard

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

16.2 Regulatory Compliance

This study is to be conducted in compliance with the IRB approved **protocol** and according to the following considerations:

- US Code of Federal Regulations (CFR) governing clinical study conduct
  - Title 21 Part 11 – Electronic Records; Electronic Signatures
  - Title 21 Part 50 – Protection of Human Subjects
  - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
  - Title 21 Part 56 – Institutional Review Boards
  - Title 21 Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies
  - Title 21 Part 312 – Investigational New Drug Application
  - Title 45 Part 46 – Protection of Human Subjects
- US Federal legislation, including but not limited to
  - Health Insurance Portability and Accountability Act of 1996
  - Section 801 of the Food and Drug Administration Amendments Act
- State of California Health and Safety Code, Title 17
- COH policies and procedures

16.3 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent document will be in the possession of the investigator before the study is initiated.

The IRB will be informed of revisions to other documents originally submitted for review; serious unexpected or unanticipated adverse experiences occurring during the study, and any additional adverse experiences in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

Any amendment to the protocol document and accompanying informed consent document/template, as developed and provided by the PI, will require review and approval by the COH IRB before the changes are implemented in the study.

16.4 Informed Consent

The Principal Investigator or IRB approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Prospective participants will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Prospective participants will be afforded sufficient time to consider whether or not to participate in the research.

After the study has been fully explained, written informed consent will be obtained from either the prospective participant or his/her guardian or legal representative before study participation. The method of obtaining and documenting the informed consent and the contents of the consent must comply with the ICH-GCP and all applicable regulatory requirements.

Before implementing any study procedure, informed consent shall be documented by the use of a written consent form approved by the IRB and signed and dated by the prospective participant or his/her legally authorized representative at the time of consent. A copy of the signed informed consent will be given to the participant or his/her legally authorized representative. The original signed consent must be maintained by the investigator and available for inspection sponsor designated representatives, or regulatory authority at any time.

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation.

16.5 Participant Withdrawal

Participants may withdraw from the study at any time and for any reason without prejudice. The withdrawal must be documented per institutional policies.

Participant withdrawal may consist of any of the following with regard to study procedures and data collection:

- Withdrawal from study treatment, but agreement to continue with active study procedures and chart review and follow-up procedures.
- Withdrawal from study treatment and all active procedures, but agreement for chart review and follow-up procedures.
- Withdrawal from study treatment, all active procedures, and any future data collection.

16.6 Special and Vulnerable Populations

16.6.1.1 Inclusion of Women and Minorities

The study is open anyone regardless of gender or ethnicity. Efforts will be made to extend the accrual to a representative population, but in a trial which will accrue approximately 29 participants, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

Pregnant women are excluded because the study drugs may have adverse effects on a fetus in uterus.

16.6.1.2 Exclusion of Children

Children (< 18 years old of age) are excluded from this study because the disease does not primarily affect children.

16.6.1.3 Inclusion of HIV Positive Individuals

Participants with a history of HIV are excluded from receiving protocol therapy due to concerns about inadvertent augmentation of infectious and/or inflammatory activity.

16.6.1.4 Vulnerable Populations

45 CFR §46.111 (a)(3) and 45 CFR §46, Subparts B-D identifies children, prisoners, pregnant women, mentally incapacitated persons, or economically or educationally disadvantaged persons as vulnerable populations.

Adults lacking capacity to consent are not excluded from participation. This study does not pose additional risks for adults lacking capacity than for the general population. In such instances, informed consent will be sought and documented from the prospective participant's legally authorized representative in agreement with institutional policies and COH IRB approval.

16.7 Participant Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to any study information relating to participants.

This research will be conducted in compliance with federal and state requirements relating to protected health information (PHI), including the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). HIPAA regulations require a signed participant authorization informing the participant of the nature of the PHI to be collected, who will have access to that information and why, who will use or disclose that information, ant the rights of a research participant to revoke their authorization for use of their PHI. In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. For participants that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the participant is alive) at the end of their scheduled study period.

Release of research results should preserve the privacy of medical information and must be carried out in accordance with Department of Health and Human Services Standards for Privacy of Individually Identifiable Health Information, 45 CFR 164.508. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed and no identifiers will be used.

