SUMMARY OF PROTOCOL CHANGES

For Protocol Amendment # 5 to: **# 6**
UCCC Protocol #: UCCI-HN-19-01
Protocol Date: 04 Mar 2024

#	Section	Change
1	4 Eligibility	Updated Inclusion and exclusion criteria to reflect current NCI eligibility requirements with respect to brain metastases. Specifically, we have added inclusion criteria "Patients with new or progressive brain metastases (active brain metastases) are eligible if the treating physician determines that immediate CNS specific treatment is not required and is unlikely to be required during the first cycle of therapy." And we have removed exclusion criteria "Patients with known active brain metastases, given that there is a chance for increasing edema with immunotherapy and active brain metastases. See inclusion #9 for requirements for participation for patients with treated brain metastases."
2	10.4 Randomization	Added clarification that if screening labs are to be obtained on same day as treatment randomization may be performed at the time all other eligibility criteria are informally confirmed to allow adequate time for treatment planning
3	SOE	Removed text "Baseline evaluations are to be conducted within 10 days prior to start of protocol therapy. Scans and x-rays must be done <4 weeks prior to the start of therapy. Following registration, patients should begin protocol treatment within 28 days." From before SOE as it is duplicative with other areas of the protocol.
4	Various	Other grammatical and formatting changes throughout
5	10.2 Sample Size/Accrual Rate	Updated the overall accrual from 20 subjects to 25 subjects due to the higher than expected withdraw rate before starting or reaching steady state of metformin on arm 2. The stats and enrollment report have been updated as well to reflect this increase in accrual.
6	Various	Although Protocol Amendment v5 dated 05Feb2024 has been IRB approved, these changes to the protocol were based upon updates made to Protocol v1 dated 22Jun2020 rather than the most recently approved prior version Protocol Amendment v4 dated 16 Sept 2022. Protocol v6 is being submitted to incorporate the previously IRB approved update to increase the overall accrual from 20 subjects to 25 subjects into the protocol v4's content.

Local Protocol #: UCCI-HN-19-01
Local IRB #: 2020-0365

ClinicalTrials.gov Identifier: NCT04414540

TITLE: A Phase 2 Feasibility Study Combining Pembrolizumab and Metformin to Harness the Natural Killer Cytotoxic Response in Metastatic Head and Neck Cancer Patients

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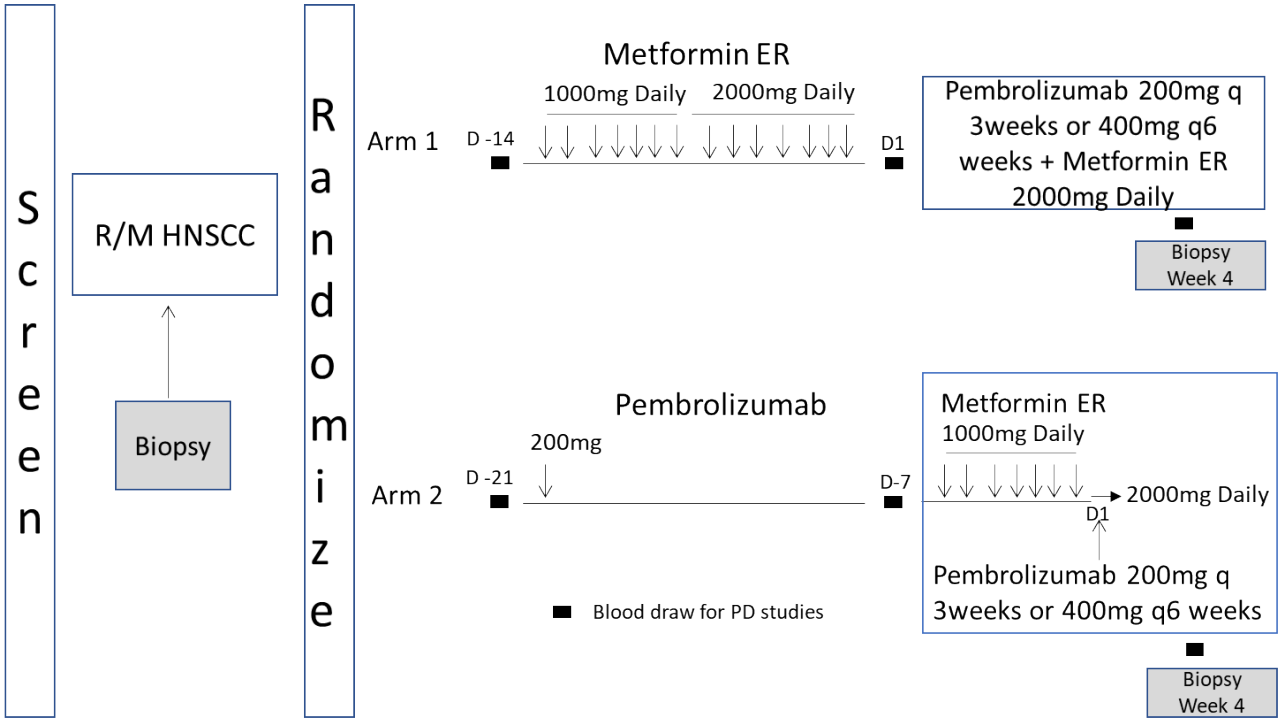
Study Exempt from IND Requirements per 21 CFR 312.2(b)

Protocol Type / Version # / Version Date: Original / Version #6 / 04 MAR 2024

SCHEMA



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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE: Adverse event

CTCAE: Common Terminology Criteria for Adverse Events

HNC: Head and Neck Cancer

HNSCC: Head and neck squamous cell carcinoma

iRECIST: immune Response Evaluation Criteria In Solid Tumors

LAHNSCC: Locally advanced head and neck squamous cell carcinoma

NK: Natural Killer

OS: Overall survival

PD-1: Programmed death –1

PD-L1: Programmed death ligand-1

PFS: Progression free survival

RECIST: Response Evaluation Criteria In Solid Tumors

SAE: Serious adverse event

STAT-3: Signal transducer and activator of transcription number 3

OBJECTIVES

2.1 Primary Objective

(1) To determine anti-tumor activity by measuring overall response rate by RECIST 1.1 and iRECIST in recurrent and/or metastatic HNSCC patients receiving the combination of metformin and pembrolizumab.

2.2 Secondary Objective

(1) To observe and record safety and toxicity of combination, progression free survival and overall survival in recurrent and/or metastatic HNSCC patients receiving the combination of metformin and pembrolizumab.

2.3 Exploratory Objectives

(1) Characterize and compare peripheral blood immune cell phenotypes before and after metformin, pembrolizumab and combination treatment with a particular focus on Natural Killer (NK) cells and innate immunity.
(2) Characterize and compare tumor infiltrating NK cells before and after metformin, pembrolizumab and combination treatment.
(3) Determine cytokine levels before and after metformin, pembrolizumab and combination in the plasma.
(4) Determine NK effector functions before and after metformin, pembrolizumab and combination.
(5) Determine Stat-3 RNA levels and phosphorylation status in NK cells and tumor tissue before and after metformin, pembrolizumab and combination treatment.
(6) Evaluate NKG2D soluble ligands before and after metformin, pembrolizumab and combination in the plasma.

BACKGROUND

3.1 Recurrent and Metastatic Head and Neck Squamous Cell Carcinoma (HNSCC)

Approximately 64,000 new cases of head and neck cancer (HNC) were estimated to be diagnosed in 2018 and approximately 13,000 patients were estimated to die from this cancer type last year in the United States indicating a significant disease burden[1]. Approximately 90% of all HNC is squamous cell carcinoma in origin (HNSCC). Early stage tumors are often cured with single modality treatment, however approximately 60% of newly diagnosed patients present as locally advanced (stage III/IV) squamous cell head and neck cancer (LAHNSCC)[2]. Progression free survival (PFS) after definitive treatment for LAHNSCC remains around 60-70%, and upon recurrence and/or metastasis, survival is dismal at around 15% necessitating the development of new treatments. The standard of care for first line treatment of incurable recurrent and/or metastatic HNSCC (R/M HNSCC) prior to 2019 was the extreme regimen (see NCCN guidelines) including

a combination of a platinum (cisplatin or carboplatin), fluorouracil and Cetuximab (an epidermal growth factor receptor inhibitor). Although adding Cetuximab to platinum-based chemotherapy significantly extended median overall survival (OS) to 10.1 months from 7.4 months with chemotherapy alone, the rate of grade 3 or 4 adverse events was high at 82% for the combination[3]. Therefore, other platinum-based regimens such as carboplatin and paclitaxel are often used to reduce toxicity. Success in the second line setting after failure of platinum therapy is even more dismal with median OS rates around 5-6 months. More recently, immunotherapy has been shown to significantly increase survival rates in a handful of R/M HNSCC patients. In fact, the PD-1 checkpoint inhibitors, Pembrolizumab and Nivolumab, are both now FDA approved after failure of platinum therapy in the recurrent/metastatic setting. Keynote 40, comparing Pembrolizumab to standard of care chemotherapy (methotrexate, docetaxel or Cetuximab) after failure to platinum therapy, demonstrated that although the primary endpoint was not reached, there was a clinically meaningful increase in overall survival for Pembrolizumab at 8.4 months (95% CI 6.4–9.4) compared to 6.9 months (5.9–8.0) with standard of care (hazard ratio 0.80, 0.65–0.98; nominal p=0.0161)[4]. Nivolumab was also found to be superior in overall survival compared to standard of care chemotherapy in Checkmate 141 (hazard ratio for death, 0.70; 97.73% CI, 0.51 to 0.96; P=0.01) establishing immunotherapy as the new standard of care after platinum failure in HNSCC[5]. Recently, Pembrolizumab was found to be superior to the extreme regimen in the first line setting of metastatic/recurrent HNSCC as well[6]. Pembrolizumab alone is now FDA approved for first line R/M HNSCC patients with a CPS score of ≥ 1 and combination of pembrolizumab, platinum and 5-FU for those with CPS <1. Importantly, combination of chemotherapy and immunotherapy had similar rates of toxicity to the Extreme regimen limiting its use. Despite the progress and impressive durable responses seen with immunotherapy in recurrent and metastatic HNSCC, the response rates remain low at 18-22%. Several subsequent studies have been performed to show that patients with high tumor mutational burden (TMB), increased PD-L1/L2 expression and Interferon gamma (IFN-γ) signatures are more likely to respond to immunotherapy[7,8]. However, mechanisms of resistance and more importantly, therapies likely to overcome immunotherapy resistance with low toxicity are lacking.

