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DEPARTMENT OF MEDICAL ONCOLOGY AND THERAPEUTICS RESEARCH

TITLE: A Phase 1b/2 Trial of the IRX-2 Regimen and Pembrolizumab in Patients with Advanced Gastric and Gastroesophageal Junction (GEJ) Adenocarcinoma

CITY OF HOPE PROTOCOL NUMBER:	**18069**	**Protocol Version Date: 07/06/2020**

Initial Approval	Protocol dated 09/19/18	Version: 00
Amendment 01	Protocol dated 01/11/19	Version: 01
Amendment 02	Title Page dated 05/23/19	Version: 02
Amendment 03	Protocol dated 08/23/19	Version: 03
Amendment 04	Protocol dated 04/22/2020	Version: 04
Amendment 05	Protocol dated 07/06/2020	Version: 05
Amendment 06	Protocol dated 07/06/2020 (tp)	Packet: 06

DISEASE SITE:	Gastric and Gastroesophageal Junction Adenocarcinoma
STAGE (if applicable):	
MODALITY:	Immunotherapy
PHASE/TYPE:	Phase Ib/II
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Clinical Trial Protocol

A Phase 1b/2 Trial of the IRX-2 Regimen and Pembrolizumab in Patients with Advanced Gastric and Gastroesophageal Junction (GEJ) Adenocarcinoma

Version Date:	July 6, 2020
Protocol Version:	04
City of Hope #:	18069
Agents:	IRX-2, pembrolizumab
IND Number:	141722
Sponsor/IND Holder:	City of Hope
Funding Support:	Brooklyn ImmunoTherapeutics, Inc.
Industry Partner:	Brooklyn ImmunoTherapeutics, Inc.
Industry Partner Protocol ID:	Pending
NCT Number:	Pending
Participating Sites:	City of Hope (Duarte) Honor Health Research Institute (Scottsdale, AZ) Texas Oncology-Baylor Charles A. Sammons Cancer Center (Dallas, TX)

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Protocol Synopsis

Protocol Title:
A Phase 1b/2 Trial of the IRX-2 regimen and Pembrolizumab in Patients with Advanced Gastric and Gastroesophageal Junction (GEJ) Adenocarcinoma
Brief Protocol Title for the Lay Public (if applicable):
IRX-2 and Pembrolizumab Treatment in Gastric Cancer
Study Phase:
Phase 1b/2
Participating Sites:
City of Hope (Duarte, CA) Honor Health Research Institute (Scottsdale, AZ) Texas Oncology-Baylor Charles A. Sammons Cancer Center (Dallas, TX)
Rationale for this Study:
Advanced gastric cancer continues to account for a large proportion of worldwide causes of cancer-related mortality. Systemic and targeted therapies remain limited, though the immune checkpoint inhibitor pembrolizumab became FDA-approved in September of 2017 as third-line and beyond therapy based on results of the KEYNOTE-059 trial.[1] However, durable objective responses remains limited to a small proportion of patients. The IRX-2 Regimen is an immunomodulatory regimen in development by Brooklyn ImmunoTherapeutics that includes a novel primary cell-derived biologic (IRX-2) that contains several active cytokines and cyclophosphamide. This treatment regimen has been demonstrated to bolster several facets of immune response, including tumor infiltration by lymphocytes.[2-7] Therefore, it is possible that the combination of the IRX-2 regimen with pembrolizumab will improve treatment outcomes. This phase 1b/2 study will seek to investigate the safety and tolerability of the combination of the IRX-2 regimen with pembrolizumab. It will be used to describe the safety profile of combination IRX-2 regimen and pembrolizumab and determine the maximum tolerable dose of combination IRX-2 regimen with pembrolizumab in patients with gastric or GEJ adenocarcinoma who have progressed on or are intolerant to two lines of systemic therapy. Clinical efficacy will also be evaluated with assessment of the objective clinical response rate of IRX-2 regimen combined with pembrolizumab using RECIST 1.1 and immune modified RECIST criteria, and resultant median progression-free and overall survival. Exploratory analyses will seek to evaluate the following: circulating T cell profile in patients before and after treatment with the IRX-2 regimen; the baseline and post-treatment tumor tissue for Nanostring gene expression profiling and correlation of these profiles to putative response; multiplex assays (MHC-PepSeq) in combination with genomic and transcriptomic sequencing to identify mutations associated with tumor neo-antigens; and other circulating biomarkers (other immune cell populations and circulating tumor DNA) in peripheral blood to develop hypotheses for response to these therapies.
Objectives:
Primary Objectives

- To determine the safety profile of the combination IRX-2 regimen and pembrolizumab

Secondary Objectives

- To evaluate the overall response rate of IRX-2 regimen combined with pembrolizumab using RECIST 1.1 and immune modified RECIST criteria.
- To evaluate initial median progression-free and overall survival in these patients treated with combination IRX-2 regimen and pembrolizumab.

Exploratory Objectives

- To evaluate the circulating T cell profiles in patients before and after therapy with the combination IRX-2 regimen and pembrolizumab
- To evaluate the baseline and post-treatment tumor tissue immune gene expression profiling using the Nanostring platform
- To explore identification of tumor tissue neoantigens through a multiplex proteomic assay (MHC-PepSeq) paired with tumor genomic and transcriptomic sequencing
- To explore putative biomarkers (including circulating tumor DNA and immune cell profiles) in peripheral blood to generate hypotheses for response to treatment with combination IRX-2 regimen and pembrolizumab.

Study Design:

The study will consist of two phases – a safety phase and a dose expansion phase:

- Safety phase (phase 1b): A standard 3+3 dose finding rule will be employed with two dose levels. Patients will start at dose level 1 [IRX-2 regimen at dose of 2 mL (230 units/day)]. Patients will be enrolled in cohorts of 3, separated by at least 1 week. Initially, 3 patients will be treated with expansion to 3 more patients in the event of 1/3 dose limiting toxicities (DLTs) as observed during the first 3 weeks of treatment, escalation if 0/3 or 1/6 DLTs, and termination of escalation if ≥ 2 DLTs. Once ≥ 2 patients experience a DLT at a dose level, the next lower dose level will be expanded to 6 evaluable patients, if fewer than 6 evaluable patients have been treated at the dose level. The maximum tolerated dose (MTD) is defined as the highest dose tested in which only 0 or 1 out of 6 evaluable patients experience a DLT. The Recommended Phase 2 Dosing (RP2D) will not exceed the MTD but may be lower based on cumulative toxicities and dose modification patterns. If the rules suggest escalation at the highest proposed dose, that dose will accrue to 6 patients where <2 DLTs will determine that as the MTD, and 2 or more DLTs will require de-escalation. These rules will be used to determine the IRX-2 regimen dosing for the expansion phase.
- Dose expansion phase (phase 2): 8-14 patients will be enrolled at the recommended dose level from the safety phase, for a total enrollment of 20 patients. The 6-12 patients treated in the safety lead-in portion of the study will be counted as part of the dose expansion patient population.

Endpoints:

<u>Primary Endpoint</u>

- Occurrence and severity of adverse events including dose limiting toxicities, with severity determined according to NCI Common Terminology Criteria for Adverse Events version 5.0.

<u>Secondary Endpoints</u>

- Progression Free Survival (PFS), Overall Survival (OS), and Overall Response Rate (ORR) as assessed using RECIST version 1.1 and irRECIST.

Exploratory Endpoints

- Description of circulating T cell profiles in patients before and after therapy with combination IRX-2 regimen and pembrolizumab.
- Correlation between baseline and post-treatment tumor tissue Nanostring gene expression profiling and clinical response.
- Identification of tumor neoantigens using the MHC-PepSeq assay.
- Development of hypotheses for response based on circulating immune cell profiles circulating tumor DNA from peripheral blood.

Sample Size:

Phase I: 6-12 patients

Phase II: 20 patients (11 + 9 or 8 + 12)*

* Patients treated at all dose levels in the phase I portion of the study will be counted as a part of the phase II patient population.

Estimated Duration of the Study:

42 Months

Summary of Subject Eligibility Criteria:

Inclusion Criteria:

1. Patients with histologically or cytologically confirmed recurrent or metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma progressed or intolerant to ≥ 2 lines of systemic therapy.
2. Patients must have recurrent or metastatic gastric/GEJ adenocarcinoma that are not amenable to local therapy with curative intent (surgery or radiation therapy with or without chemotherapy).
3. Willing and able to give informed consent and adhere to protocol therapy; written informed consent and any locally required authorization must be obtained from the patient prior to performing any protocol-related procedures, including screening evaluations.
4. At least 18 years of age.
5. No prior exposure to PD-1/PD-L1 inhibitor therapy.
6. Patients are deemed eligible for pembrolizumab therapy with tumors demonstrating PD-L1 expression by the Combined Positive Score (CPS) being ≥ 1 as per the FDA-approved Dako PD-L1 IHC 22C3 PharmDx assay.
7. ECOG 0-1
8. Body weight must be >30 Kg.
9. Adequate normal organ and marrow function as defined below:
10. Hemoglobin >8 g/dL
11. Absolute neutrophil count (ANC) >1,200 x 10^9/mL
12. Platelet count >75 x 10^9/mL
13. Serum bilirubin ≤1.5 × institutional upper limit of normal (ULN) **OR** Direct bilirubin ≤ ULN if total bilirubin levels > 1.5 x ULN

14. Aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) ≤ 5 x ULN
15. Prothrombin time (PT) and partial thromboplastin time (PTT) < 1.5x the ULN.
16. Serum creatinine ≤ 1.5 x ULN **OR** Measured creatinine clearance (CL) >40 mL/min or calculated creatinine clearance CL>40 mL/min by the Cockcroft-Gault formula [8] or by 24-hour urine collection for determination of creatinine clearance:

Males:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}}$$

Females:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age}) \times 0.85}{72 \times \text{serum creatinine (mg/dL)}}$$

17. Palliative radiation therapy is allowed to non-target lesions at the discretion of the treating physician.
18. Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) or evaluable disease as outlined in RECIST version 1.1.
19. Life expectancy of greater than 3 months in the opinion of the treating physician.
20. Female patients of childbearing potential must have a negative serum pregnancy test within 7 days prior to enrollment.

Exclusion Criteria:

1. Prior exposure to the IRX-2 regimen and/or PD-1/PD-L1 inhibitors are excluded.
2. Radiation therapy with a curable intent within 30 days of first dose of study treatment is excluded. However, radiation therapy with a palliative intent is allowed as long as treatment with study medication occurs ≥14 days after last dose of radiation.
3. Any medical contraindications or previous therapy that would preclude treatment with the IRX-2 Regimen or pembrolizumab.
4. Known allergies to ciprofloxacin or phytohemagglutin given trace amount of these agents are contained in IRX-2.
5. Patients with ongoing chronic myelosuppression, myelodysplasia, or hemorrhagic cystitis which would contraindicate receipt of cyclophosphamide.
6. Any unresolved toxicity NCI CTCAE Grade ≥ 2 from previous anticancer therapy with the exception of alopecia, vitiligo, and the laboratory values defined in the inclusion criteria.
7. Patients with Grade ≥ 2 neuropathy will be evaluated on a case-by-case basis after consultation with the study physician.
8. Patients with irreversible toxicity not reasonably expected to be exacerbated by treatment with IRX-2, pembrolizumab may be included only after consultation with the study physician.
9. Active or prior documented autoimmune or inflammatory disorders (including inflammatory bowel disease [e.g., colitis or Crohn's disease], diverticulitis [with the exception of diverticulosis], systemic lupus erythematosus, Sarcoidosis syndrome, or Wegener syndrome [granulomatosis with polyangiitis, Graves' disease, rheumatoid

arthritis, hypophysitis, uveitis, etc]). The following are exceptions to this criterion:

- Patients with vitiligo or alopecia.
- Patients with hypothyroidism (e.g., following Hashimoto syndrome) stable on hormone replacement.
- Any chronic skin condition that does not require systemic therapy.
- Patients without active disease in the last 2 years may be included but only after consultation with the study physician.
- Patients with celiac disease controlled by diet alone.

10. Current or prior use of immunosuppressive medication within 14 days prior to Cycle 1, Day 1. The following are exceptions to this criterion:
    - Intranasal, inhaled, topical steroid or local steroid injections (e.g., intra articular injection).
    - Systemic corticosteroids at physiologic doses not to exceed 10 mg/day of prednisone or its equivalent.
    - Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication).
11. Major surgical procedure (as defined by the Investigator) within 28 days prior to Cycle1, Day 1. Note: Local surgery of isolated lesions for palliative intent is acceptable.
12. History of allogeneic organ transplantation.
13. Symptomatic cardiopulmonary disease (including congestive heart failure and hypertension), coronary artery disease, serious arrhythmia or chronic lung disease. Patients with these conditions who are stable with relatively minor symptoms and who are appropriate candidates for systemic treatments need not be excluded.
14. Myocardial infarction within the last 3 months.
15. Has a known history of human immunodeficiency virus (HIV) (HIV 1/2 antibodies).
16. Has a history of active Hepatitis B requiring ongoing antiviral therapy or a history of untreated Hepatitis C.
17. Has received a live vaccine within 4 months of planned start of study therapy (Cycle 1, Day 1).
    Note: The killed virus vaccines used for seasonal influenza vaccines for injection are allowed provided not within 4 weeks of planned start of study therapy (Cycle 1, Day 1); however intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.
18. Signs or symptoms of systemic infection (use of antibiotics to treat superficial infection or contamination of tumor shall not, by itself, be considered evidence of infection).
19. Stroke or other symptoms of cerebral vascular insufficiency within the last 3 months.
20. Previous diagnosis of invasive cancer from which the individual is not disease-free AND that has required treatment within the past 3 years, except for superficial skin, cervical cancer in-situ, or early stage prostate or bladder cancer (i.e. treatment with curative intent and long term disease-free expectations).
21. History of leptomeningeal carcinomatosis
22. Known allergy or hypersensitivity to any of the study drugs or any of the study drug excipients.
23. Female patients who are pregnant or breastfeeding or male or female patients of reproductive potential who are not willing to employ effective birth control from

screening to one year after last dose of cyclophosphamide or 180 days after the last dose of pembrolizumab therapy, whichever is longer.