Medical records of participants will be securely maintained in the strictest confidence, according to current legal requirements. Data will be entered, analyzed and stored in encrypted, password protected, secure computers that meet all HIPAA requirements. All data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number.

The investigator/institution will permit direct access to source data and documents by sponsor representatives, the FDA, and other applicable regulatory authorities. The access may consist of trial-related monitoring/ auditing, IRB reviews, and FDA/regulatory authority inspections. The participant's

confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

16.8 Future Use of Specimens Collected for this Trial

Left-over specimens will be stored for up to 10 years.

16.9 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study Sponsor (City of Hope) prior to participation in this study. All City of Hope investigators will follow the City of Hope conflict of interest policy.

16.10 Financial Obligations, Compensation, and Reimbursement of Participants

The study drugs pembrolizumab and GTx-024 will be provided by the manufacturer free of charge to study participants.

The research participant nor the insurance carrier will be responsible for the research procedures related to this study.

The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at the City of Hope to the injured research participant, however, financial compensation will not be available.

The research participant will not be paid for taking part in this study.

16.11 Publication/Data Sharing

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by City of Hope for the purposes of performing the study, will be published or passed on to any third party without the written approval of the PI. Any investigator involved with this study is obligated to provide City of Hope with complete test results and all data derived from the study.

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the City of Hope and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

In accordance with the U.S. Public Law 110-85 (Food and Drug Administration Amendments Act of 2007 or FDAAA), Title VIII, Section 801, this trial will be registered onto ClinicalTrials.gov and results will be reported on ClinicalTrials.gov within 12 months of the estimated or actual completion date of the trial, whichever date is earlier.

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APPENDIX A: ECOG PERFORMANCE STATUS

ECOG Performance Status [64]	
Grade	**Descriptions**
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.

APPENDIX B: CONTRACEPTION GUIDELINES

Pembrolizumab and GTx-024 may have adverse effects on a fetus in uterus. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

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

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

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

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

has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

1. Practice abstinence† from heterosexual activity; **OR**
2. Use (or have their partner use) acceptable contraception during heterosexual activity.

<u>Single method (one of the following is acceptable)</u>:

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

<u>Combination method (requires use of two of the following):</u>

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

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

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

APPENDIX C: GTX-024 PILL DIARY

Subject ID#:	Patient Initials (F, M, L):	
Institution:	Cycle #:	Cycle start date:

Study drug Instructions – When and How:

- Take GTx-024 **once a day** with water
- Swallow pills; do not chew them or crush them
- Take the drugs at approximately the same time each day with or without food
- Do not skip any doses

What if I miss a scheduled dose?

- If **less than 12 hours** have passed from the scheduled time, then **take the missed dose** as soon as you remember.
- If more than 12 hours have passed from the scheduled time, then skip the missed dose. Wait for your next scheduled dose. Do not take extra medicine to make up the missed dose.

What if I vomit a dose?

- If you vomit your pills, write this down in your pill diary.
- Wait until the next scheduled dose; do not take extra medicine to make up the vomited dose.

Additional Instructions:

- Keep your study drug in the original container until you take it.
- Do NOT throw away empty pill bottles or unused pills.
- Bring this diary, all pill bottles, and any unused pills to each clinic visit.
- Contact your study doctor or nurse if you are having any new or worsening side effects.

Contact Information for Study Doctor or Nurse

Phone:

Name:

How to fill the Pill Diary (Example Only)

To be filled by study team *# of pills to take: 1*

Participant Only	*Week 1*					
	Cycle Day	*Week Day*	*Date*	*Time*	*# of pills taken*	*Comments*
	1	*Mon*	*4/10/17*	*10:10 AM*	*1*	
	2	*Tues*	*4/11/17*	*10:30 AM*	*1*	

Subject ID#:	Patient Initials (F, M, L):	
Institution:	Cycle #:	Cycle start date:

To be filled by study team # of pills to take:_____

Week 1					
Cycle Day	**Week Day**	**Date**	**Time**	**# of pills taken**	**Comments**
1			___:___AM/PM		
2			___:___AM/PM		
3			___:___AM/PM		
4			___:___AM/PM		
5			___:___AM/PM		
6			___:___AM/PM		
7			___:___AM/PM		