3.2 Experimental Agents

3.2.1 Pembrolizumab

Immunotherapeutic approaches for cancer treatment have existed for several decades. Recent advances in immunotherapeutics have recognized key regulatory pathways that are involved in the anti-tumor response. Multiple immune checkpoint molecules are up regulated during the immune response in an attempt to prevent autoimmune damage to normal tissue by maintenance of tolerance to self-antigens. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) as well as programmed death-1 (PD-1) and its ligands are key inhibitors of the anti-tumor response. CTLA-4 acts a dampener in lymph nodes preventing early activation of T cells whereas PD-1 is induced on T cells after activation by immune stimulation either by infection or tumor progression. Interestingly,

the negative immune cell regulator, PD1 ligand-1 (PD-L1), has been found to be up regulated on many tumors including HNSCC.

PD-1 (or CD279), a 55-kilodalton Type 1 transmembrane protein is a member of the CD28 family of T-cell co-stimulatory receptors that include immunoglobulin super family members CD28, CTLA-4, ICOS, and BTLA. PD-1 is highly expressed on activated T cells, B cells and NK cells. Two ligands specific for PD-1 have been identified: PD-L1 (also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273). PD-L1 and PD-L2 have been shown to down-regulate T-cell activation upon binding to PD-1 in both murine and human systems. The interaction of PD-1 with its ligands, PD-L1 and PD-L2, which are expressed on antigen-presenting cells (APCs) and DCs, transmits negative regulatory stimuli to down-modulate the activated T-cell immune response. The absence or inhibition of PD-1 in murine models has resulted in the development of various autoimmune phenotypes and autoimmune diseases. Taken together, these results suggest that inhibition of PD-1 binding to its ligands has the potential to activate at least T-cell responses. Since these responses are variable and dependent upon various host genetic factors, PD-1 deficiency or inhibition is not accompanied by a universal loss of tolerance to self-antigens.

Tumors can express tumor-specific antigens as a result of mutational burden, and ongoing immune surveillance is believed to control the development of many tumors. Tumor progression may depend on the acquisition of mechanisms that permit them to evade an effective immune response. One such mechanism of evasion may be the expression of ligands, which engage inhibitory receptor(s) on anti-tumor T-cells of many tumors. PD-L1 expression has been found on a number of tumors and may be a mechanism by which tumors can directly engage PD-1 to evade an effective anti-tumor immune response. Expression of IFN-γ by activated T cells is known to induce PD-L1 expression in tumors. PD-1 engagement on T-cells by PD-L1-positive APC or PD-L1-positive tumor cells in the tumor microenvironment may limit effective immune responses. Conversely, PD-L1 expression may be a positive prognostic factor as it may indicate infiltration of tumor-specific T cells that secrete IFN-γ, which up regulates PD-L1 expression. Consistent with this hypothesis is the co-localization of lymphoid cell infiltrates and PD-L1 staining observed in human melanoma lesions.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. The PD-1 cell surface membrane receptor is a negative regulatory molecule expressed by activated T and B-lymphocytes as well as NK cells. Pembrolizumab has been approved in the metastatic setting for lung cancer, renal cell cancer, melanoma, and metastatic HNSCC among others as mentioned above. Several studies are now ongoing to study PD-1 inhibitor combinations in the upfront and adjuvant setting. The safety profile of pembrolizumab is well-established. Immunotherapy is unique in that auto-immune type of adverse events (AEs) can occur and must be managed appropriately most often with steroids. In keynote 48, the most common AEs in the pembrolizumab group were fatigue, rash, diarrhea and nausea with 6.3%

Grade 3 or higher immune related AEs and 54.7% Grade 3 or higher AEs overall, which was significantly lower than the extreme regimen (83.3%). Here, we propose to use the FDA approved every 3 week schedule of 200mg flat dose or the most recently FDA approved dose of 400mg every 6 weeks (https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-new-dosing-regimen-pembrolizumab).

3.2.2 Metformin

Activation of the PI3K, Akt and the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway was identified as being a prevalent molecular signature in many HNSCCs[9,10], resulting in potential strategies to target this pathway. In fact, activating mutations of the mTOR pathway are now recognized as drivers of some HNSCCs. However, targeting the mTOR pathway with rapamycin analogues, such as everolimus, results in undesirable side effects including mucositis, thrombocytopenia, and increased infection risk making combinatorial treatment with systemic agents such as chemotherapy and radiation difficult. Interestingly, metformin has been shown to inhibit the mTOR pathway. Metformin is a biguanide and an oral antihyperglycemic agent, which is currently FDA approved for the treatment of type II diabetes. Specifically, metformin inhibits mTORC1 activity, through activation of the AMPK pathway resulting in decreased proliferation, metabolic synthesis and metastases in breast and colon cancer[11,12]. Additionally, metformin inhibits HNSCC cell growth *in vitro* and prevents development and progression of oral cavity tumors in a HNSCC mouse tumor model supporting a role for metformin as a cancer preventative[13]. The latter study demonstrated that metformin may also inhibit mTORC1 activity independent of AMPK activation. In line with these animal studies, retrospective population case-control cohort studies have demonstrated a decreased HNSCC risk in diabetic patients treated with metformin[14]. In addition, metformin use resulted in a better overall survival in diabetic patients diagnosed with laryngeal squamous cell carcinoma[15]. The potential impact of metformin use in non-diabetic HNSCC patients is unknown.

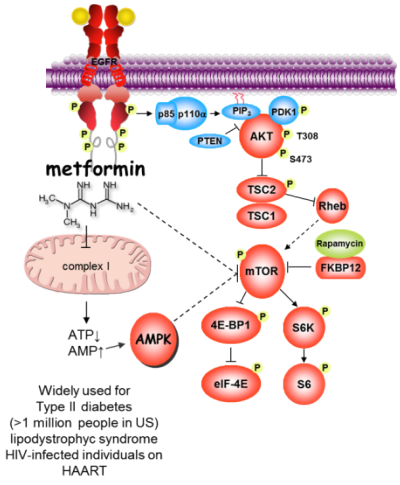


Figure 1. Effect of Metformin on mTOR pathway.

Metformin is widely used in many diabetic patients and overall well tolerated. Unlike other antihyperglycemic agents such as the sulfonylureas, metformin does not usually cause hypoglycemia. Adverse effects are relatively minimal with diarrhea being the most common cause of discontinuation. However, lactic acidosis, although rare (estimated incidence of 4.3 cases per 100,000 person-years in metformin users), is a serious adverse event resulting in up to 50% mortality when it occurs[16,17]. Lactic acidosis has been associated with high plasma concentrations of metformin (>10mg/ml) which can occur in the presence of renal dysfunction. Therefore, careful

monitoring of renal function of patients on metformin is imperative to reduce the incidence of this rare side effect. However, a recent retrospective study from the United Kingdom, demonstrated that there was no increase in lactic acidosis in patients taking metformin even in patients with severe kidney dysfunction (eGFR <30ml/hr)[3]. The latter study did not measure serum metformin levels. In our phase I study using escalating doses of metformin in combination with cisplatin and radiation in locally advanced HNSCC, no incidence of increased lactic acid was seen and peak plasma concentrations of metformin at highest dose of 3000mg daily was 2585 +/- 1007 ng/ml[18]. However, it should be noted that Metformin was held for Cr >1.5 in the latter study. No incidence of hypoglycemia was observed. Survival outcomes in this small phase I study were impressive with 90% 2 year overall survival. Importantly, many patients discontinued Metformin due to nausea and/or diarrhea most often at the highest dose of 3000mg daily but also at doses of 2550mg daily. Peak concentrations of patients from cohort one (2000mg daily) was 2045 +/- 1022 ng/ml with interpatient variability. However, given the range of peak concentrations between the three cohorts and the increase in toxicity of higher cohorts, in this current study, we have chosen to use the 2000mg daily dose. Metformin ER which is an extended release form of metformin will be used on this study as it has been shown to cause less side effects and will allow for better treatment compliance. Therefore, the standard dose will be 2000mg daily of metformin ER.