Investigational Product and Reference Therapy:

IRX-2 is supplied as a pale yellow, sterile liquid for subcutaneous injection in a 2.0 ml single dose vial.
Pembrolizumab as a 200 mg IV Q3weeks as a 30 minute infusion

Cycle 1 through 35

Pembrolizumab as a 200 mg IV Q3weeks as a 30 minute infusion (every Cycle)
IRX-2 regimen every 12 weeks as below starting on Cycle 1:

Agent	Dose	Route of Administration
Cyclophosphamide (Day 1)	300 mg/m^2	IV
IRX-2: For 10 days starting on Day 4; the 10 days are not required to be consecutive (e.g. dosing not mandatory on weekends/holidays) provided dosing is completed by Day 15	Dose Level 1: 230 units total daily (2 injections daily comprised of 115 units in each injection) Dose Level 2: 460 units total daily (4 injections daily comprised of 115 units in each injection)	Dose Level 1: Subcutaneous at or near the supraclavicular lymph node regions bilaterally (substitute axillary if supraclavicular nodes have been removed). Dose level 2: Subcutaneous at or near the supraclavicular and axillary node regions bilaterally (if any of these nodes have been removed, can inject at or near mastoid insertion of both sternocleidomastoid muscles).

Statistical Considerations:

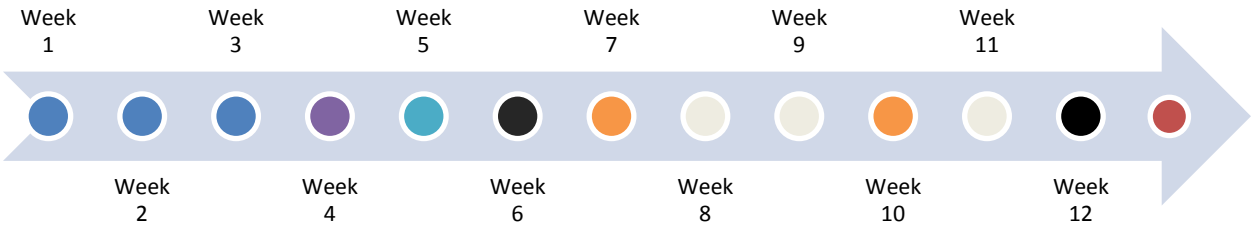
The safety phase of the study will enroll 3 to 6 patients per dose level. The dose expansion phase will enroll 8 to 12 patients at the recommended dose level from the safety phase for a total enrollment of 20 patients. The 6 to 12 patients treated in the safety phase of the study will be counted as a part of the dose expansion patient population.

Historical data with single agent PD-1 inhibitors in gastroesophageal cancer indicate median PFS at 1.5 months. With an alpha of .05, hypothesized median PFS improved to 3.0 months with the combination IRX-2 regimen and pembrolizumab, study accrual of 30 months, and 12-month follow-up evaluation timeframe, a sample size of 20 patients will have 80% power to detect median differences in PFS. Assuming that dose level 1 will be used for the dose expansion phase, these individuals will be included in phase II to reach the needed sample of 20 patients evaluable for immune activity.

Sponsor/Licensee:

City of Hope/Brooklyn ImmunoTherapeutics, Inc.

Study Schema



IRX-2 Regimen* + Pembrolizumab

Pembrolizumab

Imaging for RECIST assessment***

Pembrolizumab administration , DLT Assessment Completed**

Post-Treatment Biopsy*

Additional 12 Week Sequences

*IRX-2 Regimen: IRX-2 x 10 days, Cyclophosphamide x 1 dose on Day 1; given every 12 weeks

Pembrolizumab: 200 mg; IV administration; given every 3 weeks

**Cycle 1 only

***Imaging scans performed every 6 weeks for the first 18 weeks, then every 9 weeks afterwards

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1.0 Goals and Objectives (Scientific Aims)

Primary Objectives

- To determine the safety profile of combination IRX-2 regimen and pembrolizumab

Secondary Objectives

- To evaluate the overall response rate of IRX-2 regimen combined with pembrolizumab using RECIST 1.1 and immune modified RECIST criteria.
- To evaluate initial median progression-free and overall survival in these patients treated with combination IRX-2 regimen and pembrolizumab.

Exploratory Objectives

- To evaluate the circulating T cell profiles in patients before and after therapy with combination IRX-2 regimen and pembrolizumab
- To evaluate the baseline and post-treatment tumor tissue immune gene expression profiling using the Nanostring platform
- To explore identification of tumor tissue neoantigens through a multiplex proteomic assay (MHC-PepSeq) paired with tumor genomic and transcriptomic sequencing
- To explore putative biomarkers (including circulating tumor DNA and immune cell profiles) in peripheral blood to generate hypotheses for response to treatment with combination IRX-2 regimen and pembrolizumab.

2.0 Background

Gastric Cancer

2.1.1 Epidemiology

In 2012 nearly 1 million new cases of gastric cancer were diagnosed globally with an estimated 723,100 deaths, reflective of the high mortality rate associated with this disease.[9]

2.1.2 Current Treatment Options

A number of chemotherapy agents have demonstrated activity in advanced gastric and gastroesophageal junction (GEJ) cancer. These include platinum drugs, such as cisplatin and oxaliplatin, and fluoropyrimidines, such as intravenous 5-fluorouracil and oral capecitabine. Multi-agent chemotherapy regimens are able to improve by nearly 7 months the approximately 4 months median survival seen with best supportive care alone.[10] In terms of predictive biomarkers to date that guide treatment decisions in front-line therapy, evidence of HER2 overexpression and/or gene amplification supports addition of HER2 blockade with trastuzumab to combination platinum and fluoropyrimidine chemotherapy based on the phase III ToGA trial.[11] Despite initial activity seen with multi-agent chemotherapy, development of drug resistance and invariable disease progression still accounts for high mortality rates associated with advanced disease.

Second-line chemotherapy with taxanes or irinotecan after platinum and fluoropyrimidine chemotherapy have demonstrated modest activity in randomized trials.[12-14] More recently, evidence that angiogenesis inhibition can induce tumor responses and/or delay disease progression was demonstrated with delivery of the VEGFR2-inhibitory antibody ramucirumab in the phase III REGARD and RAINBOW trials, when utilized as a single agent or added to paclitaxel, respectively.[15, 16] Median overall survival improved from 7.4 to 9.6 months (p=0.017) when ramucirumab was added to paclitaxel,

representing a stepwise improvement for this patient population, but still indicating more options are needed for this diagnosis.

Immune checkpoint inhibitors have recently become an option for treatment-refractory advanced gastric cancer. KEYNOTE 059 was a multi-cohort phase 2 trial, in which in its largest cohort, Cohort 1, examined pembrolizumab in 259 gastric and GEJ adenocarcinoma patients who had failed at least 2 prior systemic regimens.[1] Pembrolizumab was continued until completion of 35 cycles (approximately 2 years) in patients without demonstration of progressive disease, intolerable toxicity, withdrawal of consent, or investigator decision to stop therapy. Fuchs et al. reported those with tumor PD-L1 expression had an overall RR of 15.5% with 2.0% complete responses, and 13.5% partial responses by RECIST1.1. Evidence of PD-L1 expression by a combined positive score (CPS) was defined as a cutoff ≥ 1 tumor cells and/or immune cells out of 100 viable tumor cells with PD-L1 expression by immunohistochemistry using the 22C3 pharmDx assay. Microsatellite instability was also tested as a biomarker, and 4% of the study population harbored tumors with high microsatellite instability (MSI-H). ORR was more encouraging at 57.1% in the MSI-H subset vs. 9.0% in tumors defined to be non-MSI-H. Ultimately 13.3% of patients with non-MSI-H or undetermined MSI, PD-L1 CPS positive tumors demonstrated objective tumor responses which were durable with median durations of response between 2.3+ to 19.4+ months. While the durability of responses are very encouraging for a disease historically with dismal prognosis, the low proportion of patients having such clinical benefit speaks to the need to augment immunotherapy responses. This was further highlighted by recent reporting of the phase III KEYNOTE-061 trial, in which 395 metastatic gastric or GEJ cancer patients with PD-L1 CPS positive tumors were randomized to single agent pembrolizumab or paclitaxel.[17] In this trial, a median PFS of 1.5 months was observed with pembrolizumab versus 4.1 months with paclitaxel, indicating the vast majority of patients demonstrate disease progression early despite pembrolizumab therapy.

Immunotherapy

2.1.3 PD-1 and Cancer

Developing immunity to a particular cancer can be viewed as a cyclic process in which immune-stimulatory and inhibitory factors compete against each other to modulate the immune response.[18] This relationship is further complicated by the ability of the tumor to down-regulate stimulatory signals and up-regulate inhibitory signals. Some tumors express Programmed death receptor ligand 1 (PD-L1) the ligand for programmed death-1 (PD-1), a cell surface molecule that regulates the adaptive immune response.[19] PD-1 is found on the surface of T cells and sends inhibitory signals that inactivate T cell receptor (TCR) signaling upon the binding of PD-L1.[20] The interaction of PD-L1 with PD-1 has been shown to inhibit the killing activity of cytotoxic T cells as well as interfere with the role of regulatory T cells.[21] Tumors take advantage of immune checkpoints such as the PD-1/PD-L1 pathway to evade immune destruction, resulting in disease progression. It is necessary to overcome multiple immunosuppressive mechanisms in order to establish an effective antitumor immune response. [22] Anti-PD1 and Anti-PD-L1 are designed to block immune checkpoints thereby decreasing immune inhibitory pathways and increasing the antitumor effect. [23, 24] In targeting tumors that express PD-L1, immunotherapy has been used to produce an antibody blockade to the PD-1 receptor on T cells.[25]

Pembrolizumab is a fully human, IgG4 (kappa) monoclonal antibody that binds PD-1, which is primarily expressed on activated T cells, B cells and myeloid cells.[26-29] The PD-1 receptor-ligand interaction is a major pathway utilized by tumors to evade the surveillance and clearance by the immune system. Atezolizumab works in similar fashion, except it is an IgG1 isotype antibody which blocks the interaction of PD-L1 with PD-1 and CD80.[30, 31] A variety of cancers have demonstrated the ability to express PD-L1

and binding of PD-1 to PD-L1 or PD-L2 has been shown to down-regulate T-cell activation.[32-35] Interactions between PD-1/PD-L1 may also indirectly modulate the response to tumor antigens through T-cell/APC interactions. PD-1 blockade by nivolumab, pembrolizumab and PD-L1 blockade by atezolizumab has been shown to reverse immune tolerance and enhance T cell effector function in several tumor types including melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), urothelial carcinoma, hepatocellular carcinoma (HCC), microsatellite instability-high (MSI-H) colorectal cancer, and head and neck squamous cell carcinoma (HNSCC).[28, 36-42] As discussed earlier, for the FDA approval of pembrolizumab in the third-line treatment of advanced gastric/GEJ adenocarcinoma, the observed response rate was 15.5%, indicating the possibility for improvement of this therapy through combination with an additional immunomodulatory regimen.

2.1.4 The IRX-2 Regimen

The IRX-2 Regimen is an immunomodulatory regimen in development by Brooklyn ImmunoTherapeutics that includes a novel primary cell-derived biologic (IRX-2) and cyclophosphamide. Preclinical studies showed that the IRX-2 biologic promotes upregulation of antigen presenting machinery, improved antigen presentation, dendritic cell (DC) maturation, improved T cell activation, and T cell protection from extrinsic and intrinsic forms of tumor-induced apoptosis.[2-7] Clinical studies evaluating a 3-week cycle of the IRX-2 Regimen in therapy-naïve subjects with squamous cell carcinoma of the head and neck (HNSCC) intended for curative surgery demonstrated modest tumor regression with increased lymphocyte infiltration into tumors when surgical specimens were compared to pre-treatment biopsies. Increase in tumor lymphocyte infiltration appeared to be associated with better overall survival.[43-46] These findings suggest that the IRX 2 Regimen may mobilize tumor-infiltrating effector lymphocytes resulting in a clinically significant anti-tumor immune response.

2.1.5 Manufacture and Composition of the IRX-2 Biologic

IRX-2 is a primary cell-derived biologic with multiple active cytokine components, produced under pharmaceutical standards from phytohemagglutinin (PHA) and ciprofloxacin stimulated donor mononuclear cells. Multiple healthy donor leukocytes (“buffy coats”) are pooled and then stimulated with PHA (to mimic a cellular immune challenge), and then locked in pre-s phase by ciprofloxacin. Subsequently, the PHA, ciprofloxacin and all cellular elements are removed and the cell-free supernatant is filter-sterilized, nanofiltered to clear viral particles, vialed and frozen as IRX-2. IRX-2 contains nanogram quantities (physiologic) of IL-1β, IL-2, IFN-γ, and TNFα, and also includes IL-6, IL-8, GM-CSF, and G-CSF. Each of these human cytokines when studied individually enhances cell-mediated immunity via different mechanisms.

2.1.6 Modifying the Lymph Node Microenvironment (IRX-2 Biologic)

The IRX-2 biologic is administered by subcutaneous injection in the peri-lymphatic area adjacent to lymph nodes draining the tumor bed to take advantage of the normal afferent and efferent pathways of lymph node activation. The immunosuppressive microenvironment contributes to incomplete or ineffective tumor antigen processing in regional lymph nodes. Administering IRX-2 near lymphatic capillaries and vessels enables the immunostimulatory cytokines to be drawn up into and potentially restore the lymph node microenvironment to facilitate DC maturation, antigen processing, and effector T cell activation.

2.1.7 Tumor-Induced Immune Suppression

Tumor-induced immune suppression ranges from tumors utilizing immune checkpoints (CTLA-4, PD-1/PD-L1) to production of soluble factors such as IL-10, TGF-β, prostaglandins, vascular endothelial growth factor, and Fas ligand which contribute to depression of cellular immunity by suppressing lymphocyte and monocyte function.[47-49] The production of these factors by the tumor results in the induction of host suppressor cells of both lymphoid and myeloid origin including regulatory T cells, myeloid suppressor cells, suppressor macrophages and DCs.[50-52]

Rationale for Combining the IRX-2 Regimen and Pembrolizumab in Gastric/GEJ Adenocarcinoma

Recently it has been shown that the degree of lymphocyte infiltration is an important prognostic factor for treatment with anti-PD-1 monoclonal antibodies in melanoma.[53] Additionally, it has been well established that the level of T cell infiltration in primary tumor biopsies is a strong predictive factor of improved outcome in multivariate analyses for many cancers.[54-56] This provides a compelling basis for incorporating treatment with the IRX-2 Regimen to increase the level of lymphocyte infiltration in tumors with anti-PD-1 treatments. In a Phase 2 trial, IRX-2 was shown to increase T cell infiltration into the tumor and this correlated with overall survival in HNSCC.[44, 45] In the Phase 2a study in patients with HNSCC, IRX-2 facilitated reductions in circulating B and NKT cell numbers, suggesting redistribution of these cells to tissues.[44]

In pre-clinical models, the IRX-2 Regimen was shown to be superior to recombinant IL-7 and IL-15 in protecting T cells from tumor- induced apoptosis and to be involved in IFN-γ+-driven T cell polarization towards Teff and suppression of Treg differentiation in the tumor microenvironment.[4] IRX-2 was subsequently shown to restore the function of dendritic cells from head and neck cancer patients.[5] A decrease in naïve T cells and unchanged numbers of Tregs (suppressor T cells) after IRX-2 therapy indicated that IRX-2 does not expand suppressor T cells thus potentially benefiting anti-tumor immune responses.[44]

As the IRX-2 regimen has been shown to work in an upstream immune-activating manner and PD-1 inhibitors work downstream by reducing tumor-induced immune suppression, a combination of such therapies would likely be synergistic. Pembrolizumab, a fully human monoclonal immunoglobulin (Ig) G4 antibody that binds to the PD-1 cell surface membrane receptor has been approved by FDA for the third-line treatment of gastroesophageal cancer although only a 13% objective response rate was observed in non-MSI-H tumors with positive PD-L1 expression by the CPS score (Fuchs ref). Therefore, it is likely that patients with evidence of immune checkpoint expression in the tumor and tumor microenvironment would benefit from additional immune system activation in conjunction with immune checkpoint inhibition. With employment of an immune checkpoint inhibitor, indomethacin and the need for omeprazole prophylaxis against NSAID-induced gastritis has been deemed unnecessary and will not be utilized in the trial. Zinc deficiency and supplementation in the HNSCC trials were unique to that patient population, and will also not be utilized in this trial for patients with metastatic gastroesophageal adenocarcinomas.