To be filled by study team # of pills to take:_____

Week 2					
Cycle Day	**Week Day**	**Date**	**Time**	**# of pills taken**	**Comments**
8			___:___AM/PM		
9			___:___AM/PM		
10			___:___AM/PM		
11			___:___AM/PM		
12			___:___AM/PM		
13			___:___AM/PM		
14			___:___AM/PM		

Participant Signature *(please sign when submitting your diary)*	**Date:**
_____	____/_____/_____

Subject ID#:	Patient Initials (F, M, L):	
Institution:	Cycle #:	Cycle start date:

To be filled by study team # of pills to take:_____

Week 3					
Cycle Day	**Week Day**	**Date**	**Time**	**# of pills taken**	**Comments**
15			___:___AM/PM		
16			___:___AM/PM		
17			___:___AM/PM		
18			___:___AM/PM		
19			___:___AM/PM		
20			___:___AM/PM		
21			___:___AM/PM		

Participant Signature *(please sign when submitting your diary)* _____	**Date:** ____/_____/_____

Study Team ONLY: # of Pill Bottles Returned: _____ # of Pills Returned: _____
Compare with drug diary entries made by participant/guardian. If there is a discrepancy (in the # of bottles or the pills returned), please reconcile (initials & date):_____

APPENDIX D: RECIST CRITERIA V1.1

The following criteria describe RECIST Criteria V1.1 [65].

Disease Parameters

Measurable disease

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

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.

Malignant lymph nodes

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

Non-measurable disease

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

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

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

Target lesions

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

The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions

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

Methods for Evaluation of Measurable Disease

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

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

Clinical lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray

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

Conventional CT and MRI

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

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

PET-CT

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

Ultrasound

Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy

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

Tumor markers

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

Cytology, Histology

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

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

FDG-PET

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

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
- FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

Response Criteria

Evaluation of Target Lesions	
Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR):	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters
Progressive Disease (PD):	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

Evaluation of Non-Target Lesions	
Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis) Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.
Non-CR/Non-PD:	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

Evaluation of Best Overall Response

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

Table D1: For Patients with Measurable Disease (i.e., Target Disease)

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

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

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

Table D2: For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

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

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

Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

APPENDIX E: IMMUNE RELATED RESPONSE CRITERIA

Immune related RECIST (irRECIST) is an adaptation of RECIST 1.1 to account for the unique tumor response characteristics to treatment with new immunotherapeutic agents, including pembrolizumab. RECIST 1.1 was developed based on treatment with cytotoxic agents. Immunotherapeutic drugs, such as pembrolizumab, may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest as clinical responses after initial increases in tumor burden or even the appearance of new lesions. Standard RECIST 1.1 may not provide an accurate assessment of response to immunotherapeutic agents such as pembrolizumab [66], and will therefore be used with the adaptations referred to as irRECIST.

Antitumor response based on total measurable tumor burden

For the irRECIST, only index and measurable new lesions are taken into account (in contrast to conventional WHO criteria, which do not require the measurement of new lesions, nor do they include new lesion measurements in the characterization of evolving tumor burden). At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (five lesions per organ, up to 10 visceral lesions) is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions (up to 5 new lesions per organ; 10 visceral lesions) are added together to provide the total tumor burden: Tumor Burden = SPD index lesions + SPD new, measurable lesions.

Table E1: Comparison WHO and irRECIST criteria

	WHO	irRECIST
New, measurable lesions	Always represent PD	Incorporated into tumor burden
New, nonmeasurable lesions	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two consecutive observations not less than 4 wk apart	Disappearance of all lesions in two consecutive observations not less than 4 wk apart
PR	≥50% decrease in SPD of all index lesions compared with baseline in two observations at least 4 wk apart, in absence of new lesions or unequivocal progression of non-index lesions	≥50% decrease in tumor burden compared with baseline in two observations at least 4 wk apart
SD	50% decrease in SPD compared with baseline cannot be established nor 25% increase compared with nadir, in absence of new lesions or unequivocal progression of non-index lesions	50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point)	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 wk apart

Time-point response assessment using irRECIST

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out irPD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening). The irRECIST were derived from WHO criteria and, therefore, the thresholds of response remain the same. However, the irRECIST response categories have been modified from those of WHO criteria as detailed in Table E1.