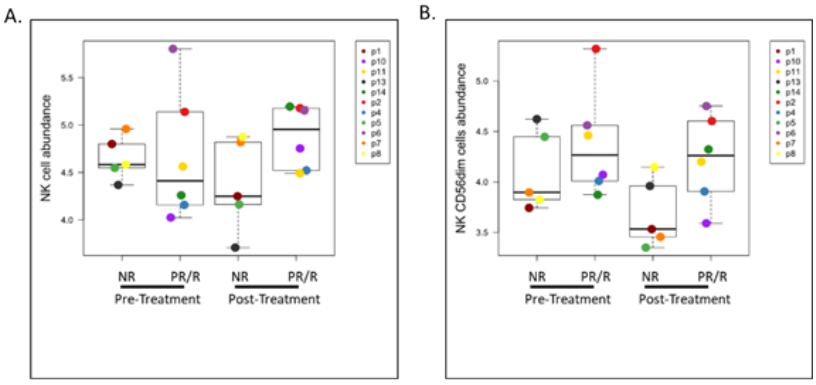
3.3 Rationale for Combination

In addition to prevention of HNSCC, several preclinical studies, in which metformin is used as an anticancer therapeutic, are also promising. Metformin has been shown to inhibit signal transducer and activator of transcription number 3 (STAT3), important for tumor growth in many tumor types including HNSCC, expression and phosphorylation which leads to inhibition of brain tumor initiating cells and has potential in enhancing cisplatin cytotoxicity in lung cancer in an AMP kinase independent pathway[19,20]. A “window of opportunity trial” in which metformin was administered to patients between biopsy and definitive surgical resection starting at a dose of 500 mg/day and escalated to 1,000 mg twice daily by day 6 after initial biopsy, showed metformin to impact HNSCC cells by promoting apoptosis as well as by affecting stromal markers of metabolism (e.g. CAV1 and GALBG)[21]. Follow-up analyses demonstrated tumor infiltration of CD8+ lymphocytes and Tregs suggesting that metformin also influences the adaptive anti-tumorigenic immune response[22]. A separate group also demonstrated that metformin results in increased CD8+ tumor infiltrating lymphocytes (TIL) in breast cancer patients and subsequently showed that metformin inhibits PDL1 localization to the membrane through activation of AMPK in a mouse model[23]. Both of these studies suggest that Metformin allows for recruitment of cytotoxic T cells into the microenvironment and that combination of immunotherapy and metformin could capitalize on this synergy.

Importantly, we have shown that treatment of HNSCC patients with metformin leads to increased

peripheral NK cells as well as increased activation and expression of the activating receptor, NKG2D[24]. In addition, NK cells express PD-1 and therefore, can potentially be inhibited by its ligands suggesting treatment with PD-1 inhibitors could lead to subsequent activation of not only cytotoxic T cells but also NK cells. Therefore, combining metformin and PD-1 inhibition may enhance NK cell function and tumor cytotoxic response leading to improved survival outcomes. In fact, Scharping et al. showed that combining PD-1 blockade and metformin did enhance tumor growth inhibition in a melanoma mouse model[25] which was thought to be due to a metformin related decrease in hypoxia allowing for immune cell infiltration. In this study, we will combine metformin and pembrolizumab in a phase II clinical trial in order to test clinical overall response in recurrent and/or metastatic HNSCC patients as well as determine the importance of NK cell infiltration, activation and possible mechanism in which the combination therapy exerts this effect.

Pembrolizumab and metformin have documented safety in several previous clinical trials and retrospective studies. A retrospective study comparing metastatic melanoma patients receiving PD-1 inhibitors versus those that were on the combination with metformin not only showed a trend towards improved clinical response, but also similar safety profiles with 60.6% vs. 59% immune related side effects respectively[26]. A phase IB study in NSCLC also demonstrated early efficacy signals for the combination and similar safety profiles between pembrolizumab alone and combination[27]. However, it is important to note that patients on these studies received metformin at doses of 500-1000mg daily. Given documented safety of the combination but not at the higher dose of metformin of 2000mg daily, we therefore feel it is reasonable to proceed with a phase II study with safety lead-in to determine dose limiting toxicity (DLTs) in this group of patients.



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Patient #	Clinical Stage	Tumor Site	% Treatment Response	% Remaining Viable Tumor	Path stage	Pathological Risk	Clinical Response
HN1501-01	T4aN0M0	Oral Cavity	< 10%	90%	T4aN1	Intermediate	Recurrence
HN1501-02	T3N2bM0	Oral Cavity	80-90%	10-20%	T3N2b	High	NED
HN1501-04	T3N2bM0	Oral Cavity	70-80%	20-30%	T2N2c	High	NED

Fig. 2. HNSCC patients enrolled on a window of opportunity study, UCCI-HN-15-01, were treated with 1 dose of pembrolizumab 1-3 weeks prior to surgery. Pre- and post- pembrolizumab tissue was subjected to nanostring immune panel. A. RNA expression for NK cell abundance (measured by NCR1 and XCL1/2 RNA levels) in responders (PR/R) and non-responders (NR) are compared in pre- and post-treatment with pembrolizumab. B. RNA expression for cytotoxic NK CD56^{dim} cells (measured by IL21R, KIR2DL3, KIR3DL1, and KIR3DL2 RNA levels). C. Patient characterization and pathological response which was characterized by >10% treatment effect (TE) or decreased viable tumor (HN1502-02). TE was defined as tumor necrosis and/or histiocytic inflammation and giant cell reaction to keratinaceous debris. TE percentage was determined by dividing estimates of area showing these features by the total area showing residual viable tumor (VT) and TE. Clinical response was assessed at three months post radiation or at time of relapse with imaging and clinical exam. NED= no evidence of disease. All pathological characterization was performed by a certified pathologist.

3.4 Correlative Studies Background

Characterization of peripheral blood immune cell phenotypes before and after metformin, pembrolizumab and combination treatment.

As discussed above, our previous work has demonstrated that metformin alone results in an increase of peripheral NK cells and expression of activating receptor, NKG2D. In addition,

NK cells are known to express PD-1 and can potentially be inhibited by its ligands. We have also shown that pembrolizumab, when given to previously untreated HNSCC patients prior to surgery in a window of opportunity study, that higher levels of cytotoxic NK cells ($CD56^{dim}$) correlate with increased pathological response (See Fig 2C). Therefore, we hypothesize that metformin will induce peripheral activation of NK cells and its activating receptors and that the combination of pembrolizumab will enhance activation of these NK cells which will correlate with clinical response.

Characterization of tumor infiltrating NK cells before and after metformin, pembrolizumab and combination treatment.

Although the focus has primarily been on cytotoxic T cell infiltration and correlation with improved prognosis as well as response to immunotherapy in cancer patients, accumulating evidence suggests that NK cell infiltration is also important. NK cells are a part of the innate immune response with intrinsic selectivity and capacity to kill cancer cells over healthy cells[28]. It has been shown that patients with dysfunctional or deficient NK cells are both more susceptible to the development of cancer and have a relatively poor prognosis[29]. Therefore, increasing NK cell infiltration and/or activation of NK cell effector function may result in enhanced immunotherapy responses. Given that metformin alone resulted in an increase in peripheral NK cells and activating receptors, and that metformin reduces hypoxia in the tumor microenvironment allowing for immune infiltration and survival, we expect to see an enrichment of NK cell infiltration after metformin exposure and increase in activation with the combination of pembrolizumab.

NK cell functional activity and cytokine levels before and after metformin, pembrolizumab and combination in the plasma.

NK cells can rapidly trigger effector functions resulting in cytolysis of cancer cells and secretion of cytokines (IFN-γ and TNF-α) without the requirement of priming and activation compared to the adaptive immune response (cytotoxic T cells). We have previously demonstrated that metformin induces an anti-tumorigenic cytokine response. In addition, PD-1 inhibition also results in enrichment of anti-tumorigenic cytokines. Here we hypothesize that the combination will result in an enhanced shift from pro- to anti-tumorigenic cytokines in the plasma, NK cell polyfunctional activity, and in addition result in enhanced NK cell directed cancer cell lysis ex vivo.

Stat-3 RNA levels and phosphorylation status in NK cells and tumor tissue before and after metformin, pembrolizumab and combination treatment.

Metformin has previously been postulated to inhibit tumor development by reducing insulin/IGF-1 signaling, in turn preventing the release of pro-inflammatory cytokines through NF-κB and enhancing the anti-cancer immune response mediated by NK cells and cytolytic T-

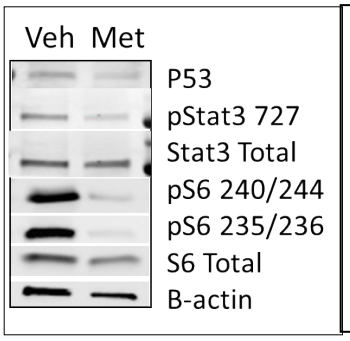

Fig. 3. HNC cell line SCC-47 was treated with either vehicle or 50mM metformin and protein was harvested at 24 hours, subjected to western blot analysis and probed with antibodies listed above.

cells[30]. In addition, STAT3 which can be inhibited by metformin as mentioned above, is important for NK cytolytic functions[31]. Our work has shown that metformin does indeed reduce STAT-3 expression and phosphorylation in a HNC cell line (SCC-47). In Fig. 3, we show that metformin reduces pS6 which is downstream from mTOR and known to be decreased by metformin but that metformin also results in pSTAT3 decrease. Our hypothesis is that metformin reduces Stat-3 expression in patient tumor cells as well as immune cells. It is unclear if pembrolizumab will result in any additive effect. Therefore, tissue will be examined for Stat-3 expression in both immune cell populations and tumor cells.

NKG2D soluble ligands before and after metformin, pembrolizumab and combination in the plasma.

NKG2D, an important activating receptor on NK cells, can be inhibited by NKG2D soluble ligands. In addition, HNSCC patients with high levels of such a ligand, sMICA, carry a poor prognosis[32]. NK cells express both inhibitory (killer cell Ig-like receptors or KIRs and CD94-NKG2A) and activation receptors (e.g., NKG2D). Inhibitory receptors bind MHC-1 which is often lost on cancer cells rendering cancer cells more susceptible to NK dependent killing. Cytokines (interleukin-2 and -15) can lower the threshold of NK activation and may be activated by singular receptors (NKG2D) in order to trigger effector functions. Understanding the interplay between activation and inhibition of NK cells is critical and may give the ability to exploit NK cells to better harness the benefits of immunotherapy. Here we hypothesize that patients able to express higher levels of NKG2D will also have lower levels of sMICA in the peripheral blood.