Overview of Study

The IRX-2 Regimen is an immunomodulatory regimen in development by Brooklyn ImmunoTherapeutics that includes a novel primary cell-derived biologic (IRX-2) that contains several active cytokines and cyclophosphamide. This treatment regimen has been demonstrated to bolster several facets of immune

response, including tumor infiltration by lymphocytes.[2-7] Therefore, it is possible that the combination of the IRX-2 regimen with pembrolizumab will improve treatment outcomes in advanced, treatment-refractory gastric and GEJ adenocarcinoma.

This phase 1b/2 study will seek to investigate the safety and tolerability of the combination of the IRX-2 regimen with pembrolizumab in this patient population. It will be used to describe the safety profile of combination IRX-2 regimen and pembrolizumab and determine the maximum tolerable dose of combination IRX-2 regimen with pembrolizumab in patients with gastric and gastroesophageal junction adenocarcinoma who have progressed on or are intolerant to ≥ 2lines of prior systemic therapy. The objective clinical response rate of IRX-2 regimen combined with pembrolizumab using RECIST 1.1 and immune modified RECIST criteria and progression free survival will also be evaluated. Exploratory analyses will seek to evaluate the following: circulating T cell profiles in patients before and after treatment with the IRX-2 regimen, the baseline and post-treatment tumor tissue for Nanostring gene expression profiling and correlation of these profiles to clinical response and evaluate additional biomarkers to develop hypotheses for response to these therapies.

3.0 Patient Eligibility

Inclusion Criteria:

1. Patients with histologically or cytologically confirmed recurrent or metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma progressed or intolerant to ≥ 2 lines of systemic therapy.
2. Patients must have recurrent or metastatic gastric/GEJ adenocarcinoma that are not amenable to local therapy with curative intent (surgery or radiation therapy with or without chemotherapy).
3. Willing and able to give informed consent and adhere to protocol therapy; written informed consent and any locally required authorization must be obtained from the patient prior to performing any protocol-related procedures, including screening evaluations
4. At least 18 years of age.
5. No prior exposure to PD-1/PD-L1 inhibitor therapy.
6. Patients are deemed eligible for pembrolizumab therapy with tumors demonstrating PD-L1 expression by the Combined Positive Score (CPS) being ≥ 1 as per the FDA-approved Dako PD-L1 IHC 22C3 PharmDx assay .
7. ECOG 0-1
8. Body weight must be >30 Kg.
9. Adequate normal organ and marrow function as defined below:
10. Hemoglobin >8 g/dL
11. Absolute neutrophil count (ANC) >1,200 x 10^9/mL
12. Platelet count >75 x 10^9/mL
13. Serum bilirubin ≤1.5 × institutional upper limit of normal (ULN) **OR** Direct bilirubin ≤ ULN if total bilirubin levels > 1.5 x ULN
14. Aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) ≤ 5 x ULN
15. Prothrombin time (PT) and partial thromboplastin time (PTT) < 1.5x the ULN.
16. Serum creatinine ≤ 1.5 x ULN **OR** Measured creatinine clearance (CL) >40 mL/min or

calculated creatinine clearance CL>40 mL/min by the Cockcroft-Gault formula [8] or by 24-hour urine collection for determination of creatinine clearance:

Males:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}}$$

Females:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age}) \times 0.85}{72 \times \text{serum creatinine (mg/dL)}}$$

17. Palliative radiation therapy is allowed to non-target lesions at the discretion of the treating physician.
18. Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) or evaluable disease as outlined in RECIST version 1.1.
19. Life expectancy of greater than 3 months in the opinion of the treating physician.
20. Female patients of childbearing potential must have a negative serum pregnancy test within 7 days prior to enrollment.

Exclusion Criteria:

1. Prior exposure to the IRX-2 regimen and/or PD-1/PD-L1 inhibitors are excluded.
2. Radiation therapy with a curable intent within 30 days of first dose of study treatment is excluded. However, radiation therapy with a palliative intent is allowed as long as treatment with study medication occurs ≥14 days after the last dose of radiation.
3. Any medical contraindications or previous therapy that would preclude treatment with the IRX-2 Regimen or pembrolizumab.
4. Known allergies to ciprofloxacin or phytohemagglutin given trace amount of these agents are contained in IRX-2.
5. Patients with ongoing chronic myelosuppression, myelodysplasia, or hemorrhagic cystitis which would contraindicate receipt of cyclophosphamide.
6. Any unresolved toxicity NCI CTCAE Grade ≥ 2 from previous anticancer therapy with the exception of alopecia, vitiligo, and the laboratory values defined in the inclusion criteria.
7. Patients with Grade ≥ 2 neuropathy will be evaluated on a case-by-case basis after consultation with the study physician
8. Patients with irreversible toxicity not reasonably expected to be exacerbated by treatment with IRX-2, pembrolizumab may be included only after consultation with the study physician.
9. Active or prior documented autoimmune or inflammatory disorders (including inflammatory bowel disease [e.g., colitis or Crohn's disease], diverticulitis [with the exception of diverticulosis], systemic lupus erythematosus, Sarcoidosis syndrome, or Wegener syndrome [granulomatosis with polyangiitis, Graves' disease, rheumatoid arthritis, hypophysitis, uveitis, etc]). The following are exceptions to this criterion:
    - Patients with vitiligo or alopecia.
    - Patients with hypothyroidism (e.g., following Hashimoto syndrome) stable on hormone replacement.

- Any chronic skin condition that does not require systemic therapy.
- Patients without active disease in the last 2 years may be included but only after consultation with the study physician.
- Patients with celiac disease controlled by diet alone.

10. Current or prior use of immunosuppressive medication within 14 days prior to Cycle 1, Day 1. The following are exceptions to this criterion:
    - Intranasal, inhaled, topical steroid or local steroid injections (e.g., intra articular injection).
    - Systemic corticosteroids at physiologic doses not to exceed 10 mg/day of prednisone or its equivalent.
    - Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication).
11. Major surgical procedure (as defined by the Investigator) within 28 days prior to Cycle 1, Day 1. Note: Local surgery of isolated lesions for palliative intent is acceptable.
12. History of allogeneic organ transplantation.
13. Symptomatic cardiopulmonary disease (including congestive heart failure and hypertension), coronary artery disease, serious arrhythmia or chronic lung disease. Patients with these conditions who are stable with relatively minor symptoms and who are appropriate candidates for systemic treatments need not be excluded.
14. Myocardial infarction within the last 3 months.
15. Has a known history of human immunodeficiency virus (HIV) (HIV 1/2 antibodies).
16. Has a history of active Hepatitis B requiring ongoing antiviral therapy or a history of untreated Hepatitis C.
17. Has received a live vaccine within 4 months of planned start of study therapy (Cycle 1, Day 1).
    Note: The killed virus vaccines used for seasonal influenza vaccines for injection are allowed provided not within 4 weeks of planned start of study therapy (Cycle 1, Day 1); however intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.
18. Signs or symptoms of systemic infection (use of antibiotics to treat superficial infection or contamination of tumor shall not, by itself, be considered evidence of infection).
19. Stroke or other symptoms of cerebral vascular insufficiency within the last 3 months.
20. Previous diagnosis of invasive cancer from which the individual is not disease-free AND that has required treatment within the past 3 years, except for superficial skin, cervical cancer in-situ, or early stage prostate or bladder cancer (i.e. treatment with curative intent and long term disease-free expectations).
21. History of leptomeningeal carcinomatosis
22. Known allergy or hypersensitivity to any of the study drugs or any of the study drug excipients.
23. Female patients who are pregnant or breastfeeding or male or female patients of reproductive potential who are not willing to employ effective birth control from screening to one year after last dose of cyclophosphamide or 180 days after the last dose of pembrolizumab therapy, whichever is longer.

4.0 Screening and Registration Procedures

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, even if the studies were done before informed consent was obtained. All screening procedures and their respective windows are detailed in the Study Calendar.

Informed Consent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject and a signed informed consent will be obtained. Documentation of informed consent for screening will be maintained in the subject's research chart and medical record and the patient will receive a copy of the signed inform consent document. All institutional, Federal, and State regulations concerning Informed Consent will be fulfilled.

COH Data Coordinating Center

Eligible participants 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)**.

- E-mail: DCC@coh.org

Registration Requirements/Process

4.1.1 Confirming, reserving a slot and dose level assignment

Staff (including physicians, protocol nurses and/or CRCs) should contact the DCC to verify the current dose level and slot availability, and to reserve a slot for a specific prospective subject. Slots can only be held for a limited time.

Eligible subjects must be registered **prior** to the 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.

4.1.2 Registration Process

Once a slot at a dose level has been reserved, the signed informed consent has been obtained, all pretreatment evaluations have been performed, and subject's eligibility has been confirmed by the Data Coordinating Center (DCC), a subject will be registered on study.

To register a subject, the treating physician should contact the protocol nurse or the responsible clinical Research Coordinator (CRC) in the Clinical Trial Office (CTO) to complete the eligibility checklist.

Allow up to 24 hours for the DCC to review eligibility. To register a participant the subsequent procedure is to be followed:

1. The study team should contact the DCC via 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 study team will email a **Complete Eligibility Packet** to the DCC, which consists a copy of the following documents:
    - Registration Cover Sheet (Appendix II)
    - Completed eligibility checklist (printed from Section 3.0 of the protocol) with required signature(s)
    - Source documents that support all eligibility criteria listed in the checklist
    - Signed Informed Consent
    - Signed HIPAA authorization form (if separate from informed consent)
    - Signed subject's bill of Rights (California only)
3. When all source documents are received, the DCC will review to verify eligibility, working with the study team to resolve any missing required source elements. Any missing documents may delay review and registration. A participant failing to meet all protocol eligibility requirements will not be registered and the study team will be immediately notified.
4. Once eligibility is confirmed, the DCC will send a Confirmation of Registration Form and signed Eligibility Checklist, including the Subject Study Number and cohort assignment to:
    - The study team: Site Lead Investigator, treating physician/sub-investigator, protocol nurse, CRC and pharmacy (as needed).
    - The COH Study PI and sponsor team designees (including but not limited to study monitor(s) and statistician(s)).
5. Upon receipt of the Confirmation of Registration Form, COH study team will register the patient in OnCore. The DCC will register non-COH patients in OnCore.

A subject failing to meet all protocol requirements will not be registered.

Patients must begin protocol treatment within 1 week following registration date.

Screen Failures and Registered Participants Who Do Not Begin Study Treatment

Notify the DCC immediately if the participant screen fails after registration or if the participant does not start treatment. For non-COH sites, the reason for screen failure will be documented in the registration coversheet (Appendix II) and submitted to the DCC.

5.0 Treatment Program

Treatment Overview

For a detailed tabular view of the treatment, monitoring, and follow-up schedule, see the Study Calendar (Section 10)

Treatment cycle definition

Each cycle will consist of 3 weeks coinciding with timing of pembrolizumab infusions. Cycle 1, Day 1 will start with the 1st day of IRX-2 regimen and a dose of pembrolizumab.

Treatment Plan

Treatment on study will continue up to 24 months or until confirmed disease progression, patient refusal, patient lost to follow up, unacceptable toxicity, whichever occurs first, or the study is terminated by the Sponsor.

The study will consist of two phases – a safety phase and a dose expansion phase:

Safety phase: A standard 3+3 dose finding rule will be employed with two dose levels. Patients will start at dose level 1 [IRX-2 regimen at dose of 2 mL (230 units/day)]. Patients will be enrolled in cohorts of 3, separated by at least 1 week. Initially, 3 patients will be treated with expansion to 3 more patients in the event of 1/3 dose limiting toxicities (DLTs) as observed during the first 3 weeks of treatment, escalation if 0/3 or 1/6 DLTs, and termination of escalation if ≥ 2 DLTs. Once ≥ 2 patients experience a DLT at a dose level, the next lower dose level will be expanded to 6 evaluable patients, if fewer than 6 evaluable patients have been treated at the dose level. The maximum tolerated dose (MTD) is defined as the highest dose tested in which only 0 or 1 out of 6 evaluable patients experience a DLT. The Recommended Phase 2 Dosing (RP2D) will not exceed the MTD but may be lower based on cumulative toxicities and dose modification patterns. If the rules suggest escalation at the highest proposed dose, that dose will accrue to 6 patients where <2 DLTs will determine that as the MTD, and 2 or more DLTs will require de-escalation. These rules will be used to determine the IRX-2 regimen dosing for the expansion phase.

Dose expansion phase: 8-14 patients will be enrolled at the recommended dose level from the safety phase, for a total enrollment of 20 patients. The 6-12 patients treated in the dose escalation portion of the study will be counted as part of the dose expansion patient population. Two doses of IRX-2 will be considered (Table 5-1). There will be no intra-patient dose escalation; participants will undergo dose modification due to toxicity (Section 6).