Overall response using the irRECIST

The sum of the products of diameters at tumor assessment using the immune-related response criteria (irRECIST) for progressive disease incorporates the contribution of new measurable lesions. Each net Percentage Change in Tumor Burden per assessment using irRECIST criteria accounts for the size and growth kinetics of both old and new lesions as they appear.

Definition of Index Lesions Response Using irRECIST

irComplete Response (irCR):	Complete disappearance of all index lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
irPartial Response (irPR):	Decrease, relative to baseline, of 50% or greater in the sum of the products of the two largest perpendicular diameters of all index and all new measurable lesions (i.e. Percentage Change in Tumor Burden). Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SPD increases by ≥25% when compared to SPD at nadir
irStable Disease (irSD):	Does not meet criteria for irCR or irPR, in the absence of progressive disease.
irProgressive Disease (irPD)	At least 25% increase Percentage Change in Tumor Burden (i.e., taking sum of the products of all index lesions and any new lesions) when compared to SPD at nadir

Definition of Non-Index Lesions Response Using irRECIST

irComplete Response (irCR):	Complete disappearance of all non-index lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria
irPartial Response (irPR) or irStable Disease (irSD):	Non-index lesion(s) are not considered in the definition of PR, these terms do not apply
irProgressive Disease (irPD)	Increases in number or size of non-index lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (i.e., the SPD at nadir of the index lesions increases by the required amount)

Impact of New Lesions on irRECIST

New lesions in and by themselves do not qualify as progressive disease. However their contribution to total tumor burden is included in the SPD which in turn feeds into the irRECIST criteria for tumor response. Therefore, new non-measurable lesions will not discontinue any subject from the study.

Definition of Overall Response Using irRECIST

Overall response using irRECIST will be based on these criteria (Table E2):

irComplete Response (irCR):	Complete disappearance of all tumor lesions (index and non-index together with no new measurable/unmeasurable lesions) for at least 4 weeks from the date of documentation of complete response
irPartial Response (irPR):	The sum of the products of the two largest perpendicular diameters of all index lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the sum of the products of the two largest perpendicular diameters of all index lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline of the irSPD compared to the previous SPD baseline, of 50% or greater is considered an immune Partial Response (irPR).
irStable Disease (irSD):	irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease
irProgressive Disease (irPD)	It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute progressive disease: • At least 25% increase in the sum of the products of all index lesions over nadir SPD calculated for the index lesions. • At least a 25% increase in the sum of the products of all index lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the index lesion.

Table E2. Derivation of irRECIST overall responses

Measurable response	Nonmeasurable response		Overall response
Index and new, measurable lesions (tumor burden),*%	Non-index lesions	New, nonmeasurable lesions	Using irRECIST
↓100	Absent	Absent	irCR†
↓100	Stable	Any	irPR†
↓100	Unequivocal progression	Any	irPR†
↓≥50	Absent/Stable	Any	irPR†
↓≥50	Unequivocal progression	Any	irPR†
↓<50 to <25↑	Absent/Stable	Any	irSD
↓<50 to <25↑	Unequivocal progression	Any	irSD
≥25	Any	Any	irPD†

*Decreases assessed relative to baseline (scan prior to start of any protocol therapy), including measurable lesions only

†Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart.

Immune-Related Best Overall Response Using irRECIST (irBOR)

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

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

Appendix F: Stool Collection Procedure

STOOL COLLECTION KIT GENERAL INSTRUCTIONS

As a part of your participation in the current study, we have some specific instructions related to collection of stool. Please abide by these instructions, as they are essential for the proper conduct of the study.

You are being asked to collect samples at the following times:

- 7 days prior to Cycle 1 Day 1
- Cycle 5 Day 1 (+/-7 days), every 4 cycles (+/-7 days) and end of treatment (+/-7 days)

If you have any questions about sample collection, please call this number: _____

USING THE STOOL COLLECTION KIT

Before you begin, review the following:

a. Make sure you have a *collection hat* and collection tube
b. Make sure you are able to deliver the sample to City of Hope within 1 week.