PATIENT SELECTION

4.1 Eligibility Criteria

1. Patients must have histologically or cytologically confirmed recurrent or metastatic non-cutaneous HNSCC for which there are no surgical or radiation curative options.

2. Patients may have received up to 3 prior lines of therapy for metastatic or recurrent disease.

3. Age ≥18 years.

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

5. Patients must have adequate organ and marrow function as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,000/mcL
– platelets	≥75,000/mcL
– total bilirubin	≤ institutional 1.5x upper limit of normal (ULN)

- AST(SGOT)/ALT(SGPT) ≤3 × institutional ULN
- creatinine ≤ 1.5x institutional ULN
  OR
- glomerular filtration rate (GFR) ≥50 mL/min/1.73 m^2 (see Appendix B).

6. Human immunodeficiency virus (HIV)-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial.

7. For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.

8. Patients with a history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load.

9. Patients with treated brain metastases are eligible if follow-up brain imaging after central nervous system (CNS)-directed therapy shows no evidence of progression for at least 4 weeks.

10. Patients with new or progressive brain metastases (active brain metastases) are eligible if the treating physician determines that immediate CNS specific treatment is not required and is unlikely to be required during the first cycle of therapy.

11. Patients with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial.

12. Patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the New York Heart Association Functional Classification. To be eligible for this trial, patients should be class 2B or better.

13. Female subjects of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

14. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 4 months after the last dose of study medication. Subjects of childbearing potential are those who have not been surgically sterilized, or have not been free from menses for > 1 year.

15. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 4 months after the last dose of study therapy.

16. Must have adequate archival baseline tissue available (see tissue requirements) or new biopsy must be performed (section 6.2).

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

4.2 Exclusion Criteria

1. Patients with nasopharyngeal HNSCC will be excluded.

2. Patients who have had chemotherapy or radiotherapy within 2 weeks prior to entering the study. Palliative radiotherapy is allowable and does not require washout. Palliative radiotherapy on study is allowed as long as it is not to target lesions.

3. Patients who have not recovered from adverse events due to prior anti-cancer therapy (i.e., have residual toxicities > Grade 1) with the exception of alopecia. Grade 2 neurotoxicity is allowable as long as clinically stable.

4. Patients who are receiving any other investigational agents.

5. Patients who have previously received PD-1 or PD-L1 inhibitors for metastatic/recurrent disease. Treatment in the curative setting is allowable.

6. Patients currently receiving metformin or who have received metformin in the last 6 months.

7. History of allergic reactions attributed to compounds of similar chemical or biologic composition to pembrolizumab or metformin.

8. Patients may not receive any medication or substance that is known to be strongly associated with lactic acidosis (ex. NRTIs, see Appendix C) within 14 days of initiating metformin ER treatment.

9. Patients with uncontrolled intercurrent illness that in the opinion of the investigator will prohibit compliance with the study.

10. Patients with psychiatric illness/social situations that would limit compliance with study requirements in the opinion of the investigator.

11. Requirement of any use of steroids greater than the equivalent of 10mg prednisone daily is not allowed.

12. Patients with history of autoimmune diseases currently requiring systemic immunosuppressive treatment in addition to, or instead of, steroids are excluded.

13. Pregnant women are excluded from this study because there is an unknown but potential

risk for adverse events in nursing infants secondary to treatment of the mother with pembrolizumab. Breastfeeding should be discontinued if the mother is treated with pembrolizumab.

14. Patients that are unable to swallow pills will be excluded as metformin ER cannot be crushed. PEG tube is allowable as long as patients are able to swallow pills.

15. Patients cannot receive live vaccines within 30 days prior to the first dose of trial treatment. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

4.3 Inclusion of Women and Minorities

Women and minorities will be included.

REGISTRATION PROCEDURES

5.1 Patient Registration

To register a patient, the following documents must be completed by the sub-site research team or UC research team and securely e-mailed to the UC Study Project Manager and UC PI:

- Copy of all baseline tests required per the protocol calendar. Tests must be within the protocol specified windows and assessed for clinical significance.
- Signed consent.
- Consent documentation note.
- Source documents sufficient to support verification of every inclusion & exclusion criteria.

Upon UC PI confirmation of eligibility, to complete the registration process, the Project Manager will:

- Assign a patient study number (see 7.3.4 Assignment of Screening/Study Number)
- Register the patient on the study.
- Assign the patient into one of the two arms after using R software
- E-mail the patient study number and arm to the participating site and ensure site is notified of patient's eligibility status.

5.2 General Guidelines

Following registration, patients should begin protocol treatment within 28 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Project Manager should be notified of cancellations as soon as possible.

BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

6.1 Biomarker Plan

List of Biomarker Assays in Order of Priority

Priority	Biomarker Name	Biomarker Assay	Biomarker Type and Purpose	M/O	Timing	Specimen	Quantity Needed	Laboratory
1	Peripheral Blood Immune Phenotypes	Flow Cytometry	Exploratory To determine the change in peripheral blood immune populations in response to metformin, pembrolizumab or combination.	M	Pre-treatment Post-lead-in During combination	Peripheral blood in EDTA tubes	20 mls	CTO/Borchers/ Wise-Draper
2	NK cell infiltration and anti-tumor activity	Immunofluorescence	Integrated To determine NK cell infiltration after treatment with metformin and pembrolizumab.	O	Pre-treatment During combination	FFPE slides	5 slides each or block	Wise-Draper
3	NK Effector Functions	NK Cytotoxicity Assays	Exploratory To determine NK cell activation and cytotoxic activity after treatment with metformin and pembrolizumab.	O	Pre-treatment Post-lead-in During combination	NK cells isolated from EDTA tubes	40 mls	Wise-Draper
4	NK Effector Functions	Isolight Analysis	Exploratory To determine NK cell polyactivation after treatment with metformin and pembrolizumab.	O	Pre-treatment Post-lead-in During combination	PBMCs/ NK cells isolated from EDTA tubes	20 mls (can be combined with #3)	CTO

Priority	Biomarker Name	Biomarker Assay	Biomarker Type and Purpose	M/O	Timing	Specimen	Quantity Needed	Laboratory
5	Cytokine Levels	ELISA	Exploratory To determine the cytokine response before and after metformin, pembrolizumab and combination	O	Pre-treatment Post-lead-in During combination	Plasma isolated from EDTA tubes and 5mls from red top serum tube	10 mls (5 mls can be collected from samples for which PBMCs are isolated)	CTO/Wise-Draper
6	STAT-3 Expression	Nanostring Technology	Exploratory To determine if metformin and pembrolizumab result in decreased STAT-3 in tumor and immune cells.	O	Pre-treatment During combination	FFPE scrolls and 5 slide	1 scroll each, 5 FFPE slides	Wise-Draper
7	NKG2D soluble ligands	ELISA	Exploratory To determine if metformin and pembrolizumab result in release of NKG2D soluble ligands.	O	Pre-treatment Post-lead-in During combination	Plasma isolated from EDTA tubes	5 mls (can be collected from samples for which PBMCs are isolated)	Wise-Draper
8	Tissue Biomarkers	IHC	Integrated To determine if PD-L1 and other potential biomarkers predict response to metformin and pembrolizumab combination	O	Pre-treatment During combination	FFPE slides	5-10 slides	Wise-Draper

Specimen Collection Schedule

Specimen Type	Pre-treatment Day -14 (Arm 1) Day -21 (Arm 2)	Cycle 1 Day 1 (Arm 1); Day -7 (Arm 2)	Cycle 2 Day 1 (Week 4)
Peripheral Blood 60mls in 6 EDTA tubes and 5mls in 1 red top serum tube	X	X	X
FFPE slides x 20 and 1 tissue scroll	X		X

6.2 Integrated Correlative Studies

Please consult the study lab manual for more details.

NK Cell Infiltration and Tissue Biomarkers

- Collection of Specimen(s): Tumor block (if block is unavailable, 20 FFPE slides may be permissible but need verification from PI) will be collected at screening and Week 4; Archival is acceptable for pre-treatment
- Handling of Specimens(s): Normal operating procedures
- Shipping of Specimen(s): Ship per standard operating procedures; Notify CTO lab by email at ctolabuccc@ucmail.uc.edu, ***the day of shipping the sample***.
- Site Performing Correlative Study: University of Cincinnati, Wise-Draper Laboratory

6.3 Exploratory/Ancillary Correlative Studies

Peripheral Blood Immune Phenotypes/NK Effector Functions

- Collection of Specimen(s): 6 EDTA 10ml tubes will be collected at
  - Day -21 prior to treatment that day (Arm 2), Day -14 prior to treatment that day(Arm 1), and
  - Day 1 (Arm 1) or Day –7 (Arm 2), and
  - Week 4 both arms
- Handling of Specimens(s): Do not shake or freeze tubes;
- Label each tube as follows:
- Clinical trial study number (HN1901)
- Subject's (ID) (site number, patient ID example: HN02-01)
- Date the tube was drawn (example: 2/2/2018)
- Time of blood draw (example: 15:00)
- Study time-point (example: Day 1)
  - Isolation of PBMCs to be performed after delivery to laboratory below

Shipping of Specimen(s):

- Place sample in absorbent pack and biohazard bag along with blood sample requisition form.

- Wrap gel pack around biohazard bag with sample and place into *ambient* shipping box.
- Follow IATA shipping instructions and standards by properly labeling all shipping boxes to prevent delays.
- Attach provided FedEx Airbill to the shipping box.
- Ship per standard operating procedures; Notify CTO lab by email at ctolabuccc@ucmail.uc.edu, ***the day of shipping the sample***.
- Samples to be shipped Monday-Thursday only via FedEx First Overnight.