Table 5-1: Dose Levels of IRX-2

Dose Level	IRX-2 Dose
Starting Dose Level: 1	230 units (2 mL) total daily for 10 days: 2 injections daily comprised of 115 units in each 1 mL injection; 1 injection administered into the left and 1 injection into the right supraclavicular lymph node regions (substitute axillary regions if supraclavicular nodes have been removed)
2	460 units (4 mL) total daily for 10 days: 4 injections daily comprised of 115 units in each 1 mL injection; 1 injection each into both supraclavicular and 1 injection each into both axillary lymph node regions (substitute mastoid insertion if supraclavicular or axillary nodes have been removed)

Definition of Dose Limiting Toxicity

A DLT will be defined as any Grade 3 or higher toxicity that occurs during the DLT evaluation period of 3 weeks (between Cycle 1 Day 1 and Cycle 1 Day 21) and are considered **related to study treatment**. Toxicity that is clearly and directly related to the primary disease or to another etiology is excluded from this definition. A patient is evaluable for DLT if he/she receives the cycle 1 dose of pembrolizumab (100%) and

80% of the planned doses of IRX-2 during cycle 1 OR he/she experiences a DLT. If this minimum amount of treatment is not reached due to treatment related AEs, this is considered a DLT. However, patients who fail to achieve this minimum amount of treatment due to reasons that are clearly unrelated to treatment (e.g. error on part of patient or healthcare provider) will be replaced. The following will be DLTs:

- Any Grade 4 adverse event deemed to be immune-related
- Any ≥Grade 3 colitis
- Any Grade 3 or 4 non-infectious pneumonitis irrespective of duration
- Any Grade 2 pneumonitis that does not resolve to ≤Grade 1 within 3 days of the initiation of maximal supportive care
- Any Grade 3 irAE, excluding colitis or pneumonitis, that does not downgrade to Grade 2 within 3 days after onset of the event despite optimal medical management including systemic corticosteroids or does not downgrade to ≤Grade 1 or baseline within 14 days
- Liver transaminase elevation > 8 × ULN or total bilirubin > 5 × ULN
- Any unresolved toxicity necessitating a delay in the delivery of cycle 2, day 1 of pembrolizumab by > 3 weeks
- Any ≥Grade 3 non-irAE, except for the exclusions listed below

The definition excludes the following conditions:

- Grade 3 fatigue lasting ≤7 days
- Grade 3 endocrine disorder (thyroid, pituitary, and/or adrenal insufficiency) that is managed with or without systemic corticosteroid therapy and/or hormone replacement therapy and the patient is asymptomatic
- Grade 3 inflammatory reaction attributed to a local antitumor response (e.g., inflammatory reaction at sites of metastatic disease, lymph nodes, etc.)
- Concurrent vitiligo or alopecia of any AE grade
- Grade 3 infusion-related reaction (first occurrence and in the absence of steroid prophylaxis) that resolves within 6 hours with appropriate clinical management
- Grade 3 or 4 neutropenia that is not associated with fever or systemic infection that improves by at least 1 grade within 3 days. Grade 3 or Grade 4 febrile neutropenia will be a DLT regardless of duration or reversibility
- Grade 3 or 4 lymphopenia
- Grade 3 thrombocytopenia that is not associated with clinically significant bleeding that requires medical intervention, and improves by at least 1 grade within 3 days

- Isolated Grade 3 electrolyte abnormalities that are not associated with clinical signs or symptoms and are reversed with appropriate maximal medical intervention within 3 days

Agents Administration

5.1.1 IRX-2 Regimen

The IRX-2 regimen will be given every 12 weeks starting with Cycle 1. Cyclophosphamide will be administered as an intravenous infusion on Day 1 of each IRX-2 regimen at a dose of 300 mg/m^2.

IRX-2 will be administered subcutaneously into a maximum of 4 lymph node-bearing regions, i.e. the bilateral supraclavicular and axillary regions. For dose level 1, 1 ml (115 units/ml) will be injected into each of the 2 lymph node regions (total 2 ml (230 units/day)) corresponding to the bilateral supraclavicular areas only. If dose level 2 is reached, 1 ml (115 units/ml) will be injected into each of the 4 lymph node regions (total 4 ml (460 units/day)).

IRX-2 Regimen includes one dose of cyclophosphamide on Day 1 of each designated cycle, and subcutaneous IRX-2 injections in bilateral supraclavicular (and axillary regions if applicable) for 10 days between Days 4 and 15. The 10 days are not required to be consecutive (e.g. dosing not mandatory on weekends/holidays) provided dosing is completed by day 15.

5.1.2 Pembrolizumab

Pembrolizumab will be given at the approved dose of 200 mg every 3 weeks beginning with Day 1 of Cycle 1.

Pembrolizumab will be administered as an intravenous infusion over 30 minutes every 21 days (+/- 3 days).

Study Procedures

For a detailed list of all study procedures including timing and windows, see the Study Calendar.

Duration of Therapy and Criteria for Removal from Study Treatment

Patients will receive study treatment until disease progression, unacceptable toxicity, or other criteria for removal from study treatment are satisfied. Participants may be removed from treatment for any of the following reasons:

- Evidence of disease progression
- Patient is deemed intolerant to study treatment because of toxicity, despite dose modification/delay.
- Intercurrent illness that prevents further administration of treatment
- Participant withdraws from the treatment phase of the study
- General or specific changes in the participant's condition, including non-compliance, which renders the participant unacceptable for further treatment in the opinion of the treating investigator.

Documentation of the reason for discontinuing therapy and the date effective should be made in the medical record and appropriate eCRF. The participant should then proceed to off-treatment procedures for safety monitoring, and follow-up procedures. The participant's status is to be modified in the MIDAS system once the off-treatment period is completed. Alternative care options will be discussed with the participant.

End of Study Follow Up

In *End of Study Follow-Up* participants will be followed for survival information which may be obtained by reviewing the medical record, contacting the participant and/or a review of outside medical records (note: this is not an all-inclusive list).

The schedule and windows for assessments and data collection points are further detailed in the Study Calendar.

Criteria for Completion of Study Participation

Participants may be removed from the study as a whole at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reasons for discontinuing study participation should be documented and may include:

- Participant completes all study procedures including all study follow-up procedures.
- Participant withdraws consent for follow-up.
- Participant is determined to be lost to follow-up. All attempts to contact the subject must be documented.

The reason for study removal and the date the participant was removed 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 MIDAS system once the participant completes the study.

Supportive Care and Other Concomitant Therapy

All concomitant medications taken during the study treatment will be recorded in the Case Report Form. The minimum requirements are that drug name and the dates of administration are to be recorded. All prescription and over-the-counter medications that have been taken within 1 month prior to the first dose of investigational treatment should also be reported in the Case Report Form.

Patients should receive full supportive care during the study, including treatment with antibiotics, antiemetics, blood transfusions, antidiarrheals, and analgesics as appropriate.

5.1.3 Included concomitant medications

Supportive medications:

Supportive medication/class of drug:	**Usage:**
Concomitant medications or treatments (e.g., acetaminophen or diphenhydramine) deemed necessary to provide adequate prophylactic or supportive care, except for those medications identified as "prohibited".	To be administered as prescribed by the Investigator
Best supportive care (including antibiotics, nutritional support, correction of metabolic disorders, optimal symptom control, and pain management [including palliative radiotherapy to non-target lesions, etc.])	Should be used, when necessary, for all patients

5.1.4 Excluded concomitant medications

Prohibited concomitant medications

Prohibited medication/class of drug:	**Usage:**
Any investigational anticancer therapy other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment
mAbs against CTLA-4, PD-1, or PD-L1 other than pembrolizumab	Should not be given concomitantly whilst the patient is on study treatment
Any concurrent chemotherapy, radiotherapy, immunotherapy, or biologic or hormonal therapy for cancer treatment other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment. (Concurrent use of hormones for non-cancer-related conditions [e.g., insulin for diabetes and hormone replacement therapy] is acceptable. Local treatment of isolated lesions, excluding target lesions, for palliative intent is acceptable [e.g., by local surgery or radiotherapy])
Immunosuppressive medications including, but not limited to, systemic corticosteroids at doses exceeding 10 mg/day of prednisone or equivalent, methotrexate, azathioprine, and tumor necrosis factor-α blockers	*Should not be given concomitantly, or used for premedication prior to the immunotherapy infusions.* *The following are allowed exceptions:* • *Use of immunosuppressive medications for the management of immune-related AEs,* • *Short-term premedication for patients receiving combination agents where the prescribing information for the agent requires the use of steroids for documented hypersensitivity reactions,* • *Use in patients with contrast allergies.* • *In addition, use of inhaled, topical, and intranasal corticosteroids is permitted.* *A temporary period of steroids will be allowed if clinically indicated and considered to be essential for the management of non-immunotherapy related events experienced by the patient (e.g., chronic obstructive pulmonary disease, radiation, nausea, etc.).*

6.0 Anticipated Toxicities and Dose Delays/Modifications for Adverse Events

Anticipated Toxicities

Immune checkpoint inhibitors are known to cause autoimmune toxicities including pneumonitis, colitis, hepatitis, endocrinopathies, nephritis, rash, encephalitis.

In prior Phase 2 studies the IRX-2 Regimen was tolerated with acceptable toxicity. The most common adverse events (AEs) were headache (30%), injection site pain (22%), nausea (22%), constipation (15%), dizziness (15%), fatigue (11%), aspiration pneumonia (11%), anemia (11%) and myalgia (7%). All were grade 1-2 except for the aspiration pneumonias (one Grade 3, one Grade 4) and all resolved without sequelae. There were only minor (grade 1) alterations in post-treatment laboratory values. Eight serious adverse events (SAEs) in 7 subjects were reported during treatment and the 30-day post-operative period: aspiration pneumonia (n = 3), respiratory tract infection, asthma exacerbation, wound infection, neck abscess and alcohol withdrawal (n = 1 each); only the postoperative wound infection was considered related to the study treatment (42). During treatment, several subjects noted decreased pain or improved swallowing and no significant progressive symptoms were noted.

6.1.1 Potential Overlapping Toxicities

BOTH: diarrhea, nausea, abdominal pain, decreased appetite, fever, headache, infusion related reaction (injection site reaction), arthralgia, shortness of breath, asthenia

Pembrolizumab only: fatigue, itching rash, increased creatinine, increased blood sugar changes, low sodium, increased lipase, alterations in thyroid hormones, increased alkaline phosphatase, ALT, amylase, and AST, chills, constipation, cough, dizziness, dry mouth, dry skin, inflammation of color, or mouth, loss of color pigmentation, lung inflammation, musculoskeletal pain, nausea, redness, swelling, vomiting

IRX-2 only: lymphopenia, anemia, difficult swallowing, myalgia, rhinorrhea, neutrophilia, hypoalbuminemia, dehydration, hypotension, painWith elimination of indomethacin, omeprazole, and zinc supplementation, we aim to see fewer toxicities that may be associated with indomethacin-induced gastritis. To still carefully assess overlapping toxicities, dose escalation will start with dose level 1 as per Section 5.0.

6.1.2 General Information

The study will use the NCI Common Terminology for Adverse Events (CTCAE) Version 5.0 to grade toxicities.

Intra-patient dose escalation is never permitted in this study. Rules for dose modification are found in Appendix I.

Baseline values are from the last values obtained prior to treatment.

For holds due to toxicities related to study agent, if the participant does not meet the criteria to resume treatment within 14 days, the participant must permanently discontinue study treatment.

6.1.3 Dose Modifications

Guidelines for the management of immune-mediated reactions for pembrolizumab are provided in the Dosing Modification and Toxicity Management Guidelines in Appendix I. Patients should be thoroughly evaluated and appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of the immune related AE. Serologic, immunologic, and histologic (biopsy) data, as appropriate, should be used to support an immune related AE diagnosis. In the absence of a clear alternative etiology, events should be considered potentially immune related.

In addition, there are certain circumstances in which pembrolizumab should be permanently discontinued (see the Dosing Modification and Toxicity Management Guidelines in Appendix I).

Dose reductions for pembrolizumab are not permitted. In case of doubt, the local investigator should consult with the study principal investigator and Sponsor.

Patients should be thoroughly evaluated and appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of the immune related AE. Serologic, immunologic, and histologic (biopsy) data, as appropriate, should be used to support an immune related AE diagnosis. In the absence of a clear alternative etiology, events should be considered potentially immune related.

All toxicities will be graded according to NCI CTCAE, Version 5.

Dose reductions for IRX-2 injections are not permitted. In case of doubt, the local investigator should consult with the study principal investigator.

7.0 Data and Safety Monitoring and Adverse Event Reporting

7.1.0 Adverse Events and Unanticipated Problems

7.1.1 Definitions

7.1.1.1 Adverse Event (AE)

An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

7.1.1.2 Serious Adverse Event (SAE)

A serious adverse event is any expected or unexpected adverse events 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
- Secondary malignancy*

- 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).

*Modified from 21 CFR 312.32

7.1.1.3 Unanticipated Problems Involving Risks to Subjects or Others

An unanticipated problem is 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.

7.1.1.4 Adverse Events of Special Interest (AESI)

Specific adverse events, or groups of adverse events, will be followed as part of standard safety monitoring activities. These events, regardless of seriousness, will be reported.

7.1.2 Assessment of Adverse Events

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

7.1.2.1 Assessment of Adverse Event Name and Grade

Adverse events will be characterized using the descriptions and grading scales found in the most recent CTCAE v 5.0. A copy of the scale can be found at NCI/ CTEP web site. The determination of severity for all other events not listed in the CTCAE v 5.0 should be made by the investigator based on medical judgment and the severity categories of Grade 1 to 5 as defined below:

- Grade 1 (mild) – An event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Grade 2 (moderate) – An event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.

- Grade 3 (severe) – An event that requires intensive therapeutic intervention. The event interrupts usual activities of daily living, or significantly affects the clinical status of the subject.
- Grade 4 (life threatening) – An event, and/or its immediate sequelae, that is associated with an imminent risk of death or with physical or mental disabilities that affect or limit the ability of the subject to perform activities of daily living (eating, ambulation, toileting, etc).
- Grade 5 (fatal) – Death (loss of life) as a result of an event.

7.1.2.2 Assessment of Attribution

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

- **Unrelated –** The event is clearly related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant medications administered to the participant.
- **Unlikely –** The event is doubtfully related to the investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible –** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Probable –** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions, or concomitant drugs.
- **Definite –** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions, or concomitant drugs, AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

7.1.2.3 Assessment of Expectedness

The following definitions will be used to determine the expectedness of the event:

- **Unexpected**– 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. *Modified from 21 CFR 312.32 (a)
- **Expected –** An adverse event is expected if it 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.

7.1.3 Reporting of Adverse Events

7.1.3.1 Routine Reporting of Non-Serious Adverse Events by Site Investigators

Routine AE recording will occur via data entry into the study eCRF. Recording of adverse events will begin once the patient is consented and will continue until 30 days after the last dose of treatment. Adverse events will be monitored by the Protocol Management Team (PMT). Adverse events that do not meet the criteria of serious OR are not unanticipated problems do not require expedited reporting. AEs reported through expedited processes (i.e. reported to the IRB, DSMC, FDA, etc.) must also be reported in routine study data submissions.

7.1.3.2 Expediting Reporting Requirements of SAEs and UPs by Site Investigators

7.1.3.2.1 Criteria for Reporting SAEs/UPs to the Coordinating Center

Serious Adverse Events meeting the criteria specified below will be reported to the Coordinating Center within **24 hours** of notification that the event occurred.