STEP ONE: Please place the *collection hat* around the rim of your toilet seat for stool collection. 	
STEP TWO: Unscrew the collection tube cap and use the spoon to scoop one spoonful of feces (about the size of a quarter) from a sample. Place the sample in the collection tube. Tighten the cap and shake to mix the contents thoroughly (invert 10 times) to create a suspension. Note: Some fecal material may be difficult to re-suspend. As long as the material is suspended, the sample is stabilized. Foaming/frothing during shaking is normal.	 Scoop a portion of the stool sample into the DNA/RNA Shield™ Fecal Collection Tube

STEP THREE: Wash hands well, and write today's date on the label.	
STEP FOUR: Place the plastic tube in the bag, and seal the back using the adhesive tape already present on the bag.	
STEP FIVE: Bring the sample to your City of Hope appointment.	**THANK YOU FOR YOUR PARTICIPATION!**

Appendix G: Diet and Stool Frequency Log

DIET and STOOL FREQUENCY LOG - GENERAL INSTRUCTIONS

As a part of your participation in the current study, we are requesting that you complete a study log every day.

General pointers:

- When you come to the clinic, bring your logs with you.
- Each page has room for seven days – one row should be completed for each day.
- Please **avoid any intake of yogurt, yogurt-containing foods, or other bacteria-fortified foods**.

Example of how the top part of the log will look:

- A study team member will complete the information in this box before you leave the clinic.

COMPLETED BY STUDY TEAM	Participant Initials: JSM	Participant Research Number: 1001	Group: A

Example of how the information you enter might look:

- You or someone close to can complete the log for you, so long as the information is correct.
- List all prescription and non-prescription medications.
- The person who completes that day's entry should write his or her initials in the last column.

Day and Date	General description of food I ate:	*Did I eat yogurt or take probiotics?*	*How was my stool frequency?*	*Was a stool sample collected?*	*Medications taken*	*Initials of person filling information*
	Eggs, toast, juice Ham sandwich, coke, potato chips Steak, mashed potatoes, wine	○ Yes ☒ No	☒ Seems like baseline ○ 1-3 stools more than baseline ○ 4-6 stools more than normal ○ 7 or more than baseline, or incontinence	○ Yes ☒ No	Vitamin C, Lipitor	JSC
			○ Seems like baseline			

Example of the signature line:

- When you hand over the document to the study team, they will ask to sign and date at the bottom of each log if you agree that the information is complete and correct.

At the time of handing over the document -- Participant Signature: Joseph Black Smith Date 12/12/2002

COMPLETED BY STUDY TEAM	Participant Initials:	Participant Research Number:

Day and Date	General description of food I ate:	*Did I eat yogurt or take probiotics?*	*How was my stool frequency?*	*Was a stool sample collected?*	*Medications taken*	*Initials of person filling information*
	Eggs, toast, juice Ham sandwich, coke, potato chips Steak, mashed potatoes, wine	○ Yes ☒ No	☒ Seems like baseline ○ 1-3 stools more than baseline ○ 4-6 stools more than normal ○ 7 or more than baseline, or incontinence	○ Yes ☒ No	Vitamin C, Lipitor	JBC
		○ Yes ○ No	○ Seems like baseline ○ 1-3 stools more than baseline ○ 4-6 stools more than normal ○ 7 or more than baseline, or incontinence	○ Yes ○ No		
		○ Yes ○ No	○ Seems like baseline ○ 1-3 stools more than baseline ○ 4-6 stools more than normal ○ 7 or more than baseline, or incontinence	○ Yes ○ No		
		○ Yes ○ No	○ Seems like baseline ○ 1-3 stools more than baseline ○ 4-6 stools more than normal ○ 7 or more than baseline, or incontinence	○ Yes ○ No		
		○ Yes ○ No	○ Seems like baseline ○ 1-3 stools more than baseline ○ 4-6 stools more than normal ○ 7 or more than baseline, or incontinence	○ Yes ○ No		
		○ Yes ○ No	○ Seems like baseline ○ 1-3 stools more than baseline ○ 4-6 stools more than normal ○ 7 or more than baseline, or incontinence	○ Yes ○ No		

Day and Date	General description of food I ate:	*Did I eat yogurt or take probiotics?*	*How was my stool frequency?*	*Was a stool sample collected?*	*Medications taken*	*Initials of person filling information*
		◯ Yes ◯ No	◯ Seems like baseline ◯1-3 stools more than baseline ◯4-6 stools more than normal ◯7 or more than baseline, or incontinence	◯ Yes ◯ No		

At the time of handing over the document -- Participant Signature: _____ Date _____

APPENDIX H: AT HOME SAMPLE COLLECTION KIT CONTENTS

Contents of each kit to be provided to participants for at home collection:

[] Copy of Appendix F: Instructions for Stool Specimen Collection

[] Stool collection hat

[] Specimen tube with label attached

Label should have participant identifier added; the participant will be asked to add the date himself/herself.