Site(s) Performing Correlative Study: University of Cincinnati, Wise-Draper and Borchers Laboratory

Cytokine Levels/ NKG2D soluble ligands

- Collection of Specimen(s): 6 EDTA 10ml tubes and 1 red top serum 5ml tube will be collected at:
    - Day -21 prior to treatment that day (Arm 2), Day -14 prior to treatment that day(Arm 1), and
    - Day 1 (Arm 1) or Day –7 (Arm 2), and
    - Week 4 both arms

Handling of Specimens(s): Do not shake or freeze tubes;

- Label each tube as follows:
- Clinical trial study number (HN1901)
- Subject's (ID) (site number, patient ID example: HN02-01)
- Date the tube was drawn (example: 2/2/2018)
- Time of blood draw (example: 15:00)
- Study time-point (example: Day 1)
- Separation of plasma to be performed after delivery to laboratory below

Shipping of Specimen(s):

- Place sample in absorbent pack and biohazard bag along with blood sample requisition form.
- Wrap gel pack around biohazard bag with sample and place into ambient shipping box.
- Follow IATA shipping instructions and standards by properly labeling all shipping boxes to prevent delays.
- Attach provided FedEx Airbill to the shipping box

Ship per standard operating procedures; Notify CTO Lab by email at ctolabuccc@ucmail.uc.edu **the day of shipping the sample**.

Site(s) Performing Correlative Study: University of Cincinnati, CTO Laboratory and Wise-Draper Laboratory

STAT-3 Expression

- Collection of Specimen(s): Tumor block or 20 FFPE slides will be collected at screening and Week 4; Archival is acceptable for pre-treatment
- Handling of Specimens(s): Normal operating procedures; RNA to be extracted by

laboratory below
- Shipping of Specimen(s): Ship per standard operating procedures; Notify CTO lab by email at ctolabuccc@ucmail.uc.edu ***the day of shipping the sample***.
- Site(s) Performing Correlative Study: University of Cincinnati, Wise-Draper Laboratory

TREATMENT PLAN

The Study Calendar summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

7.1 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history; this will also include a history of alcohol and tobacco use. Medical history should be graded per CTCAE v.5 to aid in assessment of potential grade changes with respect to adverse events.

7.2 Prior and Concomitant Medications Review

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

Concomitant Medications
The investigator or qualified designee will record medication, if any, taken by the subject during the trial, including all medications used in the treatment of any adverse events.

7.3 Disease Details and Treatments

7.3.1 Disease Details

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

7.3.2 Prior Treatment Details

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

7.3.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30-day Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

7.3.4 Assignment of Screening/Study Number

All consented subjects will be given a unique screening number that will be used to identify the subject for all procedures that occur prior to eligibility being confirmed. Each subject will be assigned only one screening number. Screening numbers must not be re-used for different subjects. The University of Cincinnati will provide any sub-sites with instructions for the methods to be used in assigning screening information to potential subjects.

Any subject who is screened multiple times will retain the original screening number assigned at the initial screening visit. The screening number will be their study number as well once they are allocated to treatment.

7.3.5 Trial Compliance

Interruptions from the protocol specified treatment plan for greater than 4 weeks delay of pembrolizumab or metformin doses require consultation between the investigator and the University of Cincinnati PI (if not the same) and written documentation of the collaborative decision on subject management.

The total volume of pembrolizumab infused will be compared to the total volume prepared to determine compliance with each dose of pembrolizumab administered. Treatment with standard therapies will be prepared and administered as per the approved product label.

7.4 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in the Investigators Brochure for Pembrolizumab and the package insert for Metformin. Appropriate dose modifications are described in Section 8. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Pembrolizumab	No premedications; No steroids	200mg or 400mg	IV over 30 minutes	Days 1, week 1 (for 400mg	21 days (3 weeks)

				dose, therapy only given q6 weeks and on odd cycles)	
Metformin*	No premedications	1000mg for first 7 days followed by 2000mg daily	Oral	Daily	Continuous**
* The patient will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to research staff at the end of each course. ** Metformin will be escalated per schema and then given continuously throughout pembrolizumab cycles.					

7.4.1 Metformin

Treatment:
Patients will receive metformin ER starting on day -14 for arm 1 and Day -7 for arm 2. The starting dose will be 1000mg PO daily to be administered with evening meal. The tablet should be swallowed whole, and patients will be instructed not to crush, cut or chew medication. On day -7 (or Day 1 for arm 2), patients will increase to 2000mg daily. Metformin will be continued daily with pembrolizumab.

Metformin should continue throughout treatment and even through pembrolizumab delays unless patient is experiencing adverse effects attributed to metformin. Metformin should be taken with food at approximately the same time daily. Patients will be instructed to check their blood sugar if they have any symptoms of hypoglycemia including but not limited to chills/shakiness, cold feeling, lightheadedness, dizziness, etc. Patients will be given a diary to record each dose. They should also be warned that the shell of the ER tablet may be visible in stool and not to be alarmed.

NOTE: Temporarily discontinue metformin in patients undergoing radiologic studies in which intravascular iodinated contrast media are utilized. It is generally recommended that metformin be temporarily discontinued prior to or at the time of intravascular administration of iodinated contrast media (potential for acute alteration in renal function). Continue to withhold metformin for 48 hours after the radiologic study.

7.5 Clinical Procedures/Assessments

7.5.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Study Calendar and more frequently if clinically indicated. Adverse events will be graded and recorded throughout the study and during the follow-up period

according to NCI CTCAE Version 5.0 and Section 11 of this protocol. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to Section 11 for detailed information regarding the assessment and recording of AEs.

7.5.2 Full Physical Exam

The investigator or qualified designee will perform a full physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. Full physical exam requires assessment of major organ sites (Constitutional, Head and Neck, Cardiovascular, Pulmonary, Abdominal, Musculoskeletal, Lymph, Neurological, and Skin).

7.5.3 Directed Physical Exam

Except for at screening, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

7.5.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Study Calendar. Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.5.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Appendix A) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Study Calendar.

7.5.6 Tumor Imaging and Assessment of Disease

Pre-operative imaging will be performed at each institution and only site investigators (PI or Sub-PIs) may determine the assessment of disease recurrence.

7.5.7 Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 1.

Table 1. Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	
Platelet count	Alanine aminotransferase	Protein	Total triiodothyronine (T3) if TSH is outside

Hematology	Chemistry	Urinalysis	Other
	(ALT)		normal range
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	Free thyroxine (T4) if TSH is outside normal range
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Thyroid stimulating hormone (TSH)
Absolute Neutrophil Count	CO_2 or bicarbonate	Urine pregnancy test †	Prothrombin time PT/INR
Absolute Lymphocyte Count	Uric Acid		Partial Thromboplastin Time (PTT)
	Calcium		C- Peptide
	Chloride		Blood for correlative studies
	Glucose		Vitamin B-12
	Phosphorus		Lactate (Lactic acid)
	Potassium		
	Sodium		
	Creatinine		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		
† Done on all women of child-bearing potential. Urine or serum is acceptable.			

7.5.8 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events, which are present at the time of discontinuation/withdrawal, should be followed in accordance with the safety requirements outlined in Section 11. Patients have the option of withdrawing from treatment only (therefore entering survival follow-up) or withdrawing from study. This should be documented clearly in REDCap.

7.6 Visit Requirements

Visit requirements are outlined in Section 12.0 - Study Calendar.

7.6.1 Screening

Potential subjects will be evaluated to determine that they fulfill the entry requirements as set forth in eligibility requirements. Visit requirements are outlined in the Study Calendar.

Results of a test performed prior to the subject signing consent as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame. Screening procedures are to be completed within 28 days prior to the first dose of trial treatment except for the following:

- Laboratory tests and ECOG PS are to be performed within 10 days prior to the first dose of trial treatment.
- For women of reproductive potential, a serum pregnancy test will be performed within 72 hours prior to the first dose of trial treatment. A urine test may be considered if serum test is not appropriate.

Subjects may be rescreened after initially failing to meet the inclusion/exclusion criteria and they have not yet started treatment, however, re-screening tests will not be covered by study. Results from assessments performed during the initial screening period are acceptable in lieu of a repeat screening test if performed within the specified time frame and the inclusion/exclusion criteria is met.

7.6.2 Treatment Period

Visit requirements are outlined in Section 12.0 – Study Calendar.

7.6.3 On Treatment Imaging

Patients will undergo imaging (CT of affected target areas- ideally CT with contrast unless contraindication; MRI also acceptable) every 12 weeks +/- 5 days. If patient has a PR or CR, confirmation scans are required at 4 weeks after scan in which response was first observed.

The specific method of imaging to use (CT, CT with contrast, MRI) may change per the subject's clinical needs, and this determination for which imaging method to use will rely on the treating investigator's clinical discretion.

7.6.4 Post-Treatment Visits/Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 that is at least possibly attributed to study treatment will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

7.6.5 Survival Follow-up

Once a subject experiences confirmed progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone or seen in clinic every 12 weeks +/- 4 weeks to assess for survival status until death, withdrawal of consent, or at the end of the study, whichever occurs first. Patients will be followed up to 5 years.

7.7 Definition of Dose-Limiting Toxicity

7.7.1 Lead-in Safety Analysis

The first nine patients will be enrolled as the lead-in population. Safety analysis will be performed with this population and will be analyzed on a rolling basis. Monthly review with all active sites will be held to determine if any of these first 9 patients have experienced a dose limiting toxicity, DLT, (described below) to evaluate the safety of the combination. Safety analysis will be considered complete after the ninth patient has completed 28 days of combination treatment. Patients will continue to be enrolled past the initial safety cohort, but if at any time, safety analysis reveals >3 out of the 9 patients in the safety cohort have developed a DLT, then no further patients may be enrolled due to excess toxicity with this combination.