Adverse events that require expedited reporting include:

- AEs or SAEs that meet the definition of an unanticipated problem
- All deaths that occur within 30 days of active treatment
- All deaths that occur after 30 days of active treatment that are unexpected and possibly, probably, or definitely related to the study agent or procedure
- All serious adverse events, regardless of relationship to study agent or study procedure, that occur within 30 days of the last day of treatment
- All serious adverse events that occurred after 30 days of active treatment/therapy that are considered possibly, probably, or definitely related to the study agent or procedure

Note: Follow-up reports must be submitted for all events that require expedited reporting when the status of the event changes and until the resolution or stabilization of the event.

Reportable serious adverse events must be followed until the event is resolved, stabilized, or determined to be irreversible by the participating investigator; for ongoing reportable adverse events that are unrelated to study agent, the follow-up period may end at the 30-days post study-drug assessment. The Coordinating Center should be consulted prior to ending the follow-up of events that have stabilized.

7.1.3.2.2 Non-COH Sites: Procedure for Reporting SAEs/UPs to the COH Data Coordinating Center

1. 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 COH Data Coordinating Center copies of the IRB submission and corresponding IRB response.
2. Document/describe the SAE/UP on each of the following:

a. MedWatch 3500A

i. Downloadable form at http://www.fda.gov/medwatch/getforms.htm

b. UP/SAE Coversheet

i. SAE Coversheet is found in Appendix III. A modifiable Microsoft Word document is also available from the Data Coordinating Center. An electronic signature on the document will be accepted.

3. Scan and email above documents to DCC@coh.org with the subject title as "IRB # SAE".
    a. All SAE reports received at this account are forwarded immediately to study Principal Investigator, and to Coordinating Center personnel.
    b. While not required, if available and applicable, please also include the local IRB submission for this event in the submission.
4. If an email receipt from Coordinating Center personnel is not received within one working day, please call 626-256-4673 x 83968 and/or email DCC@COH.org.

7.1.3.2.3 COH Investigative Sites: Procedure for Reporting SAEs/UPs to the Coordinating Center

1. Email the following information to DCC@coh.org and jchao@coh.org:
    a. Participant ID, date the event met criteria for reporting, whether the event meets the definition of serious, whether the event is an unanticipated problem, grade of event, attribution of event, whether the event is a known expected toxicity to study agent.
2. Complete the iRIS AE/UP reporting form per COH reporting timeline.

7.1.3.3 Additional Reporting Requirements of the Study Principal Investigator

7.1.3.3.1 Reporting to COH IRB and DSMC

The study PI (or designee) will report to COH IRB and DSMC via iRIS all reportable serious adverse events that occur at COH and non-COH sites and meet COH IRB and DSMC expedited reporting criteria according to City of Hope's Institutional policy. The study PI will also submit a Protocol Management Team (PMT) report to the COH DSMC at the frequency outlined in Section 3.6. This report will include a review of aggregate adverse event data.

7.1.3.3.2 Reporting to the FDA

The study 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.

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)]

In addition, the study PI will submit annually within 60 days (via COH OIDRA) of the anniversary date of when the IND went into effect, an annual report to the FDA which is to include a narrative summary and analysis of the information of all FDA reports within the reporting interval, a summary report of adverse drug experiences, and history of actions taken since the last report because of adverse drug experiences.

7.1.3.3.3 Reporting to Participating Investigators

The study PI (or designee) will report all reportable serious adverse events to participating investigators as an IND Safety Report occurring within 30 calendar days of receipt of sponsor (lead site) notification, and indicate whether or not a protocol and/or consent form change is required. A cover letter will indicate the protocol title, the IND#, whether the FDA was informed, and, for non-COH sites, a statement that the report should be submitted to their local IRB for review as an IND safety report if applicable per local IRB policy.

The study PI will also forward to participating sites all IND safety reports received from Brooklyn ImmunoTherapeutics (formerly IRX Therapeutics), indicating whether a consent form or protocol change is required within 30 days of notification to study PI.

7.1.3.3.4 Reporting to Brooklyn ImmunoTherapeutics

All serious adverse events and AESIs (initial and follow-up information) will be reported by the study PI to Brooklyn ImmunoTherapeutics (formerly IRX Therapeutics) within the same timeframes as required by reporting to the FDA. A copy of Medwatch Form FDA 3500A submitted to the FDA will be submitted to Brooklyn ImmunoTherapeutics.

7.2.0 Protocol Deviations and Single Subject Exceptions

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. Brief interruptions and delays may occasionally be required because of travel delays, airport closures, inclement weather, family responsibilities, security alerts, government holidays, and so forth. Delays can also extend to complications of disease or unrelated medical illnesses not related to disease progression. The PI has the discretion to deviate from

the protocol when necessary so long as such a deviation does not threaten patient safety or protocol scientific integrity. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

7.2.1 Definitions

7.2.1.1 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.
Examples include, but are not limited to: a) dose adjustments based on excessive patient weight; b) alteration in treatment schedule due to non-availability of the research participant for treatment; and c) laboratory test results which are slightly outside the protocol requirements but at levels that do not affect participant safety.

7.2.1.2 Single Subject Exceptions (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.

7.2.2 Reporting of Deviations and SSEs

7.2.2.1 Reporting Deviations

For any deviation, the Investigator will notify the COH DSMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy. A list of deviations from all participating sites will be submitted along with the Protocol Management Team (PMT) progress report to the COH DSMC.

For non-COH sites:

- The local IRB and/or DSMC must be notified according to local institutional policies.
- The study Principal Investigator must be notified as soon as practical (within 24 hours of notification of the event) via email to jchao@coh.org and dcc@coh.org. This email should provide input on the following:
    - Description of the event
    - Impact on participant safety or the safety to others
    - Impact on the study design
    - A corrective and preventative action plan

7.2.2.2 Reporting Single Subject Exceptions

The SSE must be submitted as a "Single Subject Exception Amendment Request" via iRIS in accordance with 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.

All non-emergency planned deviations from the protocol must have **prior** approval by the Study Principal Investigator, the Site Principal Investigator, COH IRB, and when applicable, the local IRB. In addition, if contractually obligated, the sponsor must also approve the deviation.

7.3.0 Study Oversight, Quality Assurance, and Data & Safety Monitoring

7.3.1 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 agree 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.

7.3.2 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. Subpart D are executed in accordance with federal regulations.

7.3.3 Protocol Management Team (PMT)

The Protocol Management Team (PMT), minimally consisting of the study PI, site investigators, collaborating investigators, research nurse, clinical research associate/coordinator, and the study biostatistician, is responsible for ongoing monitoring of the data and safety of this study, including implementation of the stopping rules for safety/toxicity.

The PMT is recommended to meet (in person or via teleconference) at least monthly to review study status. This review will include, but not be limited to, reportable AEs and UPs, and an update of the ongoing study summary that describes study progress in terms of the study schema. The meeting will be a forum to discuss study related issues including accrual, SAE/AEs experienced, study response, deviations/violations and study management issues. The appropriateness of further subject enrollment and the specific intervention for subsequent subject enrollment are addressed. It is recommended that minutes of these discussions be taken to document the date of these meetings, attendees and the issues that were discussed (in a general format).

7.3.4 Monitoring

Clinical site monitoring 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 for this study will be performed by the City of Hope Office of Clinical Trials Auditing and Monitoring (OCTAM).

The site Investigator/Institution will permit the study monitors and appropriate regulatory authorities direct access to the study data and to the corresponding source data and documents to verify the accuracy of this data. The Investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

Details of clinical site monitoring are documented in the OCTAM SOP that is provided as a supplement to this document. This document specifies the frequency of monitoring, monitoring procedures, the level of clinical site monitoring activities (e.g., the percentage of subject data to be reviewed), and the distribution of monitoring reports. Staff from OCTAM will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the details described in the SOP. Documentation of monitoring activities and findings will be provided to the site study team, the site PI, study PI, and the COH DSMC.

7.3.5 Quality Assurance

The City of Hope Clinical Research Information Support will provide support for this multi-center trial as detailed in the COH DCC Operations Plan provided as a supplement to this document.

7.3.6 City of Hope Data and Safety Monitoring Committee

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 COH as the IND holder and escalates the study agent to a dose above that indicated by the FDA for the agent's currently approved indication.

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 Study Principal Investigator is required to submit periodic status reports (the PMT report) according to the guidelines outlined in the City of Hope Institutional Data and Safety Monitoring Plan. The PMT report will be submitted to the COH DSMC quarterly from the date of activation.

The COH Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The DSMC will review up-to-date participant accrual; summary of all adverse events captured via routine and expedited reporting; a summary of deviations; any response information; monitoring reports, and summary comments provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request. For Phase I studies, a Phase I Tracking Log will be utilized and reviewed by the DSMC to monitor data and safety for dose escalation. A review of outcome results (response, toxicity and adverse events) and factors external to the study (such as scientific or therapeutic developments) is discussed, and the Committee votes on the status of each study. Information that raises any questions about participant safety will be addressed with the Principal Investigator, statistician and study team. The PMT report and DSMC recommendations will be circulated to all participating sites for submission to their IRBs, in accordance with NIH guidance.

8.0 Agent Information

Drug Information for IRX-2

Mode of Action: IRX-2 is a primary cell-derived biologic containing multiple active cytokine components produced under pharmaceutical standards from phytohemagglutinin (PHA) and ciprofloxacin stimulated donor mononuclear cells. Route of administration of IRX-2 takes advantage of the normal afferent and efferent pathways of lymph node activation. By presenting the cytokine-containing biologic in the area of lymph nodes rather than systemically, there is an opportunity to facilitate the mobilization of antigen presenting cells and enhance dendritic cell function as well as directly activate T cells to proliferate and become cytotoxic lymphocytes. T cells may become activated to common tumor antigens or mutated autologous neoantigens that are found in the local tumor microenvironment. Additionally, subcutaneous administration may be less toxic since the systemic cytokine drug concentration is much lower.

8.1.1 Risks and Contraindications

Ciprofloxacin and PHA are used in the manufacture of IRX-2. Ciprofloxacin and PHA concentrations are significantly reduced by centrifugation and dilution during manufacture and only very small amounts

remain in the final product. Nevertheless, individuals who are allergic to ciprofloxacin or PHA should not be treated with IRX-2.
Cyclophosphamide is contraindicated in individuals with evidence of pre-existing myelosuppression, myelodysplasia or hemorrhagic cystitis.

8.1.2 Supplier

Brooklyn ImmunoTherapeutics

8.1.3 Dose and Administration

IRX-2 will be administered subcutaneously into a maximum of 4 lymph node-bearing regions: bilateral supraclavicular and bilateral axillary regions are preferred. IRX-2 can be administered in the bilateral mastoid lymph node-bearing region if supraclavicular or axillary nodes have been removed. Two dose levels will be considered. For dose level 1, one ml (115 units/ml) will be injected into 2 lymph node regions (total 2 ml (230 units/day)) preferentially into the supraclavicular node regions with substitution of axillary node regions if the supraclavicular lymph nodes have been removed. For dose level 2, one ml (115 units/ml) will be injected into 4 lymph node regions (total 4 ml (460 units/day)).

Other IRX-2 Regimen Medications:

Cyclophosphamide will generally be purchased by the pharmacy of the study site and will be supplied to study personnel by the study site investigational pharmacist.

IRX-2 regimen		
Agent	**Dose**	**Route of Administration**
Cyclophosphamide (Day 1)	300 mg/m^2	IV
IRX-2: For 10 days starting on Day 4; the 10 days are not required to be consecutive (e.g. dosing not mandatory on weekends/holidays) provided dosing is completed by Day 15	Dose Level 1: 230 units total daily (2 injections daily comprised of 115 units in each injection) Dose Level 2: 460 units total daily (4 injections daily comprised of 115 units in each injection)	Dose Level 1: Subcutaneous at or near the supraclavicular lymph node regions bilaterally (substitute axillary if supraclavicular nodes have been removed). Dose level 2: Subcutaneous at or near the supraclavicular and axillary node regions bilaterally (if any of these nodes have been removed, can inject at or near mastoid insertion of both sternocleidomastoid muscles).

The study personnel or other trained individuals will administer IRX-2 injections to ensure treatment compliance. IRX-2 can be given as a subcutaneous injection through a 25-guage needle.

Any non-compliance with the study treatment needs to be documented in the clinical record.

8.1.4 Formulation/Agent Preparation

IRX-2 is supplied as a pale yellow, sterile liquid for subcutaneous injection in 2.0 mL single-dose vials. All immediate study supply containers will be appropriately labeled to identify study number, lot number, and product identity. Study syringes for subcutaneous IRX-2 administration will be disposed of in accordance with biosafety procedures. Prepared drug must be used within 24 hours of removing the vial from frozen storage.

8.1.5 Storage

IRX-2 must be stored in a secure area and maintained under labeled storage conditions at a range of −15°C to –50° C.

Drug Information for Pembrolizumab (Keytruda®)

Other Names: Keytruda®

Mode of Action: Pembrolizumab is a fully human immunoglobulin (Ig) G4 monoclonal antibody which binds to and blocks the activation of PD-1, an Ig superfamily transmembrane protein. This prevents the interaction between PD-1 with its ligands programmed cell death ligand 1 (PD-L1), overexpressed on certain cancer cells, and programmed cell death ligand 2 (PD-L2), which is primary expressed on antigen presenting cells (APCs). This results in the activation of T-cells and cell-mediated immune responses against tumor cells or pathogens.

8.1.6 Risks and Contraindications

No contraindications or drug interactions

Warnings and Precautions include development of:

- Immune-Mediated Pneumonitis
- Immune-Mediated Colitis
- Immune-Mediated Hepatitis
- Immune-Mediated Endocrinopathies
- Immune-Mediated Nephritis and Renal Dysfunction
- Immune-Mediated Skin Adverse Reactions
- Immune-Mediated Encephalitis
- Other Immune-Mediated Adverse Reactions
- Infusion Reactions
- Complications of Allogeneic HSCT
- Embryo-Fetal Toxicity

Adverse Reactions: (please refer to package insert for full details)

Most common adverse reactions (≥20%) in patients treated with pembrolizumab include:

- fatigue, musculoskeletal pain, decreased appetite, pruritus, diarrhea, nausea, rash, pyrexia, cough, dyspnea, and constipation

Dose delays or discontinuations as a result of treatment expected adverse events are listed in Section 6.0 and in the package insert.

8.1.7 Supplier

Routine commercial supply of medication based on FDA approved indication for advanced gastric/GEJ adenocarcinoma.

8.1.8 Dose and Administration

Dosage Forms/Strengths: 100mg/4mL (25 mg/mL) solution in a single vial.
Administer as an intravenous infusion over 30 minutes.

- Gastric and GEJ adenocarcinoma
    - Pembrolizumab 200 mg every 3 weeks.