[] Plastic sealable bag

APPENDIX I: REGISTRATION COVERSHEET

IRB 16131: A Phase 2 Clinical Trial of the Combination of Pembrolizumab and Selective Androgen Receptor Modulator (SARM) GTX-024 in Patients with Advanced Androgen Receptor (AR) Positive Triple Negative Breast cancer (TNBC).

Data Coordinating Center:
City of Hope
1500 Duarte Road
Duarte, CA 91010
Tel: 626-256-4673 x 83968
Email: DCC@coh.org (use #secure# in subject line)

Site Principal Investigator
Name:
Address:

Phone:
Fax:
e-mail:

CRA/Study Coordinator:	Contact Number:
Patient's Initials: (F M L):	Institution:
Medical Record No:	Investigator/Treating Physician:
Patient's DOB:	IRB approval valid until (date):
Sex: _____Male _____Female	Date Informed Consent Signed:
	Projected start date of treatment:

Race		Ethnicity		Method of Payment:_____	
				Codes:	
☐	Black	☐	Hispanic		
☐	Caucasian	☐	Non-Hispanic	**01** Private	**06** Military or Veterans Adm. sponsored
☐	Asian	☐	Other_____	**02** Medicare	**07** Self-pay (no insurance)
☐	American Indian			**03** Medicare & private ins.	**08** No means of payment (no insurance)
☐	Native Hawaiian/Pacific Islander			**04** Medicaid	**09** Unknown
☐	Other_____ _____			**05** Medicaid & Medicare	

Reason for Screen Failure:

Reason for Failing to Initiate Protocol Therapy:

APPENDIX J: SAMPLE SHIPPING GUIDELINES

Follow the requirements for the proper packaging and shipping of biomedical material found in 42 CFR Part 72 - Interstate Shipment of Etiologic Agents Centers for Disease Control and Prevention, Office of Health and Safety Biosafety Branch.

1. **Samples must be de-identified with no PHI.** Aim to ship samples on a **Monday through Thursday**. If this is not feasible, advance arrangements should be made with Dr. Yuan (yuyuan@coh.org) and Dr. Susan Yost (suyost@coh.org). Notify Dr. Yuan and Dr. Susan Yost of impending shipment. Billing information can be requested at that time. Also, on the day of shipment, please email recipient the sample shipment information and a sample manifest.
2. **Peripheral Blood Samples:** For sample processing, please refer to section 9.3. Please ship to:

   Dr. Tim Synold
   Cc: Lesley Smith-Powell
   Analytical Pharmacology Core Facility (APCF)
   Shapiro 1042
   City of Hope National Medical Center
   1500 E. Duarte Road
   Duarte, CA 91010
   Tel: 626-218-2954
   tsynold@coh.org; Lsmith-Powell@coh.org

3. **Pathology Slides/Blocks:** Batch ship with frozen gel ice-packs in order to prevent the melting of paraffin-embedded tissue blocks during transit to:

   Dr. Susan Yost
   Department of Medical Oncology & Therapeutic Research
   Building 51, City of Hope National Medical Center
   1500 East Duarte Rd, Duarte, CA 91010
   Direct: 626-218-0499 Internal x 80499
   suyost@coh.org

4. **Frozen samples:** Batch ship on dry ice via FedEx. The shipment should contain enough dry ice to last at least 72 hours. When shipping frozen specimens from long distances, it is best to use a combination of dry ice and frozen gel ice-packs.