7.7.2 Dose Limiting Toxicity

Dose limiting toxicity (DLT) is defined as the appearance of side effects during treatment that are attributed to treatment and severe enough to prevent continuation of treatment or grade 3 or 4 non-hematologic toxicities other than alopecia, nausea or vomiting and that in the case of pembrolizumab, do not resolve with steroids after four weeks. If more than three of nine patients develop a DLT, then combination will be considered unsafe and the study will not proceed. Patients are assessed for DLTs during the lead-in period as well as for the first 28 days of combination treatment.

Management and dose modifications associated with the above adverse events are outlined in Section 8.

7.8 General Concomitant Medication and Supportive Care Guidelines

Metformin and pembrolizumab are not affected by p450 enzymes and therefore p450 inhibitors are allowed.

Concomitant use of furosemide results in increased levels of metformin and therefore should be avoided if possible but is allowable if needed for diuresis with close monitoring.

Cationic drugs (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, or vancomycin) that are eliminated by renal tubular secretion

theoretically have the potential for interaction with metformin by competing for common renal tubular transport systems. Therefore, these medications are to be avoided if possible while patient is on study but are allowable if felt to be necessary by patient's physician. However, it must be documented that patient was on these medications.

Nifedipine results in enhanced absorption of metformin. It must be documented if a patient is on nifedipine during the study but is allowable.

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

- Biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy
- Investigational agents other than pembrolizumab or metformin
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids should be avoided if possible. Steroids are allowed as short bursts of 5-7 days if required for clinical indication (i.e. COPD) or to modulate symptoms from an adverse event of suspected immunologic etiology. The chronic use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor Investigator.

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

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

7.9 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of child bearing potential 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 the sponsor in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by the UC PI (coordinating center PI).
- The drug manufacturer can no longer provide the study agent

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in REDCap.

7.10 Duration of Follow-Up

Patients will be followed for survival up to five years after removal from treatment or until death, whichever occurs first. Patients removed from treatment for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event but will also continue to be followed for survival up to five years.

DOSING DELAYS/DOSE MODIFICATIONS

8.1 Metformin ER

Acceptable Dose Reductions for Metformin ER	
Dose Reduction Level	**Dose Reduction**
1	1000mg
2	500mg

Diarrhea	**Management/Next Dose for Metformin ER**
≤ Grade 2	No change in dose- can use loperamide to control
Grade 3**	Hold until ≤ Grade 2. Resume at same dose level. Decrease dose to 1000mg daily. Second occurrence can decrease to 500mg daily. Third occurrence, discontinue permanently.
Grade 4	Off protocol therapy
*Patients requiring a delay of >4 weeks should go off protocol therapy. **Ensure diarrhea is not due to pembrolizumab (see criteria below).	

Diarrhea	**Management/Next Dose for Metformin ER**
Recommended management: Loperamide antidiarrheal therapy Loperamide dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used.	

Nausea	**Management/Next Dose for Metformin ER**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >4 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	
Recommended management: antiemetics.	

Vomiting	**Management/Next Dose for Metformin ER**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >4 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	
Recommended management: antiemetics.	

Hypoglycemia	**Management/Next Dose for Metformin ER**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >4 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	
Recommended management: antiemetics.	

Lactic acidosis: In a very small proportion of patients, lactic acidosis defined as lactate levels >5 can occur with metformin especially those with renal impairment. Due to the seriousness of this event, metformin should be permanently discontinued.

Renal dysfunction: Although metformin does not cause renal dysfunction, the clearance of metformin and therefore risk of lactic acidosis increases in renal dysfunction. Therefore, metformin dose should be decreased by 50% if eGFR is 30 to 45ml/min and held if eGFR is <30ml/min. Metformin may be restarted at full dose (using dose escalation as provided) once kidney function improves above these parameters.

Vitamin B12 deficiency: Metformin does not need to be held for this indication as long as B12 is replaced.

Other Toxicities: For any other grade 3-4 adverse events (besides those mentioned above), hold metformin until toxicities have recovered to grade 1 or less.

Dose Reduction for Intolerability: If patients have side effects that are considered intolerable to patient but are less than grade 3, support patients with supportive medications (loperamide, omeprazole, anti-emetics, etc.) and try alteration of their dosing schedule by reducing by 1 dose level at a time until toxicity is ≤ grade 1 for 1 week. Then attempt to re-escalate to protocol dose. Every attempt should be made to keep metformin dose at protocol dose but if unable to achieve the assigned dose, then metformin should be maintained at highest dose tolerated for remainder of treatment.

> NOTE: Temporarily discontinue metformin in patients undergoing radiologic studies in which intravascular iodinated contrast media are utilized. It is generally recommended that metformin be temporarily discontinued prior to or at the time of intravascular administration of iodinated contrast media (potential for acute alteration in renal function). Continue to withhold metformin for 48 hours after the radiologic study.
>
> NOTE: If metformin is held at any time for any reason for longer than 1 week, upon restarting the drug, it must be dose escalated again slowly with 1000mg daily for 1 week prior to escalation to 2000mg daily.

8.2 Pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per below. It will be up to investigator to determine if each AE is attributed to metformin (and follow guidelines above) or pembrolizumab to follow guidelines below.

Pembrolizumab administration should be delayed for the following:

- Any Grade ≥ 2 non-skin, drug-related adverse event, with the following exceptions:
  - Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related adverse event
- Any Grade 3 drug-related laboratory abnormality, with the following exceptions:
  - Grade 3 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis do not require a dose delay.
  - If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity

    - If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade $\geq$ 3 toxicity
- Any adverse event, laboratory abnormality, or intercurrent illness, which, in the judgment of the investigator, warrants delaying the dose of study medication

8.2.1 Management for Immuno-Oncology Agents

Immuno-Oncology (I-O) agents are associated with adverse events that can differ in severity and duration than adverse events caused by other therapeutic classes. Pembrolizumab is considered an immuno-oncology agent in this protocol. Early recognition and management of adverse events associated with immuno-oncology agents may mitigate severe toxicity. Management algorithms have been developed to assist investigators in assessing and managing the following groups of adverse events:

- Gastrointestinal
- Renal
- Pulmonary
- Hepatic
- Endocrinopathies
- Skin
- Neurological.

Investigators should use standard of care algorithms listed in the IB as well as institutional guidelines.

8.2.2 Dose Modifications for Pembrolizumab

Dose reductions or dose escalations are not permitted.

8.2.3 Criteria to Resume Pembrolizumab

Subjects may resume treatment with study drug when the drug-related AE(s) resolve to Grade $\leq$ 1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue.
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity.
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin.
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment.

If the criteria to resume treatment are met, the subject may restart treatment immediately.

Treatment may be delayed for up to a maximum of 8 weeks from the last dose; if treatment is delayed > 8 weeks, the subject must be permanently discontinued from study therapy.

8.2.4 Dose Discontinuation of Pembrolizumab

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment.
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, neurologic toxicity, hypersensitivity reactions, and infusion reactions:
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, neurologic toxicity, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation.
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:
        - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
            - AST or ALT > 8 x ULN
            - Total bilirubin > 5 x ULN
            - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
    - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
    - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset.
- Any dosing delay lasting > 8 weeks with the following exceptions:
    - Dosing delays to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing delay lasting > 8 weeks, the PI must be consulted. Tumor assessments should continue as per protocol even if dosing is delayed.
    - Dosing delays > 8 weeks that occur for non-drug-related reasons may be allowed if approved by the PI. Prior to re-initiating treatment in a subject with a dosing delay lasting > 8 weeks, the PI must be consulted. Tumor assessments should continue as per protocol even if dosing is delayed.
- Any adverse event, laboratory abnormality, or intercurrent illness, which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued

pembrolizumab dosing.

8.2.5 Management of Infusion Reactions:

Since pembrolizumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypo or hypertension, bronchospasm, or other symptoms. All Grade 3 or 4 infusion reactions should be reported within 24 hours to University of Cincinnati PI (coordinating center), as well as to the local PI and reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE (version 5.0) guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated)

Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 25-50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg at least 30 minutes before additional pembrolizumab administrations. A H1 blocker may also be administered.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g., antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for $\leq$ 24 hours).

Stop the pembrolizumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 25-50 mg IV (or equivalent) and/or acetaminophen 325 to 1000 mg; remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further pembrolizumab will be administered at that visit. Administer diphenhydramine 25-50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 25-50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg should be administered at least 30 minutes before additional pembrolizumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms: (Severe reaction, Grade 3: prolonged [i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae

[e.g., renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; presser or ventilator support indicated).

Immediately discontinue infusion of pembrolizumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. pembrolizumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

PHARMACEUTICAL INFORMATION

A listing of reported adverse events and potential risks are described in the Investigators Brochure for Pembrolizumab and the package insert for Metformin respectively. The UC Project Manager will ensure current versions of each are available to sites.

9.1 Investigational Agent

9.1.1 Metformin

Availability: Commercial Metformin ER will be used as supply and will be obtained from the site investigational drug pharmacy.

Agent Ordering and Agent Accountability: Metformin will be requested from the investigational drug pharmacy at each participating institution.

Agent Inventory Records: The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from IDS.

Investigator Brochure Availability: Metformin ER is a commercial product. Therefore, no IB will be provided.

9.2 Commercial Agent

9.2.1 Pembrolizumab

Availability: Commercial Pembrolizumab will be used.

Agent Ordering and Agent Accountability: Pembrolizumab will be used as standard of care at each participating institution.