8.1.9 Formulation/Agent Preparation

Preparation for Intravenous Infusion

- Visually inspect the solution for particulate matter and discoloration prior to administration. The solution is clear to slightly opalescent, colorless to slightly yellow. Discard the vial if visible particles are observed.
- Dilute KEYTRUDA injection (solution) or reconstituted lyophilized powder prior to intravenous administration.
- Withdraw the required volume from the vial(s) of KEYTRUDA and transfer into an intravenous (IV) bag containing 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Mix diluted solution by gentle inversion. The final concentration of the diluted solution should be between 1 mg/mL to 10 mg/mL.
- Discard any unused portion left in the vial.

8.1.10 Storage

Store non-diluted vials under refrigeration at 2°C to 8°C (36°F to 46°F) in original carton to protect from light. Do not freeze. Do not shake.

Store the diluted solution from the pembrolizumab 100 mg/4 mL vial either:

- At room temperature for no more than 6 hours from the time of dilution. This includes room temperature storage of the infusion in the IV bag, and the duration of the infusion.
- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of dilution. If refrigerated, allow the diluted solution to come to room temperature prior to administration.
- Do not freeze.

Use of IRX-2 and COVID-19

As the extent of the exposure and infection with COVID-19 grows, Brooklyn ImmunoTherapeutics (BITX) has reviewed the available literature and is providing the following as guidance for use of IRX-2 in patients at this time.

Published literature suggests that some of the deaths from COVID-19 have been linked to cytokine release syndrome, or “Cytokine storm”. It is important to note that during the development of IRX-2, we have not had any reports of Cytokine Release Syndrome to date, but, given the components, and mechanism of action of the drug, BITX feels there could be a small, but theoretically possible risk in the administration of IRX-2 to a patient who may be co-infected with COVID-19, by creating an increased risk of Cytokine Release Syndrome.

Given this, we recommend the following:

- All subjects should have a negative COVID-19 screening test within 5 days of

administration of first dose of a cycle of IRX-2.

- Subjects should be instructed to take all precautions possible to limit their exposure to COVID-19 during their treatment with IRX-2.
- Any subjects that present with symptoms of COVID-19 infection should immediately discontinue treatment with IRX-2. Once symptoms have fully resolved and the subject has demonstrated a negative COVID-19 test, resumption of IRX-2 dosing can occur at the Investigator's discretion.

9.0 Correlative/Special Studies

Tumor Tissue Studies

Radiology-guided biopsies will utilize 18 to 20-gauge core biopsy needles. Endoscopic biopsies will utilize standard 3-6 mm biopsy forceps. As feasible, the same region of the tumor will be sampled.

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	Receiving Lab	Downstream Analysis & Lab Performing Analysis
Formalin fixed (FFPE)	• Screening • Week 7	COH Pathology Core	TGen FFPE: MHC-PepSeq
			Molecular Pathology Core FFPE: Nanostring Gene Expression Profiling

9.1.1 Distribution to laboratories for analysis

The COH Pathology core will distribute tissue to internal collaborators for downstream analysis.

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).

9.1.2 Timepoints of Collection and Tube Type

Timepoint of collection	Volume per Timepoint	Tube Type	Receiving & Processing Lab	Downstream Analysis & Lab Performing Analysis
• Week 1 • Week 4 • Week 7 • Week 10 • End of Treatment	20 mL	Green-top	COH APCF (Dr. Synold)	• ctDNA analysis – TGen Tardis assay • Cytokine profile on plasma - Dr. Synold • PBMC- immuneprofiling- COH Immuno-oncology Core • Anti-Drug Antibodies Testing – Dr. Synold
	6 mL	Purple-top		
	6 mL	Red-top		

9.1.3 APCF Notification, Blood Collection and Labeling

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 Purple-top Red-top	1. Label tubes with COH protocol #, subject ID, actual collection time in 24-hour format, institution (for CP sites), and timepoint of collection (e.g. pre-chemo, pre-immuno, post-immuno, F/U). 2. Timepoints of collection are stated in Section 9.1.2. 3. Blood samples will be collected from an indwelling venous catheter or by venipuncture 4. Invert tubes eight times after collection. 5. **Green-top and Purple-top tube(s): Immediately** place the tubes **on ice**. 6. **Red-top tube(s):** Keep red-top tubes at **room temperature**.	• **Promptly** deliver the blood samples to the COH APCF, Shapiro room 1042 (Duarte Campus) for processing **within 1-2 hours (± 30 minutes) of collection.** • **Delivery temperature**: – Green-top/purple-top tubes on ice or 4°C. – Red-top tube at room temperature. • **For non-Duarte sites** process samples and store until future batched shipments.

9.1.4 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	Processing Details	
Green-top (x2) 10mL NaHep tubes (cat# BD 367874)	Plasma	1. For plasma preparation **use 20 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 (Acrodisc Filter .8/.2ucron filter. Thermo Fisher: Andwin Scientific 28139-702). 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 (Sarstedt micro tube PP 1.5ml screw cap tubes ThermoFisher: 50-809-238). 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 at City of Hope or Shipment to City of Hope for Non-COH sites.
	Peripheral blood mononuclear cells (PBMC)	1. 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. 2. PBMC will then be isolated per COH APCF procedures at City of Hope. Non COH sites will process PBMC per site standard process. 3. Isolated PBMC will be aliquoted(Thermo: 377627. Thermo Scientific™ Nunc™ Biobanking and Cell Culture Cryogenic Tubes 1.8mL) and stored at -80˚C/ liquid nitrogen until at City of Hope or Shipment to City of Hope for Non-COH sites.
Purple-top (x1) 6mL K2-EDTA (Cat# BD 367863)	Plasma	1. City of Hope subjects will process per APCF Procedures. Non-COH sites: Centrifuge the 6ml PT tube at 1000x g, 10min at 4deg. 2. Tubes should be de-identified prior to aliquoting and freezing (i.e. tubes should only contain protocol ID, study subject ID and timepoint of collection) 3. Plasma aliquots (~500 µL aliquots) (Sarstedt micro tube PP 1.5ml screw cap tubes ThermoFisher: 50-809-238).)will be stored frozen at -80˚C until shipment to TGen.
Red-top (x1) 6mL red top (Cat# BD 367815)	Serum	1. If not already done so, allow the blood to clot for at least 1 hour at room temperature. 2. Keep the red-top tubes upright and at room temperature during transport and prior to processing. 3. Centrifuge at 1000 x g at 4˚C for 15 minutes, max brakes. 4. Transfer as many 0.5 mL aliquots as possible to labeled 1.5 mL Sarstedt tubes. 4. Serum aliquots will be stored frozen at -80˚C until use at City of Hope or Shipment to City of Hope for Non-COH sites

9.1.5 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.

Stool Samples

9.1.6 Timepoints

- Screening
- Week 7

9.1.7 Collection, labeling, storage

1. Provide participant with a biospecimen bag and sterile swab (BD BBLTM CultureSwabTM Sterile, Media-free Swabs).
2. Instruct participant on proper collection from a used toilet tissue paper.
3. Stool will be collected either just before the scheduled clinic visit or during the clinic visit.
4. **Remind the participant** at least 1-2 days in advance of the protocol visit to perform stool collection and store the sample at room temperature until the day of the scheduled clinic visit.
    a. **Optional:** Participant may instead provide a stool sample at the clinic during the protocol visit provided they are reporting regular bowel movements.
5. **Labeling:** Label samples with COH protocol #, subject ID, institution (for CP sites), date of collection, and timepoint of collection (e.g. pre-chemo, pre-immuno, post-immuno, F/U).
6. **Promptly** store samples are at -20 ^{O}C until analysis.
7. **Community Practice Sites:**
    a. Ship on ice at time of blood shipment to the COH Duarte campus
    b. COH APCF will notify the study team to retrieve the sample

9.1.8 Shipping to TGen/Northern Arizona University

Samples will be batch shipped on dry ice via overnight courier by the study team designee to Dr. Caporaso.

Dr. J. Gregory Caporaso,
Northern Arizona University
1350 S Knoles Drive
Flagstaff, AZ 86011, USA

TGen Downstream Correlative Analyses

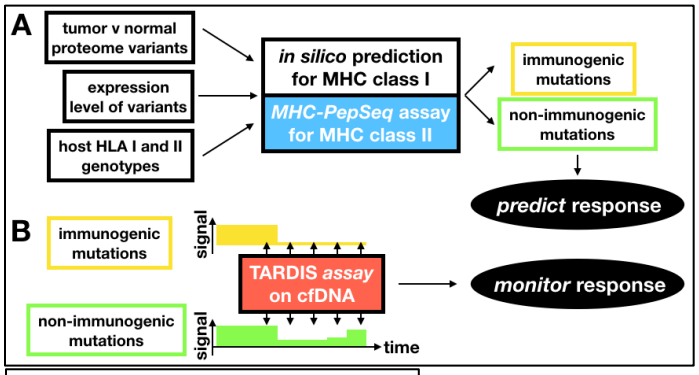

Figure 1. An integrated approach to predict and monitor neo-antigen-specific immunity. (A) Comprehensive genomic tumor analysis (exome, transcriptome sequencing, *in silico* neoantigen prediction) will be combined with the MHC-PepSeq assay to identify tumor mutations that result in variant peptides capable of binding host HLA. The landscape of such mutations may be predictive of the response to immunotherapy. **(B)** Non-invasive (ctDNA) assays measuring the abundance of different classes of the mutations characterized in (A) will be developed for serial study of each individual and used to monitor the response over time. Disproportionate changes in the abundance of immunogenic v non-immunogenic mutations may provide a sensitive measure of immune pressure in response to immunotherapy.

An overall low abundance of somatic mutations with antigenic potential is a second feature of Gastroesophageal cancers that may limit its responsiveness to immunotherapy. T cell-mediated immunity to neo-antigens (novel peptide sequences created by tumor mutations) shapes the genomic landscape of emergent tumors and mediates the efficacy of successful immunotherapies[57]. However, predicting which tumor mutations have immunogenic potential is a multi-factorial and highly-personalized inquiry, dependent upon: (i) the set of particular mutant sequences in the tumor, (ii) the expression of proteins containing those sequences, (iii) the ability of mutant sequences to be presented by autologous HLA proteins, and (iv) the activation and expansion of appropriate T cells that can eliminate the tumor. We estimate a median ~40 somatic coding mutations per GE tumor. Based on published estimates, 5-20% of these might be expected to have antigenic potential. As a consequence, even if the tumor microenvironment is modulated to become more immunogenic, the potential for a clinical response to combination immunotherapy is likely to depend upon an intrinsic capacity for T cell recognition that varies from tumor to tumor[58].

Both MHC class I-restricted CD8+ and MHC class II-restricted CD4+ T cells have been implicated in successful anti-tumor immunity[59, 60]. Athough *in silico* methods for predicting class I binding sequences have shown some utility, models of HLA class II binding are generally of limited predictive value[60]. To address this gap, we developed a novel multiplexed proteomics technology 'MHC-PepSeq' that enables large (10,000s), programmable sets of DNA-encoded peptides to be assayed for binding against a panel of human HLA class II proteins, using a digital sequencing readout. This technology will be deployed in association with comprehensive genomic and transcriptomic sequencing (**Figure 1**). Importantly, our recent validation experiments, indicate this system has proven to be highly effective at predicting the

epitope targets of naturally-arising T cell responses to influenza virus, substantially outperforming *in silico* methods.

To look for immunological predictors and correlates of therapeutic effect within the trial, we will assay tumor sections for tumor infiltrating lymphocytes (TILs). While TIL density/localization has previously been correlated with therapeutic anti-tumor immunity, it can be challenging to obtain serial samples of tumor tissue after treatment and, even when such biopsies are obtained, traditional tissue analysis affords only a fairly narrow view of the response. Therefore, in parallel, we will develop a richer view using an integrated comprehensive genomics approach to predict and monitor neo-antigen-specific immunity (outlined in **Figure 1**). This approach combines 2 novel multiplexed assays ("MHC-PepSeq" and "TARDIS") that both provide personalized information about the immunogenicity of mutations within individual tumors.

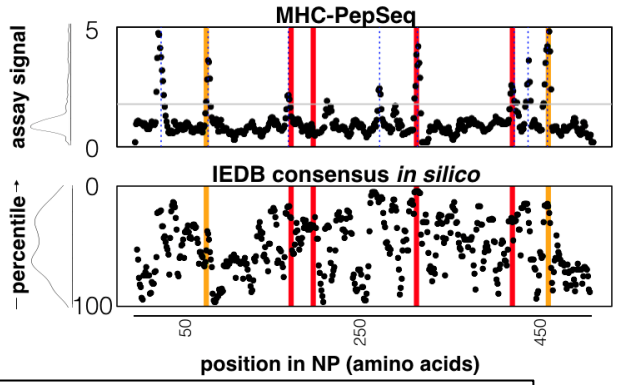

Figure 2. **Accurate T cell epitope prediction using the MHC-PepSeq assay**. A library comprising 3431 DNA-encoded peptides representing the proteome of H5N1 influenza was synthesized and tested for binding against 10 HLA-DR proteins, resulting in ~34000 multiplexed peptide-MHC measurements. The subset (~1%) of data corresponding to HLA-DRB1*01:01 binding of NP-derived peptides is shown (*upper panel*), and compared to *in silico* prediction data generated for the same peptides using the tool available at www.iedb.org (*lower panel*). Overlaid in vertical red / orange bars are the positions of all six

(i) *MHC-PepSeq* – identifying mutations with immunogenic potential

We will use a new multiplexed assay (MHC-PepSeq), together with *in silico* prediction, to assay all tumor mutations expressed in each individual (identified by both DNA and mRNA sequencing) against autologous HLA II proteins. Each mutation will be represented, alongside its wild-type counterpart, at each position of a 15mer peptide - resulting in ~30 peptides per SNV/indel.

For each patient, the assay will output a *personalized immunogenicity landscape* that represents the predicted interaction between tumor and host. We expect ~5-20% of the coding somatic mutations to have immunogenic potential restricted by at least one of the patient's HLAs, resulting in a fairly limited and variable set of potential neo-antigens in each patient. We will assess whether these neo-antigen sets are predictive of the clinical response to combination therapy across the trial cohort.