   Process tumor biopsy or large tumor tissue as follows:
   Materials Needed
   - PBS
   - Freezing media: 50% media (RPMI or DMEM), 40% FBS, and 10% DMSO

   a) Collect biopsy or large tumor tissue at time of surgery and place in PBS or RPMI media on ice. Process samples as soon as possible.
   b) For available tissue biopsies, proceed directly to “step e”.
   c) For available large tumor tissue, quickly mince tissue into ~5mm^3 (~1/4 inch) samples.

d) Aliquot available tissue by placing one tumor tissue biopsy or two 5mm^3 tumor samples per 2mL labelled cryovial containing 1mL ice-cold freezing media.
e) Immediately place vial in -80°C freezer overnight to cryopreserve at controlled rate of -1°C/min using a rate controlled container.
f) Send samples to Shabnam Pathan as follows:

Ke Cui

CC: Biospecimen Coordinator Shabnam Pathan
COH Biorepository Core
City of Hope National Medical Center
1500 E. Duarte Road
Duarte, CA 91010
626-218-1848; 626-218-0462
kcui@coh.org; spathan@coh.org

5. **Microbiome specimens: See Appendix F for stool collection and ship samples to:**

Ke Cui
CC: Biospecimen Coordinator Shabnam Pathan
COH Biorepository Core
City of Hope National Medical Center
1500 E. Duarte Road
Duarte, CA 91010
626-218-1848; 626-218-0462
kcui@coh.org; spathan@coh.org

Approximately 75-125 samples stool samples will be temporarily stored at -80^{o}C in the Analytical Pharmacology Core Facility (APCF) in Shapiro 1042 (Dr. Tim Synold/Leslie Smith-Powell) until batch shipping to TGen:

Sarah Highlander, PhD, Director

TGen Clinical Microbiome Services Center

Pathogen and Microbiome Division

Translational Genomics Research Institute

3051 W. Shamrell Blvd., Suite 106

Flagstaff, AZ 86005

shighlander@tgen.org | 928-213-6996

6. **DNA and RNA Sequencing**

Tumor DNA and RNA sequencing will be performed at Tempus. No identifiable information will be sent to Tempus and all unused specimen including DNA/RNA extract, FFPE slides and fresh frozen specimen will be returned to COH investigator. Tempus will not store nor conduct any other research with the unused or leftover tissue

Tempus xT Panel (595 genes): 15 FFPE +1 H&E for NGS and PD-L1 (20% tumor --- ratio of tumor nuclei to benign nuclei; 5-25mm^2), plus normal sample (buffy coat from 1-2 x 8 ml tubes whole blood).

Ship to:

Tempus
600 West Chicago Ave. Ste 510
Chicago, IL 60654
800.739.4137

APPENDIX K: EXPEDITED REPORTING COVERSHEET

NOTIFICATION OF UNANTICIPATED PROBLEM/SERIOUS ADVERSE EVENT

For Use by Participating Institutions Only

THIS FORM ALONG WITH A COPY OF THE MEDWATCH 3500 OR IRB REPORTING FORM MUST BE **EMAILED** TO DCC@COH.ORG WITHIN 24 HOURS OF KNOWLEDGE OF ONSET OF SERIOUS ADVERSE EVENT OR UNANCTICIPATED PROBLEM (Use #secure# in subject line)

COH IRB #16131- Participating Site IRB # _____

From:	Date:
Phone No.:	Email:
Reporting Investigator:	
Event:	
Participant ID:	Institution:
Date Event Met Reporting Criteria (as defined in protocol):	

Type of Report:	☐ Initial ☐ Follow-up
CTCAE Grade:	☐ G1/mild ☐ G2/moderate ☐ G3/severe ☐ G4/life threatening ☐ G5
Attribution to **Pembrolizumab:**	☐ Unrelated ☐ Unlikely ☐ Possible ☐ Probable ☐ Definite
Attribution to **GTx-024**	☐ Unrelated ☐ Unlikely ☐ Possible ☐ Probable ☐ Definite
Historical/Known Correlation to **pembrolizumab**:	☐ Expected ☐ Unexpected
Historical/Known Correlation to **GTx-024**:	☐ Expected ☐ Unexpected
Meets Definition of Serious AE:	☐ Serious ☐ Non-serious
Meets Definition of Unanticipated Problem:	☐ UP ☐ Not a UP
Has the event been reported to the following institution's IRB?	☐ No ☐ Yes; Date:_____/____/_____

Authorized Investigator Signature:	Date:____/____/____