STATISTICAL CONSIDERATIONS

10.1 Study Design/Endpoints

Primary Endpoint: Overall Response by RECIST 1.1 and iRECIST

Responses will be summarized as frequencies and percentages.

Secondary Endpoints:

- Measurement of Adverse Events by CTCAE v5.0
- Progression Free Survival (PFS) at 1 year
- Overall Survival (OS) at 1 year

After cleaning and preprocessing of the collected data, descriptive statistics will be used to summarize all adverse events. Toxicities will be aggregated as the number and percentage of patients with each type of toxicity. Kaplan Meier methods will be used to estimate overall survival and progression free survival generating median survival estimates with 95% confidence intervals (95% CIs). Multivariable survival modeling (overall survival and progression free survival) will be performed using Cox proportional hazards models and to generate Hazard Ratios (HR) and 95% CIs, with adjustment for potential patient confounders such as age, sex, smoking status, etc.

Exploratory Endpoint:

- Characterization of peripheral blood immune cell phenotypes before and after metformin, pembrolizumab and combination treatment with a particular focus on Natural Killer (NK) cells and innate immunity.
- Characterization of tumor infiltrating NK cells before and after metformin, pembrolizumab and combination treatment.
- Cytokine levels before and after metformin, pembrolizumab and combination in the plasma.
- NK effector functions before and after metformin, pembrolizumab and combination.
- Stat-3 RNA levels and phosphorylation status in NK cells and tumor tissue before and after metformin, pembrolizumab and combination treatment.
- Level of NKG2D soluble ligands before and after metformin, pembrolizumab and combination in the plasma.

Scatter plots and histograms will be used to examine the exploratory endpoints for the presence of unusual outliers, normality and potential non-linearity. Continuous endpoints will be log-transformed if not normally distributed. The endpoints using samples before and after treatment will be compared using paired T tests. When endpoints are compared between three or more groups, ANOVA model will be used. The ANOVA will be followed up with post-hoc pairwise comparisons. A chi-square test will be used to investigate the categorical exploratory endpoints. For IHC, the percentage of positive cells per area will be multiplied by the staining intensity for each tumor to determine quantitative expression pre- and post-treatment.

To detect a difference of 15% in %NK Cell Killed Target Cells between control and treated groups,

we will need 19 pairs of patients. In an unpaired comparison, 10 patients per group will give us enough power to detect a difference of at least 30% between the groups. We note that the average %NK Cell Killed Target Cells in controls was assumed to be around 10% (Data not shown) and the standard deviation was estimated from the range of 5 to 15%. We also note that the calculations are based on the significance threshold of 0.05 and an 80% detection power using one-sided tests.

10.2 Sample Size/Accrual Rate

We expect that metformin and pembrolizumab combination therapy will improve ORR to 32%. Our preliminary sample size calculations indicate that 25 patients should be enough to approximately estimate the proportion of around 32% with a margin of error close to 20%. We note that ORR of pembrolizumab alone based on previous clinical studies varies between 15-20% in HNSCC; and retrospective studies show metformin increased response rates by about 10-15% in other tumor types. We also note that the calculations are based on the significance threshold of 0.05 and an 80% detection power using one-sided tests. We plan to enroll twenty-five patients total with goal of 19 evaluable (20% expected drop-out rate given dysphagia seen in head and neck cancer patients). If 6/19 achieve response, we will consider study successful and worth pursuing further in a phase II/III study. The above calculations are based on the significance threshold of 0.05 and using the critical values of the normal distribution. We expect to enroll the patients over 18-24 months.

PLANNED ENROLLMENT REPORT

Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	1	2	0	0	**3**
White	7	13	0	0	**15**
More Than One Race	1	1	0	0	**2**
Total	**9**	**16**	**0**	**0**	**25**

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10.3 Early Stopping Rules:

In order to verify safety of the combination of metformin and pembrolizumab, an early stopping rule will be used if 3 out 9 (33%) of the safety lead-in develop DLTs (see definition above). The lead-in will be on a rolling basis but if three patients develop DLTs, the study will be stopped so that safety may be investigated by the DSMB. If DLTs are confirmed, the study will be closed to enrollment due to safety concerns.

10.4 Randomization:

Patients will be randomized into arms 1 and 2 using R (R Core Team. 2012)[33] at time of registration (if screening labs are to be obtained on same day as treatment randomization may be performed at the time all other eligibility criteria are informally confirmed to allow adequate time for treatment planning). The UC Project Manager will work with the statistician to ensure each patient is assigned to one of the arms correctly. Randomization of patients does not affect the primary or secondary endpoints of this study as response and safety is based on the combination treatment in which both groups will receive. The patients are randomized for the exploratory endpoints in order to better understand the difference of effects of metformin versus pembrolizumab on the immune system.

ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

11.1 Adverse event definition, attributions, and expectedness

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation participant administered study drug and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

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

The expectedness of the occurrence of an adverse event is determined by a study physician and should be used to help determine whether prompt reporting requirements to regulatory authorities (IRB, FDA etc.) are required.

- Expected – An adverse event is expected if it is described as an anticipated risk described within this protocol or research informed consent form as a known adverse event/risk.
- Unexpected – If an adverse event is not described within this protocol or consent form as an expected risk to subjects then the AE will be considered to be unexpected.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject.

11.2 Serious adverse events definition

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (See NOTE below).
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event - defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above. Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

Although pregnancy, overdose, and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

NOTE: The following hospitalizations are not considered SAEs:

- A visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- Elective surgery, planned prior to signing consent
- Admissions as per protocol for a planned medical/surgical procedure
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

- Admission for administration of anticancer therapy in the absence of any other SAEs.

11.3 Serious Adverse Event Collection and Reporting

All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study should be collected on institutional adverse event logs and reported using the applicable FDA MedWatch form within 24 hours of learning of the event to the University of Cincinnati PI and Project Manager and entered into REDCap. Sites should contact the UC Project Manager for copies of the MedWatch form.

SAEs must also be reported to the IRB of record, and/or FDA as needed based on applicable institutional and regulatory timeframes.

11.4 Adverse Event Collection and Reporting

The collection of non-serious AE information should begin at initiation of study drug, all events prior to that time should be documented as medical history (BUT all SAEs must be collected from consent, see Section 11.3). All adverse events (not just those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 90 days following the last dose of study treatment.

Adverse events must be collected using institutional adverse event logs and captured in REDCap.

AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate. Subjects with an AE of Grade > 1 that is at least possibly attributed to study treatment will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded through resolution.

11.5 Other safety considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, X-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

11.6 Evaluation of adverse events and grade changes

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 5.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded within REDCap and within institution specific AE logs.

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

11.7 Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented in REDCap. Any pregnancy occurring in a patient or patient's partner from the time of consent to 4 months after the last dose of study drug must be reported and then followed for outcomes. Newborn infants should be followed until 30 days old.

11.8 Contraception/Birth Control

Participants of childbearing potential who are sexually active and their partners must agree to the use of a highly effective form of contraception throughout their participation. Effective birth control is considered to be:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
- Oral route, Intravaginal route or Transdermal route
- Progestogen-only hormonal contraception associated with inhibition of ovulation
- Oral, Injectable, or Implantable
- Intrauterine device
- Intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Vasectomized partner
- Sexual abstinence

11.9 Breast Feeding

Participants must not breast-feed while receiving protocol therapy and for 180 days following the last dose of protocol therapy.

11.10 Secondary Malignancy

A secondary malignancy is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in Section 11 (AE reporting).

11.11 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

STUDY CALENDAR

Cycle	Screening	Lead in Treatment Before Cycle 1			1	2	3, 5, 7, 9 etc.	4, 6, 8, 10, etc.	Off Study / Safety follow-up (30 days post tx)[i]	Survival Follow-up
Day	**28 days before 1st lead-in tx[O]**	**-21[C]**	**-14**	**-7**	**1**	**1**	**1**	**1**		
Window (days)				**+/-1 day**	**+/-1 day**	**+/-3 days**	**+/-3 days**	**+/-3 days**	**+/-3 days**	**+/-4 weeks**
Arm 1										
Metformin ER daily			X[a]	X[b]	X	X	X	X		
Pembrolizumab q 3 weeks or q 6 weeks[q]					X	X	X	X		
Correlative blood draws[h]			X[h]		X	X				
Arm 2										
Metformin ER daily				X[a]	X[b]	X	X	X		
Pembrolizumab q 3 weeks or q 6weeks[p]		X			X	X	X	X		
Correlative blood draws[h]		X[h]		X		X				
Both Arms										
Informed consent	X									
Demographics	X									
Medical history	X									
Concomitant meds	X	X	X----------X							
Physical exam[d]	X	X	X	X	X		X		X	
Vital signs	X	X	X	X	X	X	X	X	X	
Height	X									
Weight	X	X	X	X	X	X	X	X	X	
Performance status	X[k]	X	X	X	X	X	X		X	
CBC w/diff, plts[f]	X[k]	X	X	X	X	X	X	X	X	
Serum chemistry[f]	X[k]	X	X	X	X	X	X	X	X	
Urinalysis	X[k]				X		X			
Coagulation tests	X[k]				X		X			
C-peptide	X[k]				X	X				
Thyroid Tests[k]	X[k]				X		X			
Vitamin B-12	X[k]				X	X				
Lactate (Lactic Acid)	X[k]				X	X				
Adverse event evaluation[m]			X----------X							
Pregnancy test[e]				X						
Radiologic evaluation[l, q]	X						X			
Correlative biopsy[g]	X					X				
Medication diary[n]	X		X	X	X	X	X	X		
Post-tx survival status[j]									X	X

a. Metformin ER starting dose 1000mg daily
b. Metformin ER escalation dose 2000mg daily