(ii) *ctDNA-based* monitoring tumor response to combination immunotherapy

We have developed a targeted multiplexed digital sequencing assay for circulating tumor DNA ('TARDIS') that enables non-invasive high-dimensional tumor monitoring by quantifying a panel of personalized tumor variant sequences in circulating cell-free DNA. Changes in the allele fraction of these variants over time are indicative of changes in tumor activity, load and/or composition – and have been shown to provide early indicators of the response to therapy[61,62]. Here, we propose to extend this concept to the detection of immune-mediated selection. Using the immunogenicity-predicting assay described above, we will identify 2 categories of mutations for each individual's tumor – putatively '*immunogenic'* and '*non-immunogenic'*. For each category/patient, a multiplexed panel of ~5-10 TARDIS assays will be designed and used to monitor serial patient plasma samples. Tumor-specific immunity is expected to manifest as the disproportionate loss of immunogenic mutations v non-immunogenic mutations over

time (represented in Fig 1b), as has been observed in serial tissue biopsies of melanomas treated with nivolumab[63]. Tracking multiple mutations in each category will afford statistical power and help to compensate for any limitations in the immunogenicity prediction step. Overall, this strategy will offer a sensitive molecular correlate of anti-tumor immunity that will be viewed alongside – and used to enrich – the interpretation of standard clinical monitoring (i.e. imaging).

10.0 Study Calendar

Study Activity Calendar

Test	Screening (Day -28 to 0)	Cycles 1-35 (+/- 3 day window starting with Cycle 2)					End of treatment (EOT) or disease progression	Safety Follow-up (30 days post-discontinuation for toxicity)	Survival Follow-up (up to 1 year after EOT)
		Weeks							
		W1	W4	W7	W10	W13+			
History and Physical[a]	X	X	X	X	X	X	X	X	X
Weight and ECOG PS	X	X	X	X	X	X	X	X	
CBC and diff [b]	X	X	X	X	X	X	X	X	
Chemistries (Na, CL, CO_2, K, BUN, Creatinine, Ca, Mg, Phos)	X	X	X	X	X	X	X	X	
Liver function panel[c]	X	X	X	X	X	X	X	X	
PT, PTT, INR[d]	X	X	X	X		X			
TSH	X	X	X	X	X	X	X	X	
ECG[e]	X								
Pembrolizumab[f]		X	X	X	X	X			
IRX-2 regimen[g]		X				X			
Tissue for Research Purposes (paraffin embedded)[h]	X			X					
Stool Collection for Research Purposes	X			X					
Blood for Research Purposes[i]		X	X	X	X		X		

Toxicity Assessment[j]	X	X	X	X	X	X	X	X	
Radiology: CT-chest, abdomen, and pelvis for tumor measurements[k]	X			X		X	X		
Serum B-HCG [l]	X								

[a] Once the patient has fully recovered from the study drug-related toxicities or the patient enrolls in a hospice, the follow up can be done by a phone call if the patient is off the study drugs.

[b] CBC and diff includes: WBC, ANC, HCT, HGB, PLT, % lymphocytes, % monocytes, % neutrophils, and other differential.

[c] Liver function panel includes: Alk Phos, Total Bilirubin, SGOT (AST), SGPT (ALT), Total Protein, and albumin.

[d] PT, PTT, and INR testing to occur week 1, week 4, and week 7 of each 12 week cycle.

[e] Resting 12-lead ECGs will be recorded at screening, and then as clinically indicated throughout the study.

[f] Pembrolizumab (200mg) administered IV Q3weeks as a 30 minute infusion for up to 2 years provided no intolerable toxicities and no progression of disease as per label of pembrolizumab (35 cycles)

[g] IRX-2 Regimen administered every 12 weeks starting week 1 corresponding to cycles 1, 5, 9, 13, 17, 21, 25, 29, and 33.

[h] If tumor paraffin blocks are not available, fresh biopsy will be obtained before the first treatment. If fresh biopsy is indicated, one core (or punch) will be collected in formalin and the second core (or punch) will be collected in liquid nitrogen.

[i] Research blood will be collected at the beginning of cycle 1 prior to patient receiving week 1 of therapy and must be processed within 2 hours of collection in order to separate PBMC and plasma. Plasma will be utilized for Pepseq based ctDNA determination

[j] Toxicity assessment: Common Toxicity Criteria (CTC) version 5.0 will be used. All toxicity grades (including grade 1) should be captured on the case report forms. All toxicities that occurred during treatment should be followed until resolution.

[k.] Tumor measurement: The same type of scan should be used for repeat measurements. Scans should be obtained every 6 weeks for the first 18 weeks, then every 9 weeks afterwards. For patients with the first documentation of progressive disease, treatment can be continued if no clinical deterioration and no grade 3-4 toxicities. Confirmatory scan will be obtained in ≥ 4 weeks. If the disease progression is confirmed, the patient will be taken off the study. If the disease progression is not confirmed and the patient does not have grade 3-4 toxicities, the patient will continue the treatment on the study. For patients who complete 35 cycles of therapy, scans can be spaced out to every 12-24 weeks at the discretion of the treating physician.

[l] Pregnancy test should be done in woman of child bearing age who are sexually active and may potentially be pregnant. Prior to study enrollment, women of childbearing potential (WOCBP) must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. In addition, men enrolled on this study should understand the risks to any sexual partner of childbearing potential and should practice an effective method of birth control. All WOCBP MUST have a negative pregnancy test within 7 days prior to enrollment. If the pregnancy test is positive, the patient must not receive investigational product and must not be enrolled in the study. In addition, all WOCBP should be instructed to contact the Investigator

immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. The Investigator must immediately notify IRX in the event of a confirmed pregnancy in a patient participating in the study.

11.0 Evaluation Criteria/Measurement of Effect

Primary Endpoint: Toxicity

The primary endpoint is toxicity. Toxicity will be captured in a standardized manner using the NCI Common Toxicity Criteria for Adverse Events (v5). Dose limiting toxicity is defined in Section 5.4 of the protocol. The MTD will be based on the assessment of DLT. All patients who are not evaluable for dose limiting toxicity will be replaced. A patient is evaluable for DLT if he/she receives the cycle 1 dose of pembrolizumab (100%) and 80% of the planned doses of IRX-2 during cycle 1 OR he/she experiences a DLT. If this minimum amount of treatment is not reached due to treatment related AEs, this is considered a DLT. However, patients who fail to achieve this minimum amount of treatment due to reasons that are clearly unrelated to treatment (e.g. error on part of patient or healthcare provider) will be replaced.

Secondary Endpoint: Response

Response is the secondary endpoint. Response and progression will be evaluated in this study using the new updated international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee version 1.1.

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

11.1.1 Definitions

- Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with IRX-2 and/or pembrolizumab.
- Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

11.1.2 Disease Parameters

- Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥10mm with conventional techniques (CT, MRI, or caliper measurement) and as ≥20mm by chest X-ray (if clearly defined and surrounded by aerated lung.) Lymph nodes greater than 10mm on short axis are considered measureable as well. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).
- Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <20 mm by chest X-ray or <10 mm using CT, MRI or caliper measurement), are considered non-measurable disease. Organomegaly, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI are all non-measurable.
- 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. Lymph nodes less than 15mm in the short axis cannot be used as target

lesions. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

- Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 10 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 or absence of each should be noted throughout follow-up.

11.1.3 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 30 days 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 when both methods have been used to assess the antitumor effect of a treatment.

- Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). 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: These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.
- Ultrasound (US): When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.
- Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.
- 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.
- 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.

Response Criteria

11.1.4 Evaluation of Target Lesions

Complete Response (CR):	Disappearance of all target lesions. Lymph node CR is when the lymph node has decreased to less than 10mm in the short axis.
Partial Response (PR):	At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started (including the baseline scan if that is the smallest), and at least a 5mm increase or the appearance of new lesions.
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

11.1.5 Evaluation of Non-Target Lesions

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level.
Incomplete Response/Stable Disease (SD):	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. However, unequivocal progression should not normally trump target disease status. It must be representative of overall disease status change, not a single lesion increase.

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

Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances.

11.1.6 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 measurement criteria, but confirmation is not necessary.

Table 10: Assessment of Best Overall Response Using Target and Non-Target Lesions

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD

* 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.

11.1.7 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 recurrent 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.

11.1.8 Disease Control Rate (DCR)

DCR is defined as the proportion of patients with the best overall response of CR, PR, or SD according to RECIST 1.1 criteria.

11.1.9 Progression-Free Survival

Progression-free survival is defined as the length of time between study enrollment and when objective evidence of disease progression is documented.

11.1.10 Overall Survival

Overall survival is defined as the length of time between study enrollment and death.

11.1.11 Tumor Imaging on Study per Immune-related RECIST (irRECIST)

RECIST 1.1 may not provide an accurate assessment of immunotherapeutic agents such as pembrolizumab. Immune-related RECIST (irRECIST) is RECIST 1.1 adapted as described below to account for the unique tumor response seen with immunotherapeutics.

Patients with 1st radiologic assessment of PD by RECIST 1.1 may continue treatment if clinically stable as per Table 11. If the repeat scan confirms PD, patients will be discontinued from treatment. Exception can be made if the Investigator feels the patient is deriving benefit from treatment and after discussion with the study PI and Sponsor. Subsequent imaging should be pursued every 6 weeks until demonstration of confirmed PR or CR in which event imaging will then be conducted every 9 weeks if a patients has completed initial 18 weeks of protocol therapy. Imaging may be more frequent if clinically indicated.

Clinically stable is defined by the following criteria:

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

Table 11. Imaging and Treatment after 1st radiologic evidence of progressive disease (PD)

	Clinically Stable		Clinically Unstable	
	Imaging	Treatment	Imaging	Treatment
1st radiologic evidence of PD per RECIST 1.1	Repeat imaging at 6 weeks to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan	Repeat imaging at ≥ 4 weeks to confirm PD if possible	Discontinue treatment

Repeat scan confirms PD	No additional imaging required	Discontinue treatment (exception is possible after discussion with the PI and Sponsor)	No additional imaging required	N/A
Repeat scan shows SD, PR, or CR	Continue regularly scheduled imaging assessments every 6-9 weeks dependent on cycle of therapy	Continue study treatment at the Investigator's discretion	Continue regularly scheduled imaging assessments every 6-9 weeks dependent on cycle of therapy	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion

12.0 Data Reporting/Protocol Deviations

Data Reporting

12.1.1 Confidentiality and Storage of Records

Electronic Data Collection will be used for this protocol. The data will be stored in encrypted, password protected, secure computers that meet all HIPPA requirements. When the results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest of confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy ACT and rules for the protection of human subjects.

12.1.2 Subject Consent Form

At the time of registration, the original signed and dated Informed Consent for, HIPPA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

12.1.3 Data Collection Forms and Submission Schedule

All data will be collected using electronic data collection, stored as indicated above, and will be submitted according to the timelines presented in the table below.

Table 12-1: 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 10 calendar days of treatment administration
Adverse Event Reporting Forms	For cycle 1: with 7 calendar days of AE assessment/notification All other cycles: within 10 calendar days of AE assessment/notification
Response Assessment Forms	With 10 calendar days of response assessment
Other Assessment Forms (Concomitant Medications)	Within 10 calendar days of the assessment
Off Treatment/Off Study Forms	Within 10 calendar days of end of treatment/study
Follow-up/Survival Forms	Within 14 calendar days of the protocol defined follow-up visit or call

Eligibility checklist

The eligibility checklist must be completed by a protocol nurse or clinical research coordinator and signed by an authorized investigator prior to registering the subject. See Section 4.3 for the registration procedure.

Protocol Deviations

12.1.4 Deviation Policy

This protocol will be conducted in accordance with COH's "Clinical Research Protocol Deviation Policy"

Deviations from the written protocol that could increase patient risk or alter the protocol integrity require prior IRB approval of a single subject exception (SSE) request. IRB pre-approved SSE protocol modifications are considered an amendment to the protocol and not a deviation. The submission of a deviation report is not required.

Brief interruptions and delays may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, government holidays, etc. This can also extend to complications of disease or unrelated medical illnesses not related to disease progression. The PI has the discretion to deviate from the protocol when necessary so long as such deviation does not threaten patient safety or protocol scientific integrity. Example include, but are not limited to: dose adjustments based on excessive patient weight, alteration in treatment schedule due to non-availability of the research participant for treatment, laboratory test results which are slightly outside the protocol requirements but at levels that do not affect participant safety. These instances are considered deviations from the protocol. A deviation report will be submitted to the DSMC/IRM within five days.

12.1.5 Reporting of Deviations

All deviations will be reported to the COH DSMC within five days. The DSMC will forward the report to the IRM following review.

12.1.6 Resolving Disputes

The COH Investigational Drug Service (IDS) cannot release a research agent that would cause a protocol deviation without approval by the PI. Whenever the protocol is ambiguous on a key point, the IDS should rely on the PI to clarify the issue.

In situations where there is misperception or dispute regarding a protocol deviation among the persons involved in implementing the protocol it is the responsibility of the PI to resolve the dispute and the PI may consult with the DSMC chair or designee to arrive at resolution.

13.0 Statistical Considerations

Evaluable Participants and Participant Replacement

All participants will be evaluable for toxicity from the time of their first treatment with IRX-2 regimen and pembrolizumab.

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13.1.1 Design

Phase I of the investigation will comprise a safety lead-in phase: A standard 3+3 dose finding rule will be employed with two dose levels. Patients will start at dose level 1 [IRX-2 regimen at dose of 2 mL (230 units/day)]. Patients will be enrolled in cohorts of 3, separated by at least 1 week. Initially, 3 patients will be treated with expansion to 3 more patients in the event of 1/3 dose limiting toxicities (DLTs) as observed during the first 3 weeks of treatment, escalation if 0/3 or 1/6 DLTs, and termination of escalation if ≥ 2 DLTs. Once ≥ 2 patients experience a DLT at a dose level, the next lower dose level will be expanded to 6 evaluable patients, if fewer than 6 evaluable patients have been treated at the dose level. The maximum tolerated dose (MTD) is defined as the highest dose tested in which only 0 or 1 out of 6 evaluable patients experience a DLT. The Recommended Phase 2 Dosing (RP2D) will not exceed the MTD but may be lower based on cumulative toxicities and dose modification patterns. If the rules suggest escalation at the highest proposed dose, that dose will accrue to 6 patients where <2 DLTs will determine that as the MTD, and 2 or more DLTs will require de-escalation. These rules will be used to determine the IRX-2 regimen dosing for the expansion phase. Occurrence and severity of adverse events including dose limiting toxicities, with severity determined according to NCI Common Terminology Criteria for Adverse Events version 5. Phase II is a single arm, non-randomized study to investigate secondary endpoints that include median progression free survival (PFS) and overall response rate (ORR) by RECIST version 1.1 criteria. Exploratory endpoints include descriptive statistics on circulating T cell profiles in patients pre and post therapeutic intervention with the combination IRX-2 regimen and pembrolizumab.

Sample Size and Accrual Rate

The safety phase of the study will enroll 3 to 6 patients per dose level. The dose expansion phase will enroll 8 to 14 patients at the recommended dose level from the safety phase for a total enrollment of 20

patients. The 6 to 12 patients treated at all dose levels in the safety phase of the study will be included as a part of the phase II dose expansion patient population.