Cycle	Screening	Lead in Treatment Before Cycle 1			1	2	3, 5, 7, 9 etc.	4, 6, 8, 10, etc.	Off Study / Safety follow-up (30 days post tx)[i]	Survival Follow-up
Day	**28 days before 1st lead-in tx[O]**	**-21[C]**	**-14**	**-7**	**1**	**1**	**1**	**1**		
Window (days)				**+/-1 day**	**+/-1 day**	**+/-3 days**	**+/-3 days**	**+/-3 days**	**+/-3 days**	**+/-4 weeks**

c. Only applies to Arm 2 as Arm 1 does not have a D -21.
d. Full physical exam at screening, directed on other visits
e. Child-bearing females only. Test within 72 hours of first treatment (See 7.6.1)
f. See table 1 in section 7.5.7
g. Archival acceptable at screening; Cycle 2 repeat biopsy if clinically feasible. Biopsy may be of a target or non-target lesion but if there is only one target lesion that is being followed, this lesion should be avoided for biopsy unless discussed with PI.
h. 60mls in EDTA tubes and 5mls in red top serum tube at Day -14 (Arm 1) or Day -21 (Arm 2); and also collect Day 1 (Arm 1) or Day –7 (Arm 2); and also collect Cycle 2 Day 1 (week 4). The Day -14 & Day -21 respective samples must be collected before any study treatment is given on those days – if this pre-treatment sample is missed then no other blood correlatives should be collected for those subjects. This first pre-treatment correlative blood sample may be obtained at another time-point after randomization but prior to first lead in treatment with the PI's approval.
i. Should be completed at 30 days post-treatment OR before new anti-cancer therapy whichever occurs first.
j. Every 3 months/12 weeks - can be completed by telephone
k. ECOG PS and screening laboratory tests to be completed within 10 days of first tx (may be performed on day 1 prior to tx)
l. Scans to be performed every 12 weeks +/- 5 days starting with C3D1
m. Adverse events should be collected for 90 days following the last dose of study treatment
n. Subjects will be provided with a medication diary at screening but will not complete entries until dispensed Metformin. The medication diary will be returned to research staff and a new diary issued at the end of each course.
o. Subjects are randomized to either Arm 1 or Arm 2 after registration (eligibility is confirmed). These arms are independent of each other. Each arm has the same amount of time 28 days, to complete screening activities before their respective first lead in treatment visits (Day -21 or Day -14).
p. Pembrolizumab may be given at 200mg q3 weeks or 400mg q6 weeks and may be changed during course of treatment per investigator discretion.
q. Temporarily discontinuing metformin in patients undergoing radiologic studies in which intravascular iodinated contrast media are utilized is left up to investigator discretion.

MEASUREMENT OF EFFECT

Although the clinical benefit of the combination of pembrolizumab and metformin has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated every 9 weeks. In addition to a baseline scan, confirmatory scans will also be obtained 4 weeks following initial documentation of an objective response.

13.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 9_weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response.

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

13.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with metformin 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.)

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

13.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the lesion has progressed after radiation treatment, then it may be considered evaluable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-

cystic lesions are present in the same patient, these are preferred for selection as target lesions.

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

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

13.1.3 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 4 weeks before the beginning of the treatment.

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

Clinical lesions: Will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which

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

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

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

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

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

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

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

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

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

13.1.4 Response Criteria RECIST 1.1

13.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (0.5 cm). (Note: the appearance of one or more new lesions is also considered progressions).

Note: Modified iRECIST is also being used in that given that immunotherapy is being administered in this protocol, patients may remain on study at first progression if investigator feels the patient is deriving clinical benefit. However, if in subsequent scans, the lesions continue to progress beyond previous scan, the patient will be considered to have progressive disease and should be taken off protocol. Any question should be discussed with the sponsor.

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

study.

13.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm [<1 cm] short axis).

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

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

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

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

13.1.4.3 Evaluation of Best Overall Response

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

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

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

PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

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

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

iRECIST Note: Given that immunotherapy is being given in this protocol, patients may remain on study at first progression if investigator feels the patient is deriving clinical benefit. However, if in subsequent scans, the lesions continue to progress beyond previous scan, the patient will be considered to have progressive disease and should be taken off protocol. Any question should be discussed with the sponsor.

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

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

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

13.1.5 Duration of Response

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

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

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

13.1.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

13.1.7 Overall Survival

OS is defined as the duration of time a patient is alive from start of treatment until time of death.

STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

14.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The UC Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events reporting. The UC Principal Investigator has access to the data at all times through REDCap. All Study Investigators at participating sites who register/enroll patients to this study are responsible for timely submission of data via REDCap and timely reporting of adverse events to the UC PI and in REDCap.

14.2 SMART IRB

The UC HRPP will utilize the Streamlined, Multisite, Accelerated Resources for Trials IRB Reliance platform (SMART IRB) master IRB reliance agreement to establish reliance arrangements with the research sites participating in this study.

Launched in 2016, SMART IRB is currently funded by the NIH Clinical and Translational Science Awards (CTSA) Program, grant number UL1TR002541-01S1. The platform serves as a roadmap for institutions to implement The National Institutes of Health (NIH) Policy on the Use of a Single Institutional Review Board for Multisite Research.

The UC IRB will act as the IRB of record for this protocol and will perform initial review and have continuing oversight of the protocol and research sites in accordance with the human subjects protection requirements of its FWA, the FWAs of relying IRBs, the federal regulations, and ethical principles referenced therein.

14.3 Data Reporting

Data collection and storage will be managed by the University of Cincinnati Cancer Center, Clinical Trials Office (UCCC CTO). The UCCC CTO will maintain storage of all clinical data in accordance with federal guidelines and GCP. Data will be entered in a secure, password protected storage databases REDCap and the UC CTMS (clinical trial management system). All hardcopies of data will be securely maintained (in a locked room or cabinet) and will only be accessible to

members of the study team or UCCC CTO personnel.

14.3.1 Data and Safety Monitoring

Any new significant finding that may affect the patient's willingness to continue in this study will be shared with patients. Immediately after the study is approved and before the first patient is enrolled, investigators will meet, develop and finalize all measurements/variables for the study. Each patient, once enrolled, will be provided a unique ID for the study. Personal information, such as name, SSN, address, phone number and DOB, will be de-identified. Confidentiality will be maintained during the phases of the trial including monitoring, preparation of interim results, review, and response to monitoring recommendations.

Exceptions may be made under circumstances where there are serious adverse events or when it is deemed appropriate for patient safety.

Study progress will be monitored regularly by the UCCC Data Safety Monitoring Board (DSMB). DSMB membership consists of persons independent of, and without any conflicts of interest with, this trial. The DSMB includes experts in the fields of relevant clinical expertise (oncology) and biostatistics.

It is the responsibility of the UC Investigator to ensure that the DSMB is apprised of all new safety information relevant to the study. Study progress & safety information will be prepared by the DSMB Coordinator with input from the UC PI as to the current status of the trial. This compiled information presented to the DSMB will include: a narrative summary from the UC PI as to trial progress and identification of any trends of significance or explanation of any SAEs or other safety related events; the accrual rate with projected completion date for the accrual phase; exclusion rates and reasons; pretreatment characteristics of patients accrued when relevant; and, the frequency and severity of adverse events.

The DSMB will function in an advisory capacity and recommendations/requests from the DSMB will be reviewed by the UC investigator and promptly addressed.

The study data from participating sub-sites will be reviewed remotely via REDCap and in person by the UCCC Study Monitor as per the Clinical Monitoring Plan (on file with UCCC CTO office).

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

Formulas to estimate renal function using serum creatinine provided by the NCI's Investigational Drug Steering Committee (IDSC) Pharmacological Task Force in table below.

1. Estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey *et al.*, 2009).

Formulae:

Race and Sex	Serum Creatinine (SCr), *µmol/L (mg/dL)*	Equation
Black		
Female	≤62 (≤0.7)	$GFR = 166 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 166 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 163 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 163 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$
White or other		
Female	≤62 (≤0.7)	$GFR = 144 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 144 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 141 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 141 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$

SCr in mg/dL; Output is in mL/min/1.73 m^2 and needs no further conversions.

2. eGFR using the Modification of Diet in Renal Disease (MDRD) Study (Levey *et al*., 2006).

175 x $SCr^{-1.154} \times age^{-0.203} \times 0.742$ (if female) × 1.212 (if black)
Output is in mL/min/1.73 m^2 and needs no further conversions.

3. Estimated creatinine clearance (ClCr) by the Cockcroft-Gault (C-G) equation (Cockcroft and Gault, 1976).

$$CLcr\ (mL/min) = \frac{[140 - age\ (years)] \times weight\ (kg)}{72 \times serum\ creatinine\ (mg/dL)} \{\times 0.85\ for\ female\ patients\}$$

Followed by conversion to a value normalized to 1.73 m^2 with the patient's body surface area (BSA).

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filtration rate. *Ann Intern Med*. 145:247-254.
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APPENDIX C DRUGS ASSOCIATED WITH LACTIC ACIDOSIS

- zidovudine (Retrovir)
- lamivudine (Epivir)
- abacavir sulfate (Ziagen)
- didanosine (Videx)
- delayed-release didanosine (Videx EC)
- stavudine (Zerit)
- emtricitabine (Emtriva)
- tenofovir disoproxil fumarate (Viread)
- lamivudine and zidovudine (Combivir)
- abacavir and lamivudine (Epzicom)
- abacavir, zidovudine, and lamivudine (Trizivir)
- tenofovir disoproxil fumarate and emtricitabine (Truvada)
- tenofovir alafenamide and emtricitabine (Descovy)