Historical data with single agent PD-1 inhibitors in gastroesophageal cancer indicate median PFS at 1.5 months. With an alpha of .05, hypothesized median PFS improved to 3.0 months with the combination IRX-2 regimen and pembrolizumab, study accrual of 30 months, and 12-month follow-up evaluation timeframe, a sample size of 20 patients will have 84% power to detect median differences in PFS.

Statistical Analysis Plan

13.1.2 Primary Objective

A standard 3+3 dose finding rule will be employed with two dose levels. Patients will start at dose level 1 [IRX-2 regimen at dose of 2 mL (230 units/day)]. Patients will be enrolled in cohorts of 3, separated by at least 1 week. Initially, 3 patients will be treated with expansion to 3 more patients in the event of 1/3 dose limiting toxicities (DLTs) as observed during the first 3 weeks of treatment, escalation if 0/3 or 1/6 DLTs, and termination of escalation if ≥ 2 DLTs. Once ≥ 2 patients experience a DLT at a dose level, the next lower dose level will be expanded to 6 evaluable patients, if fewer than 6 evaluable patients have been treated at the dose level. The maximum tolerated dose (MTD) is defined as the highest dose tested in which only 0 or 1 out of 6 evaluable patients experience a DLT. The Recommended Phase 2 Dosing (RP2D) will not exceed the MTD but may be lower based on cumulative toxicities and dose modification patterns. If the rules suggest escalation at the highest proposed dose, that dose will accrue to 6 patients where <2 DLTs will determine that as the MTD, and 2 or more DLTs will require de-escalation. These rules will be used to determine the IRX-2 regimen dosing for the expansion phase.

13.1.3 Secondary Objectives

Progression free survival is defined as the time from the first day of study drug administration to disease progression or death due to any cause. For PFS, the Kaplan-Meier estimates and corresponding 95% confidence intervals for the median and quartiles will be provided.

13.1.4 Correlative Exploratory Objectives

Descriptive statistics will be compiled on circulating T cell profiles for each patient at pre- and post-therapeutic intervention with the combination IRX-2 Regimen and Pembrolizumab. Repeated measures t-tests will be conducted to examine pre and post-intervention differences between baseline and post-treatment tumor tissue Nanostring expression profiling. Data will be screened for parametric statistical assumption and the alpha level for all statistical tests will be set at .05. The Bonferroni correction will be applied to adjust for potentially inflated family wise error rates for multiple comparisons with the same sample.

14.0 Human Subject Issues

Institutional Review Board

In accordance with City of Hope policies, as Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR50, 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.

Recruitment of Subjects

The HCC subjects will be recruited from patients undergoing treatment for HCC at the City of Hope Cancer Center and Honor Health Research Center. Any recruitment materials will be reviewed and approved by the IRB prior to their use to recruit potential study subjects.

Advertisements

Advertisements to include print, media (radio, television, and billboards), telephone scripts, etc., will be reviewed and approved by the IRB prior to their use to recruit potential study subjects.

Study Location and Performance Sites

This study will be performed at City of Hope National Medical Center in Duarte, CA and Honor Health Research Institute (Scottsdale, AZ).

Confidentiality

This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI). The study will record individual response to the study drugs and any side effects, and this will be linked to the subject's identity using a coded study number. The principal investigator, co-investigators, and laboratory technicians will have access to this information, but all information will be treated confidentially. No identifiers will be used in any subsequent publication of these results.

Financial Obligations and Compensation

The investigational drug, IRX-2 regimen will be provided free of charge by City of Hope for the duration of the trial.

The standard of care drug(s), pembrolizumab and standard of care procedures provided will be the responsibility of the research participant and/or insurance carrier. 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. However, neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at each participating institution in the injured research participant; however, financial compensation will not be available.

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

Informed Consent Processes

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. Research subjects 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 their hospital or any relationship they have with their hospital.

Should sufficient doubt be raised regarding the adequacy of comprehension, further clarifications will be made and the questionnaire repeated until a satisfactory result is obtained. Prospective research subjects who cannot adequately comprehend the fundamental aspects of the research study with a reasonable amount of discussion, education and proctoring will be ineligible for enrollment. For those subjects who do comprehend the fundamental aspects of the study, the research team will review the results of eligibility testing and determine if the subject is a candidate for study enrollment.

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16.0 Appendix I

Treatment modifications (e.g. dose delay or discontinuation) will be based on the specific laboratory and immune-related adverse event criteria per the package insert as shown in the table below:

Adverse Reaction	**Severity***	**Dose Modification**
Diarrhea/Colitis	Grade 2	Withhold dose[a]
	Grade 3	Withhold dose[a]
	Grade 4	Permanently discontinue
Pneumonitis	Grade 2	Withhold dose[a]
	Grade 3 or 4 or recurrent Grade 2 pneumonitis	Permanently discontinue
Hepatitis (non-HCC)	Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) more than 3 and up to 5 times the upper limit of normal (ULN) or total bilirubin more than 1.5 and up to 3 times ULN	Withhold dose[a]
	AST or ALT >5x ULN or total bilirubin >3x ULN	Permanently discontinue
Hepatitis (HCC)	• If AST/ALT is within normal limits at baseline and increases to more than 3 and up to 5 times the ULN • If AST/ALT is more than 1 and up to 3 times ULN at baseline and increases to more than 5 and up to 10 times the ULN • If AST/ALT is more than 3 and up to 5 times ULN at baseline and increases to more than 8 and up to 10 times the ULN	Withhold dose[b]
	If AST or ALT increases to more than 10 times the ULN or total bilirubin increases to more than 3 times the ULN	Permanently discontinue
Hypophysitis	Grade 2 or 3	Withhold dose[a]
	Grade 4	Permanently discontinue
Adrenal insufficiency	Grade 2	Withhold dose[a]
	Grade 3 or 4	Permanently discontinue
Type 1 Diabetes Mellitus	Grade 3 hyperglycemia	Withhold dose[a]
	Grade 4 hyperglycemia	Permanently discontinue
Nephritis and Renal Dysfunction	Serum creatinine more than 1.5 and up to 6 times ULN	Withhold dose[a]
	Serum creatinine more than 6 times ULN	Permanently discontinue
Skin	Grade 3 rash or suspected Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)	Withhold dose[a]
	Grade 4	Permanently discontinue
Encephalitis	New-onset moderate or severe neurologic signs or symptoms	Withhold dose[a]
	Immune-medicated encephalitis	Permanently discontinue
Other	Other Grade 3 immune-related adverse reaction:	

	First occurrence Recurrence of same grade 3 adverse reactions	Withhold dose[a] Permanently discontinue
	Life-threatening or Grade 4 immune-related adverse reaction	Permanently discontinue
	Requirement for 10mg per day or greater prednisone or equivalent for more than 12 weeks	Permanently discontinue
	Persistent Grade 2 or 3 immune-related adverse reactions lasting 12 weeks or longer after last dose of pembrolizumab (excluding endocrinopathies controlled with hormone replacement therapy)	Permanently discontinue

*Grading according to CTCAE Version 5.0.

CTCAE=Common Terminology Criteria for Adverse Events.

[a]Treatment can be resumed once adverse reaction returns to Grade 0 or 1.

[b]Treatment can be resumed once AST/ALT returns to baseline

In all cases where the subject is withdrawn due to unusual or unusually severe adverse event considered related to pembrolizumab, the investigator must report the withdrawal as a Serious Adverse Event.

APPENDIX II: REGISTRATION COVERSHEET

COH IRB# 18069: A Phase 1b/2 Trial of the IRX-2 Regimen and Pembrolizumab in Patients with Advanced Gastric and Gastroesophageal Junction (GEJ) Adenocarcinoma

Data Coordinating Center:
City of Hope
1500 Duarte Road
Duarte, CA 91010
Tel: (626)-218-7904
Email: DCC@coh.org (use #secure# in subject line)

Site Principal Investigator
Name:

CRA/Study Coordinator:	Contact Number:
Patient's Initials: (F M L):	Institution:
Medical Record No:	PI/ Sub-Investigator:
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:_____	
☐	Black	☐	Hispanic	Codes:	
☐	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 III: 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

COH IRB #18069_- 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 **IRX-2:**	☐ Not Applicable* ☐ Unrelated ☐ Unlikely ☐ Possible ☐ Probable ☐ Definite
Attribution to **pembrolizumab**:	☐ Not Applicable* ☐ Unrelated ☐ Unlikely ☐ Possible ☐ Probable ☐ Definite
Historical/Known Correlation to **IRX-2**:	☐ Expected ☐ Unexpected
Historical/Known Correlation to **pembrolizumab**:	☐ 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:_____/____/_____

*** Not Applicable should only be used if subject has not received this agent.**

Authorized Investigator Signature:	Date:____/____/____

APPENDIX IV: CORRELATIVE TISSUE FORM (FOR ALL SITES)

A copy of this form should <u>accompany the sample shipments</u> to COH Pathology Core.

Non-COH sites: refer to Appendix V for shipping instructions to COH Pathology Core.

COH IRB number: 18069	Shipping date (MM-DD-YYYY): _____/_____/_____
Subject ID (issued by DCC):	Participant Initials (F, M, L) (if applicable):
Institution:	
Date of collection/ biopsy (MM-DD-YYYY): _____/_____/_____	
Time point: ☐ Screening ☐ Week 7	
Diagnosis:	
Tissue type (FFPE scrolls):	
Number of scrolls:	Number of slides:

CRA/Study Coordinator/Nurse Printed Name:
CRA/Study Coordinator/Nurse Signature:
Contact Number:

APPENDIX V: TISSUE SHIPPING GUIDELINES TO CITY OF HOPE PATHOLOGY CORE

*These guidelines apply to **non-COH sites** only.*

All biological material must be shipped according to applicable government and International Air Transport Association (IATA) regulations.

Shipping guidelines can also be found on the FedEx website.

1. Aim to ship samples on a **Monday through Wednesday**. If this is not feasible, advance arrangements should be made with City of Hope Pathology Core (DL-PATHCORE-BiospecimenSupport@COH.org).

2. Notify City of Hope Pathology Core (DL-PATHCORE-BiospecimenSupport@COH.org) of impending shipment.

3. **Slides/ Blocks:** Batch ship at room temperature. During extreme heat, include refrigerated (not frozen) gel packs or gel insulators.

   It is recommended to ship samples via overnight (for a delivery by 3 pm or earlier the next day) or 2-day (with a morning delivery). During extreme heat, ship via overnight (for a delivery ideally by 10.30 am, or 3 pm the next day).

4. **Frozen samples** should be batch shipped on dry ice via overnight (for a delivery by 10.30 am the next day). The shipment should contain enough dry ice to last at least 72 hours.

5. On the day of shipment, email the sample shipment information to City of Hope Pathology Core (DL-PATHCORE-BiospecimenSupport@COH.org).

6. Ship samples with a **copy of the correlative tissue form (Appendix IV)** and a **copy of the pathology report** to:

Karen Miller
COH Pathology Core
City of Hope National Medical Center
1500 E. Duarte Road
Familian Science (Building 084), Room 1207
Duarte, CA 91010
Telephone: 626-218-8408
Email: DL-PATHCORE-BiospecimenSupport@COH.org

APPENDIX VI: CORRELATIVE BLOOD COLLECTION FORM FOR NON-COH SITES ONLY

Subject ID (issued by DCC):	Participant Initials (F, M, L) (if applicable):
Institution:	

To be used by **non-COH sites** for the following blood samples being sent to **COH APCF**:

Sample #	Time point of Collection *	Tube Type Used	# of aliquots	Time of Collection	Date of Collection	Indicate which sample was collected
1.	Week 1	Green-top - Plasma		____:____ AM/ PM	____/____/____	☐
		Green-top - PBMC		____:____ AM/ PM	____/____/____	☐
		Red-top - Serum		____:____ AM/ PM	____/____/____	☐
2.	Week 4	Green-top - Plasma		____:____ AM/ PM	____/____/____	☐
		Green-top - PBMC		____:____ AM/ PM	____/____/____	☐
		Red-top - Serum		____:____ AM/ PM	____/____/____	☐
3.	Week 7	Green-top - Plasma		____:____ AM/ PM	____/____/____	☐
		Green-top - PBMC		____:____ AM/ PM	____/____/____	☐
		Red-top - Serum		____:____ AM/ PM	____/____/____	☐
4.	Week 10	Green-top - Plasma		____:____ AM/ PM	____/____/____	☐
		Green-top - PBMC		____:____ AM/ PM	____/____/____	☐
		Red-top - Serum		____:____ AM/ PM	____/____/____	☐
5.	EOT	Green-top - Plasma		____:____ AM/ PM	____/____/____	☐
		Green-top - PBMC		____:____ AM/ PM	____/____/____	☐
		Red-top - Serum		____:____ AM/ PM	____/____/____	☐

A copy of this form should accompany the sample shipments to COH APCF. **Refer to the blood shipping guidelines for shipping instructions to COH APCF (Appendix VII).**

CRA/Study Coordinator/ Nurse:	Contact Number:
CRA/Study Coordinator/ Nurse Signature:	Date:

APPENDIX VII: BLOOD SHIPPING GUIDELINES TO CITY OF HOPE APCF

*These guidelines apply to **non-COH sites** only.*

All biological material must be shipped according to applicable government and International Air Transport Association (IATA) regulations.

Shipping guidelines can also be found on the FedEx website.

1. Aim to ship samples on a **Monday through Wednesday**. If this is not feasible, advance arrangements should be made with APCF (DL-APCF@coh.org).

1. **On the day of shipment**, email APCF (DL-APCF@coh.org) the Airwaybill shipment #.

2. Ship samples with a **copy of the correlative blood collection form** (Appendix VI) to:

Dr. Tim Synold
Analytical Pharmacology Core Facility
Shapiro 1042
City of Hope National Medical Center
1500 E. Duarte Road
Duarte, CA 91010

APPENDIX VIII: CORRELATIVE BLOOD COLLECTION FORM FOR NON-COH SITES ONLY

Subject ID (issued by DCC):	Participant Initials (F, M, L) (if applicable):
Institution:	

To be used by **non-COH sites** for the batched shipment of the following blood samples to **TGen**:

Sample #	Time point of Collection *	Tube Type Used	# of aliquots	Time of Collection	Date of Collection	Indicate which sample was collected
1.	Week 1	Purple-top - Plasma		____:____ AM/ PM	____/____/____	☐
2.	Week 4	Purple-top - Plasma		____:____ AM/ PM	____/____/____	☐
3.	Week 7	Purple-top - Plasma		____:____ AM/ PM	____/____/____	☐
4.	Week 10	Purple-top - Plasma		____:____ AM/ PM	____/____/____	☐
5.	EOT	Purple-top - Plasma		____:____ AM/ PM	____/____/____	☐

A copy of this form should accompany the sample shipments to TGEN.

CRA/Study Coordinator/ Nurse:	Contact Number:
CRA/Study Coordinator/ Nurse Signature:	Date: