SUMMARY OF CHANGES – Protocol

Protocol #: HCC#20-266

Protocol Date: 02/23/2024

#	Section	Change
1.		Rationale: The research agreement between the University of Pittsburgh and Zucero was terminated as of 2/3/2024 for HCC#20-266. This trial has stopped enrolling patients, and the principal investigator has decided to terminate follow up. The following language was added to various parts of the protocol. Added: Note – Patient accrual was terminated early owing to the discontinuation of pixatimod supply. Follow-up assessments will no longer be completed upon IRB approval of protocol version 02/23/2024.

Protocol Number: ZU545201 / HCC 20-266
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CLINICAL PROTOCOL ZU545201

Phase IIA Basket Study of Pixatimod (PG545) in Combination with Nivolumab in PD-1 Relapsed/Refractory Metastatic Melanoma and NSCLC and Pixatimod (PG545) in Combination with Nivolumab and low-dose Cyclophosphamide in MSS Metastatic Colorectal Carcinoma (mCRC)

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1. SYNOPSIS

Summary:	Nivolumab and other agents directed against PD-1/PD-L1 pathway are associated with improved response and survival rates in multiple malignancies including advanced melanoma and PD-L1 expressing NSCLC with objective response rates (ORR) of 35%-45%, the majority of which are durable.[1-6] However, the majority of treated patients do not respond and not all responses are durable. Pixatimod (PG545, and hereafter referred to as pixatimod) is a small molecule heparan sulfate (HS) mimetic that has natural killer (NK) cell-dependent anti-tumor effects. PG545 is not a TLR9 agonist that directly activates TLR9, rather, pixatimod results in CpG accumulation in the lysosomal compartment of DCs, leading to enhanced production of IL-12, which is essential for pixatimod-mediated NK cell activation[7,8]. In a phase Ia dose-escalation study, single agent pixatimod monotherapy at doses up to 150 mg weekly (25-150 mg weekly) demonstrated dose-proportional pharmacokinetics and innate immune cell activation.[8] In a phase Ib study, combination of pixatimod with nivolumab demonstrated promising results in uninflamed tumors including microsatellite-stable colorectal carcinoma.[9] Low-dose cyclophosphamide has several immunomodulatory properties including: depletion of regulatory T cells; increased IFN production, induction of immunogenic cell death; increased CD8+ effector T cells; increased NK cell number and function.[10] Pre-clinically, low-dose cyclophosphamide diminished immunosuppressive TGF-β and IL-10 and had synergy with intra-tumoral type B CpG SD-101 in injected and uninjected tumors.[11] Preclinical synergy with PG545 has also been reported in a murine model of established lymphoma.[12] Clinically, low-dose metronomic cyclophosphamide has primarily been used in breast cancer, castrate-resistant prostate cancer, ovarian cancer and CRC at varying doses (50-100 mg daily) and schedules (daily, 1-week-on, 1-week-off). We hypothesize that the nivolumab/pixatimod combination in PD-1 relapsed/refractory (R/R) cutaneous melanoma and NSCLC patients will be associated with anti-tumor effects. We also hypothesize that the nivolumab/pixatimod/cyclophosphamide combination in microsatellite stable (MSS) metastatic colorectal carcinoma (mCRC) patients will be associated with anti-tumor effects. We further hypothesize that nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC patients and nivolumab/pixatimod combination in PD-1 R/R melanoma or NSCLC will be associated with increased $CD8^+$ T cells *intra-tumorally* and $PD\text{-}1^+Ki67^+$ $CD8^+$ T cells *peripherally* in responders *on treatment* compared to *baseline*; and that response will be associated with evidence of antigen-specific immunity responses intra-tumorally and peripherally.
Study Population:	Men or women, ≥18 years of age, with selected solid tumors and an Eastern Cooperative Oncology Group (ECOG) performance status of ≤1 and have at least 2 lesions with measurable disease as defined by Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1.

Objectives and Endpoints:

Objective	Endpoint
Primary • To assess efficacy of nivolumab/pixatimod combination in PD-1 R/R melanoma and NSCLC. • To assess efficacy of nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC patients	• Objective response rate by RECIST v1.1 to nivolumab/pixatimod combination in PD-1 R/R melanoma and NSCLC. • Objective response by RECIST v1.1 to nivolumab/pixatimod/ cyclophosphamide combination in MSS mCRC patients
Secondary • To assess safety of nivolumab/pixatimod combination in PD-1 R/R melanoma and NSCLC; and nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC patients • To assess efficacy using alternative response endpoints. • To assess CD8+ T cell infiltrate in pre- and on- treatment tumor samples by multiplex IHC/IF. • To assess median and landmark progression-free survival (PFS) and landmark overall survival (OS) in participants treated with nivolumab/pixatimod or nivolumab/pixatimod/ cyclophosphamide	• Incidence of all-grade related adverse events (AE), serious adverse events (SAE) and dose-limiting toxicities (DLT) if any in each cohort. • Objective response by iRECIST to nivolumab/pixatimod/ cyclophosphamide combination in MSS mCRC patients and nivolumab/pixatimod combination in PD-1 R/R melanoma and NSCLC. • Summary measures of change (or % change) from baseline in the CD8+ T cell or NK cell infiltrate in the tumor and TME in tumor biopsies at pre- and on- (approximately 4 weeks) treatment timepoints. • Median PFS, landmark PFS (6 months, 1-year and 2-year), median OS and landmark OS (1-year, and 2-year) in participants treated with nivolumab/pixatimod or nivolumab/pixatimod/ cyclophosphamide
Exploratory • To explore potential association between biomarkers and clinical efficacy of nivolumab/pixatimod or nivolumab/pixatimod/ cyclophosphamide combination	• Correlation/measures of association of biomarkers and clinical effect of nivolumab/pixatimod or nivolumab/pixatimod/ cyclophosphamide combination

Overall Study Design:	This is a study of nivolumab in combination with pixatimod (PG545, hereafter referred to as pixatimod) in 3 separate cohorts: • Cohort 1: MSS mCRC in combination with low-dose cyclophosphamide • Cohort 2: PD-1 relapsed/refractory melanoma • Cohort 3: PD-1 relapsed/refractory NSCLC. Each cohort comprises a two-stage Simon phase IIA trial. The goal of this study is to assess response of PD-1 R/R melanoma/NSCLC to nivolumab and pixatimod; and MSS mCRC to nivolumab, pixatimod and low-dose cyclophosphamide.
Study Evaluation:	Participants meeting enrollment criteria will be enrolled depending on histology into one of 3 cohorts (cohort 1: MSS mCRC; cohort 2: PD-1 R/R melanoma; cohort 3: PD-1 R/R NSCLC).
Number of Participants:	In the 1st stage of cohort 1 (MSS mCRC), we will enroll 13 patients. If ≥1 response(s) are seen, 14 additional patients will be enrolled in the 2nd stage. The nivolumab/pixatimod/cyclophosphamide combination will be considered worthy of further evaluation if ≥4 responses (ORR 15%) are seen across both stages in this cohort. For cohorts 2 (PD-1 R/R melanoma) and 3 (PD-1 R/R NSCLC), we will use the same design, but separately, as follows. In the 1st stage of each cohort, will enroll 9 patients. If ≥1 response(s) are seen, 8 additional patients will be enrolled in the 2nd stage. The combination will be considered worthy of further evaluation if ≥3 responses (18%) are seen across both stages in either (or both) cohorts.
Treatment Arms and Duration:	There are 3 cohorts in this study: cohort 1: MSS mCRC; cohort 2: PD-1 R/R melanoma; cohort 3: PD-1 R/R NSCLC. Nivolumab has demonstrated clinical activity in participants with melanoma and NSCLC. However, single-agent nivolumab has negligible efficacy in PD-1 R/R melanoma/NSCLC and MSS mCRC. Treatment will be assigned based on histology. Combination therapy will be administered until toxicity/discontinuation or completion of 2 years of combination treatment.
Study Assessments and Analyses	Primary Endpoint • Objective response by RECIST v1.1 to nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC (cohort 1) patients and nivolumab/pixatimod combination in PD-1 R/R melanoma and NSCLC (cohorts 2 and 3). Secondary Endpoints • Objective response by iRECIST to nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC (cohort 1) patients and nivolumab/pixatimod combination in PD-1 R/R melanoma and NSCLC (cohorts 2 and 3). • Summary measures of change (or % change) from baseline in the CD8+ T cell and NK cell infiltrate in the tumor and TME in tumor biopsies at pre- and on- (approximately 4 weeks) treatment timepoints. • Median PFS, landmark PFS (6 months, 1-year and 2-year), median OS and landmark OS (1-year, and 2-year) in participants treated with nivolumab/pixatimod or nivolumab/pixatimod/cyclophosphamide. Exploratory Endpoints • Correlation/measures of association of biomarkers and clinical effect of nivolumab/pixatimod or nivolumab/pixatimod/cyclophosphamide combination.

	Safety Outcome Measures • Adverse events (AE) will be assessed continuously during the study and for 100 days after the last dose of study treatment. • AEs will be graded using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. Efficacy Measurements • Disease assessment with computed tomography (CT) and/or magnetic resonance imaging (MRI), as appropriate, will be performed at baseline and every 8 weeks (± 7 days) until disease progression, at the completion of follow-up, or until participants withdraw from the study. Tumor responses will be derived for evaluable participants as defined by RECIST Version 1.1 based on recorded tumor measurements. • Serologic tumor markers will be collected, as appropriate. • At the Sponsor-Investigator's discretion, scans and measurements may be collected and reviewed by independent radiologists at a later date, or at any time during the study. Biomarker Measures: • A broad translational plan to evaluate predictive biomarkers for combination therapy (see **Section 8.9.1**). Analysis of interest may include, but are not limited to: • Systemic Immunity: o Serial peripheral blood samples will be obtained for immuno-monitoring by multiplex flow-cytometry and proteomic analysis of immunologically relevant soluble factors. • Tumor Microenvironment: o Multiplex IF and IHC to evaluate tumor microenvironment and to assess relationships between baseline and on-treatment immune contexture. o Transcriptome analysis (to understand gene expression signatures and pathways that mediate response, resistance and toxicity). o Whole exome sequencing (to understand the relationship between driver mutations and response, resistance and toxicity, and to understand the relationship between mutational load and neo-antigens to response). o Peripheral blood circulating tumor deoxyribonucleic acid (ctDNA) or cell-free DNA (cfDNA; to compare this technology to findings derived directly from the tumor). • Host Status o Whole blood whole-genome sequencing (WGS) or whole exome sequencing (WGS; to assess for the relationship between germline single nucleotide polymorphisms with response, resistance and toxicity, and for making mutational calls on the tumor DNA sequencing). o Stool for microbiome (to assess the impact of fecal metagenomics on IO response, resistance and toxicity).
Statistical Considerations	<u>Sample size calculation:</u> Stage 1 (**cohort 1** – 13 patients; **cohorts 2/3** – 9 patients). Stage 2 (**cohort 1** – 14 patients; **cohorts 2/3** – 8 patients). Total enrollment for 1st stage across all 3 cohorts = 31. Total enrollment for both stages across all 3 cohorts = 61. <u>Sample size justification:</u>

	In the 1st stage of **cohort 1** (**MSS mCRC**), we will enroll 13 patients. If ≥1 response(s) are seen, 14 additional patients will be enrolled in the 2nd stage. The nivolumab/pixatimod/cyclophosphamide combination will be considered worthy of further evaluation if ≥4 responses (ORR 15%) are seen across both stages in this cohort. This study design, using Simon's 2-stage Minimax design, provides 80% power with 5% type I error to detect a response rate of 20% against a null hypothesis of 5%. For **cohorts 2** (**PD-1 R/R melanoma**) and **3** (**PD-1 R/R NSCLC**), we will use the same design, but separately, as follows. In the 1st stage, will enroll 9 patients. If ≥1 response(s) are seen, 8 additional patients will be enrolled in the 2nd stage. The combination will be considered worthy of further evaluation if ≥3 responses (ORR 18%) are seen across both stages. This study design, using Simon's optimal 2-stage design, provides 80% power with 5% type I error to detect a response rate of 25% against a null hypothesis of 5%. <u>Statistical plan:</u> In the interest of maximizing safety while minimizing exposure to a potentially ineffective combination, we have designed parallel two-stage phase II studies in three cohorts. We hypothesize that nivolumab/pixatimod combination (and low-dose Cy in MSS mCRC) will increase the frequency of responding patients in each cohort. Although the nivolumab/pixatimod combination is being evaluated in advanced studies and DLT of pixatimod has been established in a prior phase I study,[8] the nivolumab/PG545/Cy combination has not been evaluated. However, given the Cy dose being studied, no DLTs are expected. To mitigate toxicity, we will monitor toxicities continuously (cohort 1) and implement a 4-week DLT monitoring period during the enrollment of the first 3 patients across cohorts 2-3. During this period, only 1 patient will be enrolled every 4 weeks. If no DLT occurs for the first 3 patients during the DLT monitoring period, this enrollment cap will be lifted.

2. SCHEDULE OF ACTIVITIES

Procedure/ Assessment	Screening (Day -28 to -1)[A]	Treatment Cycles[B]											End of Treatment[C]	30 Day Follow-Up[D]	Post-Treatment Surveillance[E]
		Cycle 1 Day 1	Cycle 1 Day 2	Cycle 1 Day 4	Cycle 1 Day 8	Cycle 1 Day 15	Cycle 1 Day 22	Cycle 2 Day 1	Cycle 2 Day 8	Cycle 2 Day 15	Cycle 2 Day 22	Cycle 3+			
Visit Windows		None	None	None	± 2 days	± 2 days	± 2 days	± 2 days	± 2 days	± 2 days	± 2 days	± 2 days	± 7 days	± 7 days	± 14 days
Nivolumab[F]		X						X (q4 weeks)				X (q4 weeks)			
Pixatimod (PG545)[G]		X			X	X	X	X	X	X	X	X (weekly)			
Cy[H] (**Cohort 1 CRC only**)		X_H						X_H				X_H			
Informed consent	X														
Eligibility criteria assessment	X														
Physical exam including vital signs[I]	X	X						X				X (D1)	X^C	X^D	X^E
Vital signs[I]					X	X	X		X	X	X	X (D 8/15/22)			
Electrocardiogram (ECG)[J]	X	X													
Laboratory assessments including screening labs[K]	X_{K-S}	X_{K-B}				X_{K-T} (D15 only)		X_{K-B}		X_{K-T} (D15 only)		X_{K-B} (D1 only) and X_{K-T} (D15 only)		X^D	X^E
Serum/urine pregnancy test[L]	X														
Pixatimod pharmacokinetic (PK) sampling[M]		X_{M-1}	X_{M-1}	X_{M-1}	X_{M-1}										
Cy PK sampling[N]		X_{N-1}	X_{N-1}												
Tumor biopsy[O]	X_{O-S}							X_{O-O}					X_{O-P}		
Exploratory biomarker analyses[P]	X				X	X		X (C2D15, C2D22)		X (C2D15, C2D22)		X (q8 weeks)	X		
Stool sampling[Q]		X									X	X (q8 weeks)	X		
Dietary history questionnaire[R]		X													

Disease assessment[S]	X											X (q8 weeks starting with C3D1)			X[E]

Study Calendar Notes:

[A]Screening period is 28 days (≤4 weeks) prior to first dose of pixatimod/nivolumab.

- Screening studies including magnetic resonance imaging (MRI)/computed tomography (CT) imaging must be performed ≤4 weeks prior to first dose of pixatimod/nivolumab**.**
- During Screening, MRI brain with contrast (or CT equivalent) should be performed to exclude CNS metastatic disease for melanoma and NSCLC patients.
- CNS imaging is not required for MSS mCRC patients at Screening unless patient is symptomatic.
- CNS imaging need not be repeated on study unless patient is symptomatic.
- CNS imaging should be repeated at investigator's discretion in patients with a history of treated CNS metastases.

[B]Treatment cycle

- Each cycle is 28 days long.

[C]End-of-treatment (EOT) assessments to be performed within 7 days following removal of subject from pixatimod treatment. Removal of a subject from pixatimod treatment is defined as the time in which the Investigator decides to discontinue pixatimod treatment for a subject.

- Management of patients who develop unacceptable toxicity is defined in **Section 8.5.4**.
    - Patients who develop unacceptable toxicity deemed related to pixatimod (or if applicable, cyclophosphamide) but not nivolumab may continue Nivolumab (and if applicable cyclophosphamide) after pixatimod discontinuation for duration as defined in **Sections 7.3.2 and 7.4.2**.
    - Patients who develop unacceptable toxicity deemed related to nivolumab but not pixatimod must discontinue both pixatimod and nivolumab.

[D]30-day follow-up.

- This begins +30 days following completion of last dose of study treatment *or* EOT, whichever is earlier.
- Procedures to be followed during this phase are outlined in **Section 8.5.3** and will include a visit, labs (CBC, CMP, TSH, free T3, free T4) and any other studies deemed necessary at the discretion of the treating physicians and according to established Standard of Care.

[E]Post-treatment surveillance.

- This phase begins upon completion of 30 day follow up.
- Procedures to be followed during this phase are outlined in **Section 8.8.5** will include imaging (CT or PET/CT), labs (CBC, CMP, TSH, free T3, free T4) and any other studies deemed necessary at the discretion of the treating physicians and according to established Standard of Care.
- Note – Patient accrual was terminated early owing to the discontinuation of pixatimod supply. Follow-up assessments will no longer be completed upon IRB approval of protocol version 02/23/2024.

[F]Nivolumab dosing

- Nivolumab will be dosed at 480 mg q4 weeks IV.

[G]Pixatimod

- Pixatimod will be dosed 25 mg weekly IV.
- Pixatimod will be administered through either a large-bore IV line placed in the antecubital fossa or central venous catheter.
- Patients in whom pixatimod is administered through a small-bore/distal IV may developed a delayed-type hypersensitivity reaction.
    - This should not be treated as an infusion reaction and should recur upon the administration through a proximal large-bore IV line.

[H]Cyclophosphamide dosing (**Cohort 1 only**)

- 50 mg PO twice daily (D1-D7; D15-D21) with a 7-day drug free interval (D8-D14 and D22-D28).
- Cyclophosphamide dose compliance will be assessed using a pill diary.

[I]Physical exams will be conducted at Screening, each pixatimod treatment visit, EOT, 30-day follow-up and during Post-treatment surveillance.

- Screening physical exam may be performed up to 72 hours prior to the Cycle 1 Day 1 visit.
- Physical exams should be repeated on each D1 visit and should include assessment of ECOG performance status. Requirements are defined in **Section 8.4.2.**

- On D8/D15/D22 for all cycles, a formal physical exam is not required and only vital signs will be recorded.
- Height will be recorded at Screening visit only.

[J]Electrocardiograms (ECGs) should be obtained at Screening and prior to administration of pixatimod C1D1.

- ECG need not be repeated unless clinically indicated at subsequent visits.

[K]Laboratory assessments

- Laboratory assessments to be obtained at Screening (X_{K-S}): CBC, renal and liver function, coagulation parameters, infectious parameters (HIV, Hep B/C screening), thyroid function (TSH, free T3 and free T4), and urinalysis.
    - **NOTE:** In patients who are currently or have recently (within 6 months) been on heparin, low-molecular weight heparin, fondaparinux or other similar agents, anti-heparin antibodies should be obtained during **Screening.** Per **Section 6.2.3**, the presence of anti-heparin antibodies is exclusionary.
- Laboratory assessments to be obtained on Baseline (Cycle 1 Day 1; X_{K-B}): CBC, renal and liver function, coagulation parameters, fasting lipids, thyroid function (TSH , free T3 and free T4) and urinalysis.
- Laboratory assessments to be obtained on On-Treatment (X_{K-T}): CBC, renal and liver function, fasting lipids (**D1** of each cycle only starting with **C2D1**) coagulation parameters, and urinalysis.
- There is a 48-hour window to obtain Screening (X_{K-S}), Baseline (X_{K-B}) and/or On-Treatment (X_{K-T}) laboratory assessments.

[L]Pregnancy testing

- Only required at screening in women of childbearing potential (WOCP).

[M]Pixatimod PK sampling

- Pixatimod PK timepoints (relative to pixatimod infusion):
    - 1 – to be performed at **pre-dose**, **30min** (± 5 min), **2h** (± 15 min), **4h** (± 15 min), **6h** (± 30 min), **24h** (D2; ± 60 min), **72h** (D4; ± 24 hours) and **168h** (prior to C1D8; ± 24 hours) post infusion;
    - Windows:
    - Pixatimod PK sampling will be collected from **10 subjects** in **Cohort 1**, and a **total** of **10 subjects** from **Cohorts 2 and 3**.
- Samples to be collected as delineated in **Section 8.10.1** and will be processed as delineated in the **Laboratory Manual.**

[N]Cyclophosphamide PK sampling

- Cyclophosphamide PK timepoints (relative to pixatimod infusion):
    - 1 – to be performed at **pre-dose**, **10min** (± 5 min), **30min** (± 5 min), **1h** (± 10 min), **2h** (± 15 min), **4h** (± 15 min), **6h** (± 30 min), and **24h** (D2; ± 60 min) post cyclophosphamide;
    - Cyclophosphamide PK sampling will be collected from **10 subjects** in **Cohort 1 only**.
- Samples to be collected as delineated in **Section 8.10.2** and will be processed as delineated in the **Laboratory Manual.**

[O]Tumor biopsy must be performed prior to commencing Pixatimod/Nivolumab treatment.

- Punch, core (16 gauge) and/or surgical biopsies are acceptable.
  If core biopsy is performed, pathology confirmation is required to ensure adequate tissue is obtained; and a minimum of 6 cores must be obtained.
- Tumor biopsies will be obtained at Screening (X_{O-S}), on-treatment at C2D1 (X_{O-O}) and at progression (if applicable; X_{O-P}).

[P]Exploratory biomarker analyses will be obtained as follows:

- Prior to administration of pixatimod/nivolumab at Screening, on C1D1, C1D8, C1D15, C2D1, C2D15, C2D22 in Cycles 1-2.
- Cycles 3 onwards: q8 weekly.
- Samples to be collected as delineated in **Section 8.9** and will be processed as delineated in the **Laboratory Manual**.

[Q]Stool sampling will be obtained as follows:

- Prior to administration of pixatimod/nivolumab on C1D1 and C2D22.
- Cycles 3 onwards: q8 weekly.
- EOT
- Samples will be processed as delineated in the Laboratory Manual.

[R]Dietary history questionnaire will be on C1D1. Dietary questionnaire is detailed in **Appendix 6.**

[S]CT or MRI scans to assess tumor status will be obtained at Screening (baseline value should be performed ≤4 weeks prior to first pixatimod dose).

- Screening: Scans should be performed ≤4 weeks prior to first pixatimod dose. CT (or PET/CT or MRI) can be obtained at Investigator discretion, however, contrast-enhanced CT is preferred.
- Cycles 1-2 (+/- 7 days): Contrast-enhanced CT (or PET/CT) at **C3D1**.
- Cycles 3+ (+/- 7 days): Contrast-enhanced CT (or PET/CT) every 8 weeks.
- Progression: Progression should be confirmed with repeat imaging study done no sooner than 4 weeks after prior study.

3. INTRODUCTION

3.1 Study Rationale

PD-1 is a receptor expressed by activated T- and B- cells which binds to two known ligands: PD-L1 (B7-H1)[13,14] and PD-L2 (B7-DC).[15,16] PD-1 negatively regulates T cell functions through the engagement of PD-L1, which is expressed by a wide variety of tissues.[13-16] PD-L1 is also expressed by human tumors, including melanoma, either constitutively or after treatment with IFN-α.[17,18] In chronically infected mice, lymphocytic choriomeningitis virus-specific CD8+ T-cells exhibit a diminished capability to produce cytokines, lyse infected cells and proliferate in a progressive and hierarchical fashion.[19] These "exhausted" T-cells have been shown to up-regulate PD-1, and blockade of the PD-1/PD-L1 pathway led to increased cytokine production and proliferation, resulting in a significant reduction of the viral load.[19]

We have previously shown that majority of tumor antigen-specific CD8+ T cells upregulate PD-1 expression, which is associated with T cell exhaustion/dysfunction in chronic viral infections in animals and humans.[20,21] We observed that PD-1 upregulation on spontaneous tumor antigen-specific CD8+ T cells occurs along with T cell activation and is not directly associated with an inability to produce cytokines *ex vivo* upon stimulation with cognate antigen. Blockade of the PD-1/programmed death ligand 1 (PD-L1) pathway in combination with prolonged antigen stimulation with PD-L1+ antigen-presenting cells or melanoma cells augmented the frequencies of cytokine-producing, proliferating and total tumor antigen-specific NY-ESO-1 CD8+ T cells.[20,21] Collectively, these findings support that PD-1 is a regulator of antigen-specific CD8+ T cell expansion in the context of chronic antigen exposure in patients with advanced cancer; and that PD-1 blockade reverses tumor-mediated T cell dysfunction.

Immune checkpoint inhibitors (ICI) directed against PD-1/PD-L1 are associated with improved response and survival rates in multiple malignancies including melanoma, squamous/non-squamous lung cancer, bladder and renal cell carcinoma.[4,6,22-25] In advanced melanoma and PD-L1 expressing NSCLC, anti-PD-(L)1 monotherapy produces overall response probabilities of 42%-45%, the majority of which are durable.[1-6]

In Checkmate-067, dual anti-PD-1 and anti-CTLA-4 blockade with nivolumab and high-dose ipilimumab (3mg/kg every 3 weeks for 4 doses) further improved ORR (58%) in melanoma compared to either nivolumab or ipilimumab, albeit with significantly greater toxicity.[26-29] Ipilimumab/nivolumab was associated with five year progression-free survival (PFS) of 36%, compared with 29% (nivolumab) and 8% (ipilimumab).[28] Checkmate-227 compared low-dose ipilimumab/nivolumab combination (nivolumab 2mg/kg every 2 weeks; ipilimumab 1mg/kg every 6 weeks) to nivolumab monotherapy and platinum-doublet chemotherapy in PD-L1≥1% NSCLC. Ipilimumab/nivolumab combination produced slightly better response compared to chemotherapy (36% vs. 30%), with commensurately improved PFS and overall survival (OS) and led to regulatory approval.[30]

While anti-PD(L)1 blockade singly and in combination is remarkably efficacious in melanoma and NSCLC, ICI therapy is minimally efficacious in microsatellite stable (MSS) CRC. The remarkable efficacy of PD-1/PD- L1 or PD-1/CTLA-4 blockade in CRC in limited to hyper-mutated subtypes – specifically those with microsatellite instability-high (MSI-H)/mismatch repair-deficient (dMMR) CRC or those with POLE mutations.[31,32] In these patients, the response to ICI is related to increased TIL density secondary to immunogenic neoepitopes and increased tumor mutational burden (TMB). However, MSI-H tumors constitute
~3% of all CRC and ICI therapy is minimally effective in MSS mCRC.[33]

Biomarkers of response to PD-(L)1 blockade include CD8+ TIL,[34,35] interferon gamma (IFN-γ) gene expression,[36,37] high tumor mutation burden (TMB; [37-39]) or PD-L1 expression.[40,41] These observations suggest that uninflamed tumors represent the bulk of tumors that fail to respond to anti-PD(L)1 ICB; and argue for augmenting inflammation as a means to promote T cell responses in PD-1 relapsed/refractory (R/R) melanoma and PD-1 R/R NSCLC and MSS mCRC.

Tumor-associated DC are critical regulators of the balance between CD8+ T cell immunity versus tolerance to tumor antigens. There are 3 major DC subsets in humans: CD141+ myeloid/conventional DC1 (cDC1), CD1c+/CD11b+/BDCA-1+ myeloid/conventional DC2 (cDC2) and CD123+/BDCA-2+/BDCA-4+ plasmacytoid DC (pDC). Of these, both CD141+ cDC1 and CD123+/BDCA-2+/BDCA-4+ pDC are critical for CD8+ T cell priming and efficacy of PD-1 blockade.[42] TLR9 agonists activate pDCs to secrete type I IFN and to express increased levels of co-stimulatory molecules such as CD80 (B7.1) and CD86 (B7.2). TLR9 agonism results in a range of secondary effects, including secretion of cytokines such as MCP-1, IP-10/CXCL10 that activate NK cells and expand T cell subpopulations including CD8+ T cells and TH1 cells.[43] However, TLR9 agonists are limited as they have to be administered intra-tumorally as systemic administration of TLR9 agonists results primarily in liver, spleen, and RES uptake with little specific activation of pDC in draining lymph nodes; and immature tumor-associated pDC contribute to tumor growth and an adverse prognosis in patients with cancer.[44,45] Hence, systemically administered agents may overcome some of limitations of intra-tumoral innate immune agonists such as TLR9 and STING agonists, promoting antigen presentation to T cells, facilitating cross-priming and augmenting adaptive T cell responses in cancer.

3.2 Pharmaceutical and Therapeutic Background: Nivolumab

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades.[46] The inability of the immune system to control tumor growth does not appear to result from an inability to recognize the tumor as foreign. Tumor cells have been shown to evade immune destruction despite displaying recognizable antigens on their surface and despite the presence of high-avidity T cells that are specific for these antigens.[47-50] Histologic evaluation of many human cancers show extensive infiltration by inflammatory and immune cells,[51,52] suggesting that the immune system responds less effectively to malignancy. These observations have led to the hypothesis that dominant mechanisms of immune tolerance or immune suppression are responsible for the immune system's inability to effectively respond in a way that consistently results in rejection.

There are a number of inhibitory mechanisms that have been identified to be involved in tumor-mediated immune suppression and include expression of the programmed death ligand-1 (PD-L1), which can engage the inhibitory receptor PD-1 on activated T cells; the presence of the tryptophan-catabolizing enzyme IDO1, which exploits the exquisite sensitivity of T cells to tryptophan depletion and tryptophan metabolites; and infiltration with FoxP3+ regulatory T cells (Treg), which can mediate extrinsic suppression of effector T-cell function. Therefore, agents that target these negative regulatory pathways and thereby allow the expansion of effector T cells present in the tumor may be beneficial in the clinic.

The PD-1 receptor-ligand interaction is a major pathway stimulated by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. Programmed death receptor-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2).[14,15,17] The mechanism by which PD-1 down modulates T-cell responses is similar to but distinct from that of CTLA-4, as both molecules regulate an overlapping set of signaling proteins.[53,54]

Programmed death (PD) receptor-1 has been shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T cells, B cells, Tregs, and natural killer cells.[55] Expression has also been shown during thymic development on CD4-CD8- (double negative) T cells as well as subsets of macrophages and dendritic cells (DCs). The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors.[15,17,18,41,56,57] Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or

chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T cell expansion in subjects with melanoma.[20,21] This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Nivolumab is a fully human Ig G4 antibody that blocks PD-1. Nivolumab is approved for the treatment of metastatic melanoma following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor (Checkmate 037). Nivolumab is also approved for the treatment of advanced metastatic NSCLC of both squamous (Checkmate 017) and non-squamous (Checkmate 057) histologies following progression on or after platinum-based chemotherapy.

3.3 Pharmaceutical and Therapeutic Background: Pixatimod (PG545)

Pixatimod is a cholestanol-sulfotetrasaccharide conjugated small molecule compound that is a heparan sulfate (HS) mimetic with unique NK- and T cell- dependent immunomodulatory properties that is currently being evaluated in a variety of different tumors. Beyond anticoagulation, the therapeutic potential of heparin derivatives and heparan sulfate (HS) mimetics in oncology is related to their ability to bind and modulate the function of a vast array of HS-binding proteins with pivotal roles in cancer growth and progression.[7] Defining the structural and functional determinants of HS mimetics has clarified the structural requirements for the inhibitory effects of HS mimetics on heparanase, selectins, growth factor receptor signaling, immunomodulatory activities while limiting potential adverse effects.[7]

Pixatimod has an oligosaccharide backbone that is derived from starch, and retains the amylose structure of α(1 → 4)-linked glucose residues.[58] Coupling the sulfated oligosaccharide to a lipophilic cholestanol aglycone significantly increased the elimination half-life in vivo, while reducing the unwanted anticoagulant activity associated with similar compounds while retaining inhibition of HS-degrading enzyme heparanase-1 (HPSE).[58-61] As such, pixatimod has a half-life of approximately 141 hours (6 days) in humans, considerably greater than the 7-8 hours observed with most heparin mimetics - enabling weekly dosing.[58]

Pixatimod inhibits HPSE in a concentration-dependent manner; a function that is critical to its activity.[60,61] However, it is important to consider that pixatimod has significant immunomodulatory properties as well. In multiple orthotopic models, pixatimod inhibited infiltration of tumor-associated macrophages (TAMs).[62-65] TAMs contribute to cancer progression and therapeutic resistance by mediating an immunosuppressive TME and facilitating tumor metastases through multiple mechanisms including promotion of tumor cell extravasation, invasion, vascularization (see **Figure 3.3-1**).[66]

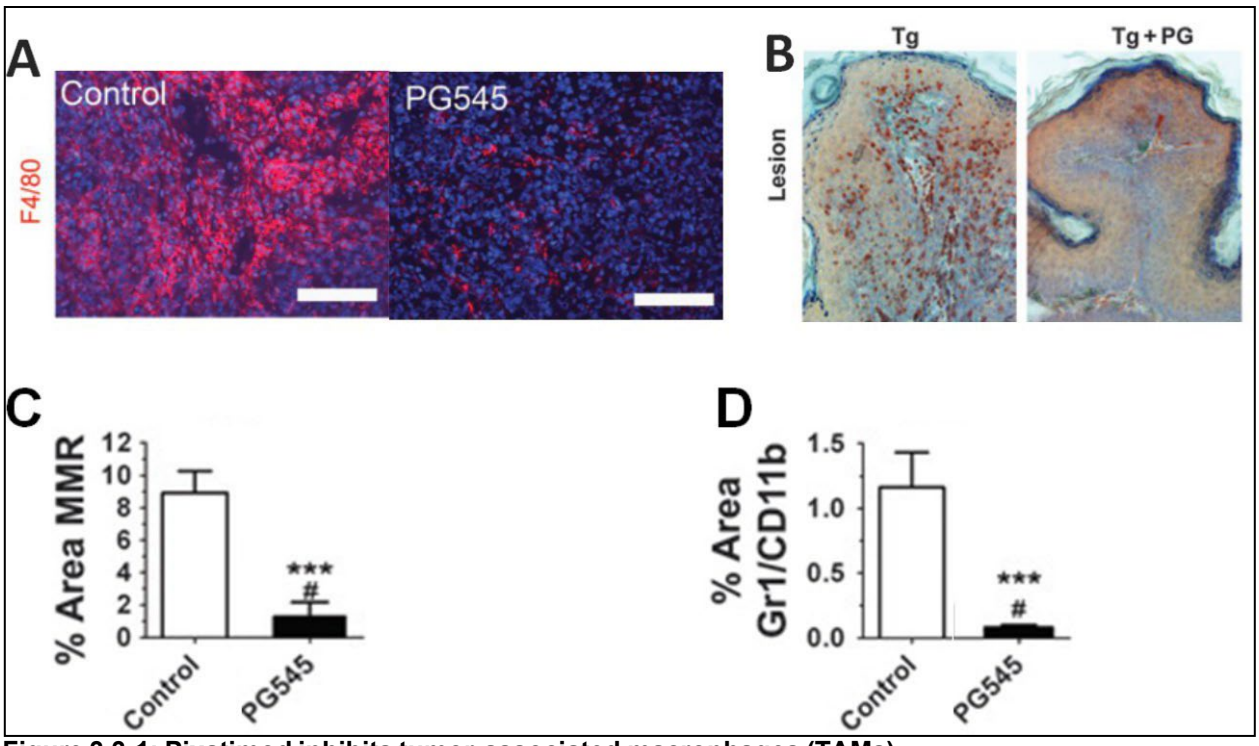

Figure 3.3-1: Pixatimod inhibits tumor-associated macrophages (TAMs).

Separately, the anti-tumor effects of pixatimod are NK-dependent, which in turn was found to be IL-12 and TLR9 dependent (see **Figure 3.3-2**).[12]

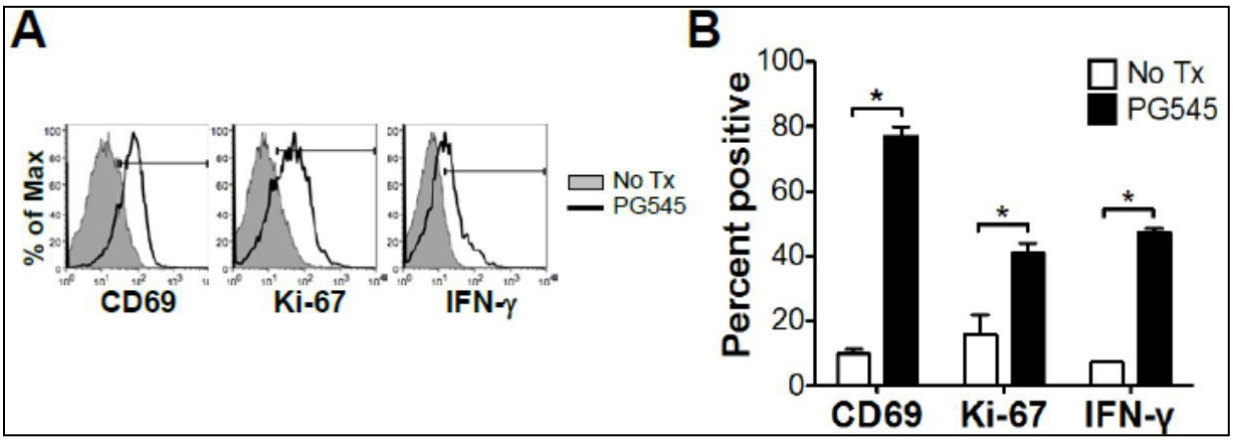

Figure 3.3-2: Activation of NK cells by pixatimod in tumor-bearing mice.

Pixatimod does not possess CpG ODN motifs and hence does not activate TLR9 directly. Rather, pixatimod increases CpG ODN accumulation in endosomal compartment of DCs, leading to enhanced production of IL-12, which is essential for pixatimod-mediated NK cell activation (see **Figure 3.3-3**).[12]

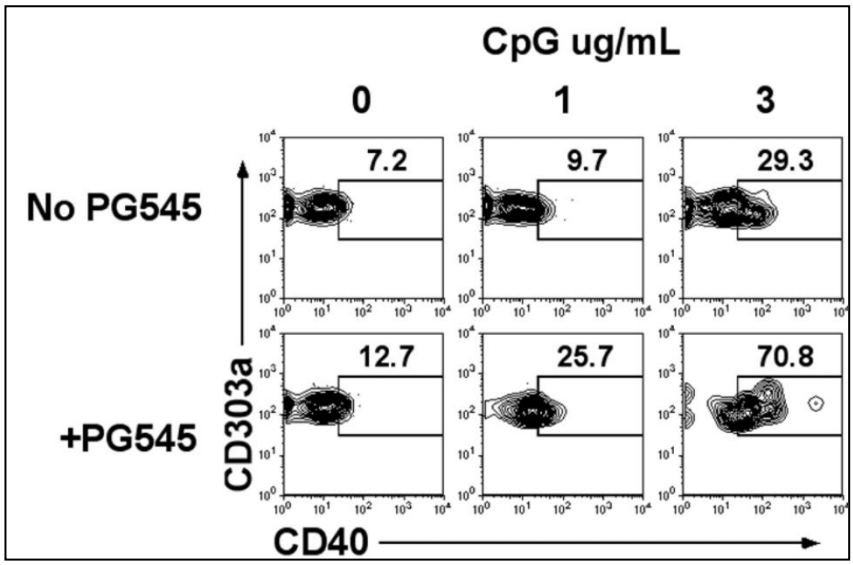

Figure 3.3-3: Pixatimod increases human PBMC-derived pDC activation (CD40) in response to 24- hour exposure to CpG ODN-2006 in vitro.

Preclinically, pixatimod potently inhibits solid tumor progression and metastasis in a number of syngeneic, orthotopic and xenograft murine models of cancer either singly[59,63,67-74] or in combination with chemotherapy such as paclitaxel or gemcitabine.[75,76] Pixatimod has demonstrated synergy with cyclophosphamide, although this may be immunodulatory given the effects of low-dose Cy on NK cell activation.[12]

3.4 Pharmaceutical and Therapeutic Background: Cyclophosphamide

Cy [*N,N*-bis (2-chloroethyl)-1, 3, 2-oxazaphosphinan-2 amine 2-oxide] is a nitrogen mustard alkylating agent from the oxazophorine group. Cyclophosphamide exists as a solid, soluble (in water), and a very weakly acidic compound (based on its pKa). It is administered orally or intravenously. Cyclophosphamide is activated in the liver, principally activated by cytochrome P450 isoforms, CYP2A6, 2B6, 3A4, 3A5, 2C9, 2C18, and 2C19, of which the CYP2B6 isoform has the highest 4-hydroxylase activity. Cy undergoes activation to eventually form active metabolites, phosphoramide mustard and acrolein.

As an alkylating agent, cyclophosphamide has three different independent effects: 1) attachment of alkyl groups to DNA bases, resulting in DNA being fragmented by repair enzymes as it attempts to replace the alkylated bases, preventing DNA synthesis and RNA transcription from the affected DNA; 2) DNA cross-linking which inhibits strand separation for replication and/or transcription; 3) induction of nucleotide mispairing. As a cytotoxic agent, cyclophosphamide is a key agent in combinations used in the treatment of breast cancer, lymphoma, leukemia, AL amyloidosis.

Pioneering work by Dr. Robert North and Dr. Judah Folkmak clarified that low-dose metronomic cyclophosphamide had immunostimulatory and antiangiogenic properties, paving the way for combinations with vaccines and other agents. While it is unclear how convergent and/or overlapping both these effects are with low-dose metronomic cyclophosphamide, what is clear is that these effects are predicated upon the following observations:

- Induction of a T_{H1}/T_{H2} to T_{H17} skew in cytokine production;[77,78]
- Depletion of CD4+ Foxp3+ regulatory T cells;[79,80]
- Enhancement of long-term survival and proliferation of lymphocytes;[81,82]
- Induction of pro-inflammatory cytokines including IL-1β, IL-7, IL-15, IL-2, IL-21, and IFN-γ;[83]

- Augmentation the function of dendritic cells (DC);[84-89] and natural killer (NK) cell number and function DC function.[79,90-92]

Low-dose cyclophosphamide has several immunomodulatory properties including: depletion of regulatory T cells;[79,80] augmentation of DC[84-89] and NK[79,90-92] cell function. The properties result in T_{H17} skew in cytokine production;[77,78] increased IFN production;[79,90-92] and induction of immunogenic cell death. [79,90-92] Pre-clinically, low-dose cyclophosphamide diminished immunosuppressive TGF-β and IL-10[93] and had synergy with intra-tumoral type B CpG SD-101 in injected and uninjected tumors.[11,93]

3.5 Preclinical and Clinical Trial Data: Nivolumab

Refer to the **Nivolumab Investigator's Brochure (IB)** for Preclinical and Clinical data.

3.6 Preclinical and Clinical Trial Data: Pixatimod (PG545)

Refer to the **Pixatimod IB** for Preclinical and Clinical data.

Preliminary safety and efficacy in advanced cancer patients

In early clinical studies, pixatimod was administered subcutaneously (SC), although following local injection site reactions, this was switched to intravenous (IV) infusion.[76] In the phase I dose-escalation study, escalating doses of pixatimod (25 mg, 50 mg, 100 mg, and 150 mg weekly) were administered IV in patients with advanced solid tumors.[8] Three dose-limiting toxicities (DLTs) were identified in the 150 mg cohort (2 instances of hypertension and 1 of epistaxis); and no DLTs were noted in the 100 mg cohort, which was identified as the maximum-tolerated dose (MTD).[8] Among 16 response-evaluable patients, no objective responses were reported; however, disease control rate (DCR) was 38% (6/16).[8] Pharmacokinetic exposure was proportional up to 100 mg with a mean serum half-life of 141h supporting once weekly administration.[8] Pharmacodynamic data revealed early increases in CD40+ pDC and NKp46+ NK cells; and increased concentration of IFNγ, TNF-α, CXCL10/IP-10 and MCP-1 in plasma.[8]

Preliminary safety and efficacy in combination with anti-PD(L)1 in advanced cancer patients

Pixatimod has been studied in combination with nivolumab in a phase IB study of advanced PD-1 naive solid tumors. In this study, patients with a variety of malignancies (pancreatic cancer, colorectal cancer, uterine adenosarcoma, squamous cell carcinoma, endometrial carcinoma, and adrenocortical carcinoma) were enrolled to receive escalating doses of pixatimod (25mg up to 50mg) IV weekly in combination with nivolumab 240mg IV q2 weekly.

A total of 58 patients were enrolled: 55 patients to 25 mg pixatimod/240 mg nivolumab and 3 patients to 50 mg pixatimod/240 mg nivolumab. Forty-eight (48) subjects withdrew due to disease progression (either confirmed per RECIST or unconfirmed clinical progression), seven due to adverse events (two in the 50 mg and five in the 25 mg pixatimod cohort), and two withdrew consent Three DLTs occurred. Two DLTs occurred in the 50 mg pixatimod cohort: multiorgan failure (assessed as related to pixatimod and nivolumab), and pulmonary oedema (assessed as related to pixatimod only). The multiorgan failure resulted in the subject's death. The 50 mg pixatimod/240 mg nivolumab dose was declared a toxic dose. One DLT occurred in the 25mg cohort: pneumonitis (assessed as related to pixatimod and nivolumab). A summary of treatment-emergent adverse events (TEAEs) is presented in **Table 3.6-1**. Given that only three subjects were recruited to the 50 mg pixatimod/240 mg nivolumab, only the 25 mg pixatimod/240 mg nivolumab is presented.

Table 3.6-1:
TEAE Events Possibly, Probably, or Likely related to Pixatimod or P ixatimod/Nivolumab – Occurring in Three or More Subjects (Safety Set)[1]

Event Type	25 mg/240 mg[2] N = 55
General disorders and administration site conditions	
Fatigue	12 (21.8) [17]
Pyrexia	7 (12.7) [8]
Chills	3 (5.5) [3]
Gastrointestinal disorders	
Nausea	14 (25.5) [19]
Diarrhoea	10 (18.2) [16]
Metabolism and nutrition disorders	
Decreased appetite	8 (14.5) [9]
Investigations	
Alanine aminotransferase increased	5 (9.1) [6]
Aspartate aminotransferase increased	4 (7.3) [6]
Skin and subcutaneous tissue disorders	
Pruritus	6 (10.9) [6]
Respiratory, thoracic and mediastinal disorders	
Pneumonitis	3 (5.5) [3]

[1] 50 mg Pixatimod/240 mg Nivolumab data not presented as only three subjects were recruited.
[2] For any given adverse event, the results are presented as the number of patients with the event: n, the proportion of patients with the event: (%), and the number of events: [e].

Preliminary data from 26 evaluable patients were previously reported: 3 MSS mCRC patients had objective responses, 7 had SD with 12% ORR and 38% disease control rate (DCR).[9] Based on this study, the recommended phase II dose of pixatimod in combination with anti-PD(L)1 blockade is 25 mg IV weekly.

A summary of severe treatment related adverse drug reactions for both cohorts is presented in **Table 3.6-2**.

Table 3.6-2:
Severe Treatment-Emergent Adverse Events, Possibly, Probably, or Likely related to Study Drug by MedDRA SOC and Prefered Term (Safety Set)

Event Type	25 mg/240 mg[1] n (%) N = 55		50 mg/240 mg[1] n (%) N = 3	
Causal Agent	**Pixatimod**	**Pix/Nivo**	**Pixatimod**	**Pix/Nivo**
General disorders and administration site conditions	0 (0%) [0]	0 (0%) [0]	0 (0%) [0]	1 (33%) [1]
Multiple organ dysfunction syndrome				
Fatigue	0 (0%) [0]	1 (2%) [1]	0 (0%) [0]	0 (0%) [0]

Gastrointestinal disorders				
Diarrhoea	0 (0%) [0]	2 (4%) [2]	0 (0%) [0]	0 (0%) [0]
Colitis	0 (0%) [0]	1 (2%) [1]	0 (0%) [0]	0 (0%) [0]
Metabolism and nutrition disorders				
Hyponatremia	0 (0%) [0]	1 (2%) [3]	0 (0%) [0]	0 (0%) [0]
Investigations				
Aspartate aminotransferase increased	0 (0%) [0]	1 (2%) [1]	0 (0%) [0]	0 (0%) [0]
Vascular disorders				
Hypertension	2 (4%) [6]	0 (0%) [0]	0 (0%) [0]	0 (0%) [0]
Nervous system disorders				
Encephalopathy	0 (0%) [0]	1 (2%) [1]	0 (0%) [0]	0 (0%) [0]
Respiratory, thoracic and mediastinal disorders				
Pneumonitis	0 (0%) [0]	1 (2%) [1]	0 (0%) [0]	0 (0%) [0]
Pulmonary oedema	0 (0%) [0]	0 (0%) [0]	1 (33%) [1]	0 (0%) [0]
Hepatobilliary disorders				
Autoimmune hepatitis	0 (0%) [0]	1 (2%) [1]	0 (0%) [0]	1 (33%) [1]
Cardiac disorders				
Cardiomyopathy	0 (0%) [0]	0 (0%) [0]	0 (0%) [0]	1 (33%) [1]

[1] For any given adverse event, the results are presented as the number of patients with the event: n, the proportion of patients with the event: (%), and the number of events: [e].

Fifty-three (53) serious adverse events were reported in 30 subjects; 13 events were assessed as being possibly/probably, likely, or certainly related to the combination. Treatment-related serious adverse events comprised: multiorgan failure, pulmonary oedema, cardiomyopathy, and autoimmune hepatitis in the 50 mg pixatimod cohort; autoimmune hepatitis, pneumonitis (three events in two subjects), encephalopathy, diarrhea, fever (two events in one subject), and transaminitis in the 25 mg Pixatimod cohort.

3.7 Preclinical and Clinical Trial Data: Cyclophosphamide

Low-dose cyclophosphamide has several immunomodulatory properties including: depletion of regulatory T cells;[79,80] augmentation of DC[84-89] and NK[79,90-92] cell function. The properties result in T_{H17} skew in cytokine production;[77,78] increased IFN production;[79,90-92] and induction of immunogenic cell death. [79,90-92] Pre-clinically, low-dose cyclophosphamide diminished immunosuppressive TGF-β and IL-10[93] and had synergy with intra-tumoral type B CpG SD-101 in injected and uninjected tumors.[11,93]

Clinically, low-dose (metronomic) cyclophosphamide has primarily been used in breast cancer, castrate-resistant prostate cancer, ovarian cancer and CRC at varying doses (50-100 mg daily) and schedules (daily, 1-week-on, 1-week-off).[94] Two single-arm studies studied cyclophosphamide and PD-1 blockade in sarcomas and reported limited activity with unusual toxicity signals.[95,96] In these studies, Cy was dosed at 50 mg twice daily, 1-week-on, 1-week-off.

3.8 Rationale

3.8.1 Rationale for Combining Pixatimod (PG545) with Anti-PD(L)1 Antibody

The activity of pixatimod is complementary to that of anti-PD(L)1 antibodies in the treatment of malignancies. Pixatimod has the dual functions of activating NK cells via DC,[12] and inactivating tumor-infiltrating

macrophages by inhibiting heparanase.[63,65] In a syngeneic breast cancer model, pixatimod in combination with anti-PD-1 improved anti-tumor control compared to anti-PD-1 antibody alone.[97] Examination of the tumors from this study revealed that the combination significantly increased CD8 and CD4 effector memory T cells and NK cell infiltration into the tumors supporting the complementarity of these agents.[97] Separately, a phase Ib study of the combination of pixatimod with nivolumab showed promising results in MSS mCRC, a cancer type that had previously demonstrated negligible response to PD-1 therapies.[9]

3.8.2 Rationale for Limiting Enrollment by an Immune-inflammatory Biomarker Cutoff

Immune-inflammatory biomarkers (IIBs) showed a prognostic relevance in patients with multiple cancers treated with immuno-oncologic agents. Several blood-based, easily obtained, IIBs have demonstrated prognostic relevance in the advanced setting in cancer patients across multiple histologies including: neutrophil-to-lymphocyte ratio (NLR),[98-100] platelet and monocyte counts,[101] and systemic immune-inflammation index (SII) (based on lymphocyte, neutrophil and platelet counts, but not monocytes).[102,103]

The pan-immune-inflammation (PIV) is a weighted interaction of both the inflammatory pro-tumor (i.e. neutrophils, platelets and monocytes) and anti-tumor (i.e. lymphocytes) cellular populations and is calculated as follows:

PIV = [neutrophil count (10^3/mmc) × platelet count (10^3/mmc) × monocyte count (103/mmc)]/lymphocyte count (10^3/mmc).

PIV is a strong predictor of survival outcomes with better performance than other well-known immune inflammatory biomarkers in patients with 1L mCRC,[104] melanoma.[105] As such, based on literature data, the PIV cutoff utilized in this study is 1200 obtained at any time during **Screening in the MSS mCRC cohort only (cohort 1)**.

3.8.3 Rationale for Adding Low-dose Cy in MSS mCRC

The addition of cyclophosphamide to the pixatimod-anti-PD(L)1 combination in MSS mCRC may provide benefit via two mechanisms. Firstly, Cy selectively targets suppressive T regs which may alleviate the immunosuppressive environment of the liver, the main site to which CRC metastases spread.[106-108] Secondly, treatment with cyclophosphamide may destroy some tumor cells releasing damage-associated molecular patterns (DAMP) and cancer antigens that serve as activating ligands or targets for NK and T cells. Evidence in support of this mechanism was seen in a preclinical lymphoma study of the pixatimod/cyclophosphamide combination where cyclophosphamide alone led to a transient decrease in A20 tumors which would have resulted in the release of tumor associated DAMP and antigens.[12] In the pixatimod/cyclophosphamide combination, there was complete tumor remission which further analysis indicated was caused by NK cells mobilized by DAMP-activated DC in a TLR9 dependent manner.[12]

3.8.4 Rationale for Dose Selection: Nivolumab

Refer to the **eIB** and the nivolumab **SmPC** or **USPI** for preclinical and clinical study data.

Nivolumab 3 mg/kg has been approved as monotherapy in melanoma and NSCLC patients. Nivolumab 3 mg/kg has been tested in MMR-deficient colorectal and non-colorectal cancers singly and in combination with low-dose ipilimumab.[109-111]

In the current study, nivolumab will be administered at 480 mg Q4W in all 3 cohorts. The choice of 480mg Q4W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of nivolumab showing that the fixed dose of 240 mg every 2 weeks (and 480 mg Q4W) will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 2 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as

associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.[112,113] A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

3.8.5 Rationale for Dose Selection: Pixatimod (PG545)

The maximum tolerated dose for nivolumab/pixatimod in combination with nivolumab has previously been determined to be 25 mg weekly IV administered as an infusion over 1 hour.[9]

3.8.6 Rationale for Dose Selection: Cyclophosphamide

Rationale for low-dose cyclophosphamide in MSS mCRC is stated in **Section 3.8.3**.

Low-dose cyclophosphamide is safe, and non-myelosuppressive with an immunomodulatory effect that is dependent upon the dose and duration of the drug-free interval.[114-116] Given recent data showing rapid anti-tumor responses mediated by CD8+ T cells following treatment with a TLR9 agonist and low-dose Cy in a colorectal cancer model,[11] a clinical investigation of nivolumab/pixatimod/cyclophosphamide is warranted in MSS mCRC.

In this context, the ideal immunological dose and schedule of low-dose cyclophosphamide which shows sustained natural killer (NK) T cell function and induction of TA- specific memory CD8+ T cells is 50 mg twice daily, 1-week-on, 1-week-off.[114-116] This dose has been explored in two studies (PEMBROSARC, and NCT02406781) in combination with PD-1 inhibitor pembrolizumab in patients with advanced osteosarcoma and soft tissue sarcomas.[95,96]

4. OBJECTIVES AND ENDPOINTS

4.1 Primary Objective, Hypothesis, and Endpoint

4.1.1 Primary Objective

To establish the efficacy of nivolumab/pixatimod combination in PD-1 R/R melanoma and NSCLC; and the efficacy of nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC.

4.1.2 Primary Hypothesis

That nivolumab/pixatimod combination produces radiographic responses in PD-1 R/R melanoma and NSCLC; and that the nivolumab/pixatimod/cyclophosphamide combination produces radiographic responses in MSS mCRC.

4.1.3 Primary Endpoint

ORR by RECIST v1.1 to nivolumab/pixatimod combination in PD-1 R/R melanoma and NSCLC; and to nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC.

4.2 Secondary Objectives, Endpoints and Hypotheses

4.2.1 Secondary Objectives

4.2.1.1 Secondary Objective 1

To assess safety of nivolumab/pixatimod combination in PD-1 R/R melanoma and NSCLC; and nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC patients

4.2.1.2 Secondary Objective 2

To assess efficacy using alternative response endpoints.

4.2.1.3 Secondary Objective 3

To assess CD8+ T cell and NK cell infiltrate in pre- and on- treatment tumor samples by multiplex IHC/IF.

4.2.1.4 Secondary Objective 4

To assess median and landmark progression-free survival (PFS) and landmark overall survival (OS) in participants treated with nivolumab/pixatimod or nivolumab/pixatimod/cyclophosphamide.

4.2.2 Secondary Hypotheses

4.2.2.1 Secondary Hypothesis 1

That nivolumab/pixatimod combination augments inflammation by increasing CD8+ T cell and/or NK cell infiltrate in on-treatment compared to pre-treatment tumor samples.

4.2.2.2 Secondary Hypothesis 2

That nivolumab/pixatimod combination produces radiographic responses in PD-1 R/R melanoma and NSCLC; and that the nivolumab/pixatimod/cyclophosphamide combination produces radiographic responses in MSS mCRC as assessed using alternative response endpoints.

4.2.2.3 Secondary Hypothesis 3

That nivolumab/pixatimod combination improves PFS and OS in PD-1 R/R melanoma and NSCLC and MSS mCRC.

4.2.3 Secondary Endpoints

4.2.3.1 Secondary Endpoint 1

Incidence of all-grade related adverse events (AE), serious adverse events (SAE) and dose-limiting toxicities (DLT) if any in each cohort.

4.2.3.2 Secondary Endpoint 2

ORR by iRECIST to nivolumab/pixatimod combination in PD-1 R/R melanoma and NSCLC; and to nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC.

4.2.3.3 Secondary Endpoint 3

Summary measures of change (or % change) from baseline in the CD8+ T cell infiltrate and/or NK cell in the tumor and TME in tumor biopsies at pre- and on- (approximately 4 weeks) treatment timepoints.

4.2.3.4 Secondary Endpoint 4

Median PFS, landmark PFS (6 months, 1-year and 2-year), median OS and landmark OS (1-year, and 2-year) in participants treated with nivolumab/pixatimod or nivolumab/pixatimod/cyclophosphamide.

4.3 Exploratory Objectives, Hypotheses, and Endpoints

4.3.1 Exploratory Objectives

4.3.1.1 Exploratory Objective 1

To explore potential association between biomarkers and clinical efficacy of nivolumab/pixatimod or nivolumab/pixatimod/ cyclophosphamide combination.

4.3.2 Exploratory Hypotheses

4.3.2.1 Exploratory Hypothesis 1

That responders to nivolumab/pixatimod combination demonstrate evidence of immune activation in tumor tissue, blood, and plasma by multiparameter flow cytometry, transcriptomic, genomic and cytokine analyses.

4.3.3 Exploratory Endpoints

4.3.3.1 Exploratory Endpoint 1

Correlation/measures of association of biomarkers and clinical effect of nivolumab/pixatimod or nivolumab/pixatimod/ cyclophosphamide combination.

5. STUDY DESIGN

5.1 Study Design

This is a phase II Simon two-stage study of nivolumab in combination with pixatimod in 3 separate cohorts:

- Cohort 1: MSS mCRC in combination with low-dose cyclophosphamide
- Cohort 2: PD-1 relapsed/refractory melanoma
- Cohort 3: PD-1 relapsed/refractory NSCLC

The study schema is outlined in **Figure 5.1-1** below.

We hypothesize that the nivolumab/pixatimod combination in PD-1 relapsed/refractory (R/R) cutaneous melanoma and NSCLC patients will be associated with anti-tumor effects. We also hypothesize that the nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC patients will be associated with anti-tumor effect. We further hypothesize that nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC patients and nivolumab/pixatimod combination in PD-1 R/R melanoma or NSCLC will be associated with increased CD8+ T cells intra-tumorally and PD-1+Ki67+ CD8+ T cells peripherally in responders on treatment compared to baseline; and that response will be associated with evidence of antigen-specific immunity responses intra-tumorally and peripherally.

The goal of this study is to assess response of PD-1 R/R melanoma/NSCLC to nivolumab and pixatimod; and MSS mCRC to nivolumab, pixatimod and cyclophosphamide.

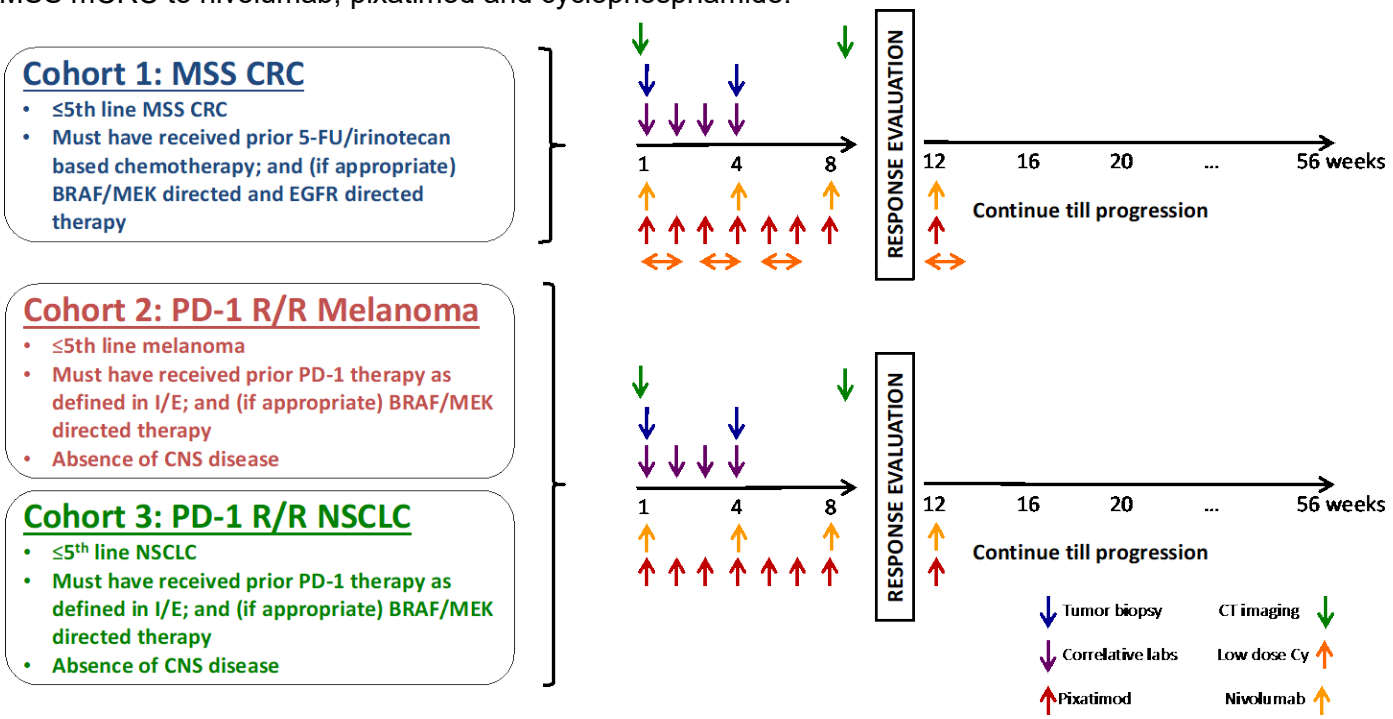

Figure 5.1-1: Clinical Trial Schema

5.2 Trial Procedures

The **Schedule of Activities** in **Section 2.0 (Schedule of Activities)** summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor-Investigator and/or

Aculeus Therapeutics for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

5.3 Number of Participants

In the 1st stage, 13 patients will be enrolled in cohort 1 while 9 patients each will be enrolled in cohorts 2 and 3. Should pre-specified efficacy boundary as defined in **Section 10.3** be met, further patients will be enrolled to one or more cohorts in 2nd stage. The total enrollment for 1st stage across all 3 cohorts is 31 response-evaluable patients. The total enrollment for both stages across all 3 cohorts is 61 response-evaluable patients.

6. STUDY POPULATION

6.1 Inclusion Criteria

6.1.1 General Inclusion Criteria

- Male/female participants who are at least 18 years of age on the day of signing informed consent with advanced/metastatic cutaneous melanoma, NSCLC or MSS mCRC who meet the following criteria will be enrolled in this study.
- Male participants:
    - A male participant must agree to use a contraception as detailed in **Appendix 3** of this protocol during the treatment period and for at least 120 days after the last dose of study treatment and refrain from donating sperm during this period.
- Female participants:
    - A female participant is eligible to participate if she is not pregnant (see **Appendix 3**), not breastfeeding, and at least one of the following conditions applies:
    - Not a woman of childbearing potential (WOCBP) as defined in **Appendix 3**;OR
    - A WOCBP who agrees to follow the contraceptive guidance in **Appendix 3** during the treatment period and for at least 120 days after the last dose of study treatment.

6.1.2 Cohort 1 (MSS mCRC)

- MSS is defined as 0-1 allelic shifts among 3-5 tumor microsatellite loci using a PCR-based assay or immunohistochemistry.
- Must have received prior therapy with a fluoropyrimidine, oxaliplatin, and irinotecan.
- Prior treatment with an anti-PD-1/anti-PD-L1 or anti-CTLA-4 antibody is not allowed.
- Prior treatment with BRAF/MEK inhibitor therapy (if BRAF mutated) and/or EGFR targeted antibody (if KRAS WT) are allowed.
- No more than 5 prior lines of therapy for metastatic disease.
- Adjuvant therapy will count as 1 prior line of therapy if received within the prior 6 months; but if not does not count towards prior line of therapy.

- PIV cutoff of 1200 as defined in **Section 3.8.2** obtained on labs obtained during **Screening**.

6.1.3 Cohort 2 (PD-1 R/R melanoma)

- **PD-1 refractory disease as defined as** progression on treatment with anti-PD-(L)1 inhibitor administered either as monotherapy or in combination with other checkpoint inhibitors or other therapies. PD-1 treatment progression is defined by meeting **all of the following criteria**:
    - Receipt of at least 2 doses of an approved or investigational anti-PD-(L)1 inhibitor.
    - Demonstrated PD after anti-PD-(L)1 inhibitor as defined by RECIST v1.1.
        - **Note:** This determination is made by the investigator. Once PD is confirmed, the initial date of PD documentation will be considered the date of disease progression.
        - **Note:** The initial evidence of PD is to be confirmed by a second assessment no less than 4 weeks from the date of the first documented PD, in the absence of rapid clinical progression (as defined by the criteria in the sub-point below).

        - Subjects who progressed on/within 3 months of adjuvant therapy with anti-PD-(L)1 inhibitor will be allowed; an adjuvant therapy will count as 1 prior line of therapy if received within the prior 6 months.
        - Progressive disease should be documented at least 12 weeks from the last dose of anti-PD-(L)1 inhibitor.
    - **Primary resistance:** Drug exposure for at least 6 weeks with best response of PD, or SD for <6 months (including PD at any time after stopping anti-PD-(L)1 for any reason unrelated to toxicity if best response while receiving anti-PDx therapy was PD or SD <6 months).
    - **Secondary resistance:** Drug exposure for at least 6 months with best response of CR or PR or SD for ≥6 months. If prior anti-PD-(L)1 was stopped prior to PD, PD must have occurred at least 12 weeks after the last dose of anti-PD-(L)1.
- Prior treatment with an anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) antibody is allowed but not required.
- Prior treatment with BRAF/MEK inhibitor therapy (if BRAF mutated) is allowed but not required.
- No more than 5 prior lines of therapy.

6.1.4 Cohort 3 (PD-1 R/R NSCLC)

- **PD-1 refractory disease as defined as** progression on treatment with anti-PD-(L)1 inhibitor administered either as monotherapy or in combination with other checkpoint inhibitors or other therapies. PD-1 treatment progression is defined by meeting all of the following criteria:
    - Receipt of at least 2 doses of an approved or investigational anti-PD-(L)1 inhibitor.
    - Demonstrated PD after anti-PD-(L)1 inhibitor as defined by RECIST v1.1.
        - **Note:** This determination is made by the investigator. Once PD is confirmed, the initial date of PD documentation will be considered the date of disease progression.
        - **Note:** The initial evidence of PD is to be confirmed by a second assessment no less than 4 weeks from the date of the first documented PD, in the absence of rapid clinical progression (as defined by the criteria in the sub-point below).
        - Subjects who progressed on/within 3 months of adjuvant therapy with anti-PD-(L)1 inhibitor will be allowed; an adjuvant therapy will count as 1 prior line of therapy if received within the prior 6 months.
        - Progressive disease should be documented at least 12 weeks from the last dose of anti-PD-(L)1 inhibitor.
    - **Primary resistance:** Drug exposure for at least 6 weeks with best response of PD, or SD for <6 months (including PD at any time after stopping anti-PD-(L)1 for any reason unrelated to toxicity if best response while receiving anti-PDx therapy was PD or SD <6 months).
    - **Secondary resistance:** Drug exposure for at least 6 months with best response of CR or PR or SD for ≥6 months. If prior anti-PD-(L)1 was stopped prior to PD, PD must have occurred at least 12 weeks after the last dose of anti-PD-(L)1.
- Prior treatment with an anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) antibody is allowed but not required.
- Prior treatment with BRAF/MEK inhibitor therapy (if BRAF mutated) must have received and progressed or have demonstrable intolerance to approved targeted therapy.

- Patients with NSCLC with known oncogenic driver (including but not limited to EGFR, ALK, ROS, MET alterations) must have received and progressed past driver-specific therapy.
- No more than 5 prior lines of therapy.

6.1.5 Other Criteria

- The participant (or legally acceptable representative if applicable) provides written informed consent for the trial.
- Have measurable disease based on RECIST 1.1. Lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions.
- Have provided newly obtained core or excisional biopsy of a tumor lesion not previously irradiated to undergo tumor biopsy (core, punch, incisional or excisional).
    - Biopsy must meet minimal sampling criteria as defined in **Schedule of Events (Section 2.0)**.
- Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1. Evaluation of ECOG is to be performed within 28 days prior to the date of enrollment.
- Adequate organ function as defined in **Table 6.1.5-1** below performed on screening labs obtained within 4 weeks of Cycle 1 day 1

Table 6.1.5–1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/μL
Platelets	≥100 000/μL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[a]
Renal	
Creatinine OR Measured or calculated[b] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥30 mL/min for participant with creatinine levels >1.5 × institutional ULN
Hepatic	
Total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN (≤5 × ULN for participants with liver metastases)
Coagulation	
International normalized ratio (INR) OR prothrombin time (PT) Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants

ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT)=aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal.

[a] Criteria must be met without erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks.

[b] Creatinine clearance (CrCl) should be calculated per institutional standard.

Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.

- Proteinuria exceeding 1gram in a 24 hour period.
- Female subject 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.
- 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 120 days after the last dose of study medication (Section 5.7.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.
- Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.
- Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

6.2 Exclusion Criteria

- Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of treatment.

- History of allergy and/or hypersensitivity and/or other clinically significant adverse drug reaction to heparin or other anti-coagulant agents, or to any monoclonal antibody.
- Use of heparin (including low-molecular weight heparin and/or fondaparinux) within 2 weeks prior to enrollment.
    - Patients who are currently receiving low-molecular weight heparin (or fondaparinux or other heparin product) for therapeutic anticoagulation may be enrolled **if they have tested negative for anti-heparin antibodies at Screening.**
- Has a diagnosis of immunodeficiency, immunosuppression and/or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.
- Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from AEs due to a previously administered agent.
    - **Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.**
    - **Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.**
    - **Note: Subjects with autoimmune disorders of Grade 4 while on prior immunotherapy will be excluded. Subjects who developed autoimmune disorders of Grade ≤ 3 may enroll if the disorder has resolved to Grade ≤1 and the subject has been off systemic steroids at doses >10 mg/d for at least 2 weeks.**
- Active (i.e., symptomatic or growing) central nervous system (CNS) metastases.
    - Note: Subjects with treated and stable CNS metastases are permitted to enroll. Stability for prior treated CNS disease should be assessed on a contrast-enhanced imaging study obtained no sooner than 14 days from data of definitive radiotherapy and/or surgery for CNS disease.
    - Note: Subjects with leptomeningeal carcinomatosis are excluded.
- Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone (or is being planned to undergo) potentially curative therapy.
- Has a systemic disease that requires systemic pharmacologic doses of corticosteroids greater than 10 mg daily prednisone (or equivalent).
    - Note: Subjects who are currently receiving steroids at a dose of ≤10 mg daily do not need to discontinue steroids prior to enrollment.
    - Note: Subjects that require topical, ophthalmologic and inhalational steroids are not excluded from the study.
    - Note: Subjects with hypothyroidism stable on hormone replacement or Sjogren's syndrome are not excluded from the study.
    - Note: Subjects who require active immunosuppression (greater than steroid dose discussed above) for any reason are excluded from the study.
- Has evidence of interstitial lung disease or active, non-infectious pneumonitis.
- Has an active infection requiring systemic therapy.
- Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
- Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

- Is pregnant or breastfeeding or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 26 weeks after the last dose of trial treatment.
- Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
    - Note: Subjects with HIV that is well controlled (undetectable viral load and CD4 count >200 cells/mm3) on anti-retroviral therapy are permitted to enroll.
- Has known active hepatitis B (e.g., HBsAg reactive) or hepatitis C (e.g., HCV RNA [qualitative] is detected.
    - Note: Subjects with treated hepatitis B and/or C with no evidence of active infection may be enrolled.
- Has a history of significant cardiac disease including but not limited to symptomatic congestive heart failure (New York Heart Association Class III/IV), uncontrolled hypertension (≥150/90mmHg) despite appropriate anti-hypertensive medication (patients with stably controlled hypertension are eligible), unstable angina pectoris or myocardial infarction (≤ 6 months prior to screening), uncontrolled cardiac arrhythmia, clinically significant cardiac valvopathy requiring treatment.
- Receipt of live vaccine(s) within 30 days prior to the first dose of trial treatment.
- Other uncontrolled intercurrent illness, including but not limited to, ongoing/active infection, active interstitial lung disease, serious chronic gastrointestinal conditions associated with diarrhea, or psychiatric illness/social situations that would limit compliance with study requirement, substantially increase risk of incurring AEs and/or compromise the ability of the patient to give written informed consent.

7. TRIAL TREATMENTS

7.1 Treatment Details

Nivolumab monotherapy (2mg/kg every 3 weeks; 240 mg every 2 weeks; 480 mg every 4 weeks) is approved for the treatment of advanced melanoma regardless of BRAF mutation status. Pharmacokinetic analyses have established flat exposure-response relationships between these various doses and schedules;[112,113] and in this study 480 mg q4 will be used.

Pixatimod monotherapy has been demonstrated to be safe and well tolerated in patients with refractory solid tumors.[8] In a phase Ib study, combination of pixatimod with nivolumab demonstrated promising results in uninflamed tumors including pancreatic cancer and microsatellite-stable colorectal carcinoma.[9] Based on this study, the recommended phase II dose of pixatimod is 25 mg IV weekly. In this study, pixatimod will be dosed at 25 mg IV weekly.

Low-dose Cy is safe, and non-myelosuppressive with an immunomodulatory effect that is dependent upon the dose and duration of the drug-free interval.[114-116] In this context, the ideal immunological dose and schedule of low-dose Cy which shows sustained natural killer (NK) T cell function and induction of TA- specific memory CD8+ T cells is 50 mg twice daily, 1-week-on, 1-week-off.[114-116] This dose has been explored in two studies (PEMBROSARC, and NCT02406781) in combination with PD-1 inhibitor pembrolizumab in patients with advanced osteosarcoma and soft tissue sarcomas.[95,96]

In **Cohort 1:**

- Nivolumab 480 mg Q4W
- Pixatimod 25mg Q1W
- Cyclophosphamide (50 mg twice daily, 1-week-on, 1-week-off).

In **Cohorts 2 and 3:**

- Nivolumab 480 mg Q4W
- Pixatimod 25 mg Q1W.

7.2 Nivolumab Dose Selection, Modification, Timing, and Duration

7.2.1 Nivolumab dose selection

Rationale for nivolumab dose selection is provided in **Section 3.8.3 - Rationale for Dose Selection: Nivolumab.**

7.2.2 Nivolumab dose modification

No nivolumab dose modification is allowed.

Nivolumab will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥ Grade 3 including laboratory abnormalities, and severe or life-threatening AEs as per **Table 7.2.2-1** below.

Table 7.2.2-1: Dose Modification Guidelines for Drug-related Adverse Events (AEs)

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
			less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	3-4	Permanently discontinue (see exception below)[a]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold nivolumab and pixatimod for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume nivolumab when patients are clinically and metabolically stable
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with nivolumab and pixatimod can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism		Therapy with nivolumab and pixatimod can be continued while thyroid replacement therapy is instituted	Therapy with nivolumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2[b]	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks Permanently discontinue for **recurrent** Grade 2 pneumonitis
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[c]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks. Exceptions may be sought for neuropathy or other AE following discussion with Principle Investigator.
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[a] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

[b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/h to 50 mL/h). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose and the infusion rate reduced by 50% for next scheduled dose. Infusion rate may be subsequently increased at investiagtor discretion.

[c] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

In case toxicity does not resolve to Grade 0-1 within 12 weeks after last infusion, trial treatment should be discontinued after consultation with the Sponsor-Investigator. With Sponsor-Investigator's agreement, subjects with a laboratory AE still at Grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic and controlled. For information on the management of AEs, see **Section 7.7.1**.

Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of nivolumab should be discontinued from trial treatment.

7.2.3 Nivolumab timing of administration

Trial treatment should be administered after all procedures/assessments have been completed as detailed on the **Schedule of Activities** in **Section 2.0**. Trial treatment may be administered up to ± 2 days before or after the scheduled Day 1 due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Nivolumab will be administered as a 30-minute IV infusion (treatment intervals may be increased due to toxicity as described). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

7.2.4 Nivolumab duration of administration

In all 3 cohorts, nivolumab will be administered at 480 mg every 4 weeks. Nivolumab 480 mg Q4 will be administered till progression and/or unacceptable toxicity.

7.3 Pixatimod (PG545) Dose Selection, Modification, Timing and Duration

7.3.1 **Pixatimod (PG545)** dose selection

Rationale for pixatimod dose selection is provided in **Section 3.8.4 - Rationale for Dose Selection: Pixatimod (PG545).**

7.3.2 Pixatimod (PG545) dose modification

No pixatimod dose modification is allowed.

Pixatimod will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥ Grade 3 including laboratory abnormalities (but excluding Grade 3 lipid elevations), and severe or life-threatening AEs.

Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of pixatimod will have this discontinued. If pixatimod is permanently discontinued for, subjects may continue to receive nivolumab.

7.3.3 Pixatimod (PG545) prophylaxis

In the initial 5 patients treated in this study, patients were premedicated with acetaminophen 1000mg prior to pixatimod infusion to prophylax against pixatimod-induced cytokine release. However, given the low incidence of cytokine-release observed, observed negative impact on vaccination response, and possible deleterious effects upon immune therapy observed in retrospective analyses[117,118], this was discontinued with protocol v6.

7.3.4 Pixatimod (PG545) timing of administration

Trial treatment should be administered after all procedures/assessments have been completed as detailed on the trial **Schedule of Activities** in **Section 2.0**. Trial treatment may be administered up to ± 2 days before or after the scheduled Day 1 due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Pixatimod will be administered as a 60-minute IV infusion (treatment intervals may be increased due to toxicity as described). Sites should make every effort to target infusion timing to be as close to 60 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

Pixatimod will be administered after nivolumab.

7.3.5 Pixatimod (PG545) duration of administration

In all 3 cohorts, pixatimod will be administered at 25 mg Q1W. Pixatimod 25 mg Q1W will be administered till progression and/or unacceptable toxicity.

7.4 Cy Dose Selection, Modification, Timing and Duration

7.4.1 Cyclophosphamide dose selection

Rationale for pixatimod dose selection is provided in **Section 3.8.2. - Rationale for Adding Low-dose Cy in MSSm CRC**.

7.4.2 Cyclophosphamide dose modification

Cyclophosphamide will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥ Grade 3 including laboratory abnormalities, and severe or life-threatening AEs.

Further, in Cohort 1, continuous toxicity monitoring will be done as delineated in **Section 9.4**. In this context, cyclophosphamide dose modification may be permitted for recurrent drug-related G4 hematologic

Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of cyclophosphamide will have this discontinued. If cyclophosphamide is permanently discontinued for, subjects may continue to receive nivolumab and pixatimod.

7.4.3 Cy timing of administration

Trial treatment should be administered after all procedures/assessments have been completed as detailed on the trial **Schedule of Activities** in **Section 2.0**. Trial treatment may be administered up to ± 2 days before or after the scheduled Day 1 due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

In **Cohort 1 only**, cyclophosphamide will be administered orally at 50 mg twice daily on D1-D7, and D15-D21 of a 28-day cycle. Pill compliance will be assessed using a pill diary.

7.4.4 Cy duration of administration

In **Cohort 1 only**, cyclophosphamide will be administered orally at 50 mg twice daily on D1-D7, and D15-D21 of a 28-day cycle. Pill compliance will be assessed using a pill diary. cyclophosphamide will be administered till progression and/or unacceptable toxicity.

7.5 Treatment Allocation

Following Enrollment and completion of **Screening**, patients will enter the **Treatment** phase.

7.6 Concomitant Medications

Medications or vaccinations specifically prohibited in the exclusion criteria and outlined in **Section 7.8.2** are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The treating Investigator should discuss any questions regarding this with the Principal Investigator. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's treating Investigator. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the treating Investigator, the Principal Investigator, and the subject.

7.6.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded in the research record including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included in the research record.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in **Section 9.2.**

7.6.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the **Screening** and **Treatment Phase** (including retreatment for post-complete response relapse) of this trial:

- Anti-cancer systemic chemotherapy or biological therapy;
- Immunotherapy not specified in this protocol;
- Chemotherapy not specified in this protocol;
- Investigational agents other than pixatimod;
- Radiation therapy.

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, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines and are not allowed.

Glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Principal Investigator.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary. However, if a patient who is otherwise deriving benefit as assessed by the treating investigator may be permitted to receive radiation therapy to a non-target lesion for the purposes of symptom control.

The **Exclusion Criteria** (**Section 6.2**) describes other medications which are prohibited in this trial.

There are no prohibited therapies during the **Post-Treatment Follow-up Phase (Section 8.5.5)**.

Agents known to have TLR9 antagonist activity are prohibited throughout the study. Known antagonists include chloroquine, hydroxychloroquine, and quinacrine.

Medications intended solely for supportive care (i.e., antiemetics, analgesics, megestrol acetate for anorexia) are allowed.

7.7 Rescue Medications and Supportive Care

7.7.1 Supportive Care Guidelines

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

Note: If after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). **Refer to Sections 7.2-7.4 for dose modification guidelines for nivolumab, pixatimod and cyclophosphamide.**

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

- **Pneumonitis**
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
    - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Diarrhea/Colitis**
    - Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

- All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
- For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.
- For **Grade 3 or 4 diarrhea/colitis,** treat with intravenous steroids followed by high dose oral steroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
  - For **T1DM** or **Grade 3-4** Hyperglycemia
    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.
- **Hypophysitis**
  - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
  - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hyperthyroidism or Hypothyroidism**
  - Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.
  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hepatic**
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids

- For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
- When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Nephritis**
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- Management of infusion reactions
  - Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 7.7.1-1 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of nivolumab.

Table 7.7.1-1: Infusion Reaction Management Guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction: infusion interruption not indicated, intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24 h	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/h to 50 mL/h). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.	Subject may be premedicated 1.5 h (± 30 min) prior to infusion of nivolumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500–1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4:	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine	No subsequent dosing

Life-threatening; pressor or ventilatory support indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. Subject is permanently discontinued from further trial treatment administration.	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

7.8 Subject Withdrawal/Discontinuation Criteria

Subjects are free to withdraw from the study at any time and without penalty or loss of future medical care, or any other benefits to which they are otherwise entitled. Subjects may discontinue study medication for any of the following conditions:

- Dose-limiting or other unacceptable toxicity considered to be related to either study medication;
    - Patients who develop unacceptable toxicity deemed related to pixatimod but not nivolumab may continue nivolumab after pixatimod discontinuation for duration as defined in **Section 7.3.2** if the patients have ongoing benefit in the assessment of treating investigator after consultation with Sponsor-Investigator.
    - Patients who develop unacceptable toxicity deemed related to cyclophosphamide but not pixatimod or nivolumab may continue pixatimod and nivolumab after cyclophosphamide discontinuation for duration as defined in **Section 7.4.2**.
    - Patients who develop unacceptable toxicity deemed related to nivolumab but not pixatimod must discontinue **both pixatimod and nivolumab (and if appropriate, cyclophosphamide).**
- PD by RECIST (Version 1.1) if accompanied by medically significant clinical deterioration, in the judgment of the Investigator. (Continuation of treatment through suspected pseudo-progression is permitted.);
- If, in the opinion of the Investigator, it is medically necessary;
- Subject withdraws consent for the study (note that subjects who withdraw consent for additional study treatment and procedures will continue to be followed for long-term
- survival unless they explicitly withdraw consent for any follow-up);
- Subject develops an intercurrent illness or AE that precludes further participation, or requires a prohibited concomitant treatment;
- Subject becomes pregnant or begins breastfeeding;
- Subject is lost to follow-up.

Patients discontinuing study medication earlier than planned or withdrawing from the study should undergo the subsequent **End of Treatment** (**EOT**) clinical and laboratory assessments as soon as possible after study medication is stopped and the requisite safety follow-up period of 30 days should also be followed. The reason for withdrawal will be recorded in the electronic case report form (eCRF) and the subject's source medical record. All subjects will continue to be followed every three months after the last nivolumab/pixatimod treatment for long-term survival follow-up until death, loss to follow-up, or withdrawal of consent for follow-up.

7.9 Subject Replacement Strategy

Patients who receive at least 1 dose each of nivolumab and pixatimod (cohorts 2 and 3); and at least 1 dose each of nivolumab, pixatimod and low-dose cyclophosphamide (cohort 1) are evaluable for safety.

All patients who met the eligibility criteria; received at least 1 dose each of nivolumab and pixatimod (cohorts 2 and 3); and at least 1 dose each of nivolumab, pixatimod and low-dose cyclophosphamide (cohort 1); and underwent restaging imaging are evaluable for response.

Patients who did not undergo restaging scans for reasons *other than disease progression* (treatment discontinuation, trial withdrawal etc.) will be deemed non-evaluable. Non-evaluable subjects may be replaced.

If in the opinion of the treating investigator and Principal Investigator, the subject's disease is rapidly progressing, restaging scans may be expedited. These patients are evaluable for objective response rate and other efficacy endpoints.

7.10 Clinical Criteria for Early Trial Termination

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

- Quality or quantity of data recording is inaccurate or incomplete.
- Poor adherence to protocol and regulatory requirements.
- Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects.
- Plans to modify or discontinue the development of the study drugs. In the event of an Aculeus Therapeutics decision to no longer supply study drug(s), ample notification will be provided so that appropriate adjustments to subject treatment can be made.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Trial Procedures

The trial **Schedule of Activities** in **Section 2.0** summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor-Investigator and/or Zuecro for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

8.2 Treatment Period

Following **Screening**, during the **Treatment Period**, patients will receive treatment delineated below. Each cycle is 28 days long. In each cycle, patients will receive pixatimod, nivolumab ± cyclophosphamide (in Cohort 1).

Cohort 1 (MSS mCRC):
- IV nivolumab 480 mg Q4W
- IV pixatimod 25 mg Q1W
- PO cyclophosphamide at 50 mg twice daily on D1-D7, and D15-D21 of a 28-day cycle

Cohort 2 (PD-1 R/R melanoma):
- IV nivolumab 480 mg Q4W
- IV pixatimod 25 mg Q1W

Cohort 3 (PD-1 R/R NSCLC):
- IV nivolumab 480 mg Q4W
- IV pixatimod 25 mg Q1W

8.3 Administrative Procedures

Informed consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion prior to enrollment.

During initial enrollment visit, study investigator (or designee) must discuss the following with patients:
- Inclusion and exclusion criteria
- Medical history – pertinently any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator.
- Prior and concomitant medications. All medications related to reportable SAEs and ECIs should be recorded as defined in **Section 8.6.**
- Disease details and treatments.

8.4 Clinical Procedures and Assessments

8.4.1 Adverse Events (AE) monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the trial **Schedule of Activities** (**Section 2.0**) and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 5.0 (see **Section 8.6**). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regards to trial treatment.

All AEs of unknown etiology associated with nivolumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (irAE). See **Section 7.7.1** regarding the identification, evaluation and management of AEs of a potential immunological etiology.

Related and clinically significant AEs occurring within 30 days after the last dose of study drug should be recorded. Patients who are discontinued from the study due to an unacceptable drug-related AE will be followed until the resolution of the AE to Grade 0-1 or stabilization or until initiation of a new therapy for their cancer, whichever occurs first. Related SAEs that occur within 90 days of the end of treatment or before initiation of a new antineoplastic treatment should also be followed and recorded.

Please refer to **Section 8.6** for detailed information regarding the assessment and recording of AEs.

8.4.2 Full physical exam

The Investigator or qualified designee will perform a complete physical exam during **Screening** and as otherwise indicated in **Schedule of Activities** (**Section 2.0**). Clinically significant abnormal findings should be recorded as medical history.

8.4.3 Vital signs

The Investigator or qualified designee will obtain vital signs during **Screening** and as otherwise indicated in trial **Schedule of Activities** (**Section 2.0**). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at **Screening** only.

8.4.4 Eastern Cooperative Oncology Group (ECOG) performance scale

The Investigator or qualified designee will assess ECOG status at **Screening**, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the trial **Schedule of Activities** (**Section 2.0**).

8.5 Visit Requirements

8.5.1 Requirements

Visit requirements are outlined in trial **Schedule of Activities** (**Section 2.0**). Specific procedure-related details are provided above in **Section 8.1 - Trial Procedures**.

8.5.2 Screening

Screening starts with the subject's provision of a written informed consent form (ICF) and should be completed within 28 days of initiation of study drug(s).

8.5.3 Thirty-day safety follow-up

The mandatory 30-day 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 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.

8.5.4 End of Treatment (EOT)

Subjects who discontinue trial treatment for disease progression will complete an EOT visit. EOT visit requirements include physical exam, medical history, AE assessment and collection of correlative samples as indicated in the trial **Schedule of Activities** (**Section 2.0**).

8.5.5 Post-treatment Surveillance Phase

Subjects who discontinue trial treatment for a reason other than disease progression (including patients who do not progress but discontinue for intolerance/toxicity) will move into the **Post-treatment Surveillance Phase**. These patients will continue to be assessed clinically and radiographically to monitor disease status. **Post-Treatment Surveillance Phase** will start 12 weeks after the last on-treatment time point for patients who have no disease progression.

These patients are evaluated per the standard follow-up schedule with imaging [contrast-enhanced CT chest/abdomen/pelvis and (if applicable) vs. PET at the discretion of the treating physician] at the following intervals. Every 3 months (±2 weeks) if patient is < 2 years from study entry, every 6 months (±4 weeks) if patient is 2-5 years from study entry, and every 12 months (±4 weeks) if patient is > 5 years from study entry for up to 15 years.

Patients who develop recurrent cancer will be followed for information on survival and for information on salvage patterns. The schedule of clinical follow up for these patients will be at the discretion of the treating Investigator and according to established guidelines for the relevant disease. AE assessment on the study will continue for all patients until 90 days after the last study drug administration.

Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study as detailed in **Survival Follow Up** (**Section 8.5.6**). Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Note – Patient accrual was terminated early owing to the discontinuation of pixatimod supply. Follow-up assessments will no longer be completed upon IRB approval of protocol version 02/23/2024.

8.5.6 Survival follow up

If a subject has confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first. This will be done every 3 months.

Note – Patient accrual was terminated early owing to the discontinuation of pixatimod supply. Follow-up assessments will no longer be completed upon IRB approval of protocol version 02/23/2024.

8.6 Laboratory Assessments

Details regarding specific laboratory assessments to be performed in this trial are provided below.

8.6.1 Screening laboratory assessments

Screening laboratory tests should be performed within 28 days of registration. Requirements are stated below in **Table 8.6.1-1**. Results must be reviewed by the Investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

There is 28-day window to obtain Screening ($\mathbf{X_{K\text{-}S}}$) laboratory assessments from signing consent.

Table 8.6.1-1: Screening laboratory assessments ($X_{K\text{-}S}$)

Blood	Urine	Other
• CBC: WBC (total and differential including absolute neutrophil count), hemoglobin and hematocrit; platelet count • Renal function: blood urea nitrogen, bicarbonate, calcium, chloride, creatinine, glucose, phosphorus, potassium, magnesium and sodium • Liver function: albumin, total protein, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (direct only if total is elevated above ULN) • Lactate dehydrogenase (LDH) • Coagulation parameters: PT and aPTT • Thyroid function studies: TSH, free T3 and free T4 • [$]Infectious serologies: HIV, hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (HBsAb) and HCV RNA	• pH • Specific gravity • Glucose • Protein • Blood • Nitrites • WBCs • Microscopic battery (RBCs, WBCs, epithelial cells, casts) - only if significant positive findings on urinalysis	• *Serum or urine pregnancy test (β-human chorionic gonadotropin) • [&]Anti-heparin antibodies (patients on heparin, low-molecular weight heparin, fondaparinux or other similar agents)
*Perform on women of childbearing potential and at **Screening** only. If urine pregnancy results cannot be confirmed as negative, serum β-human chorionic gonadotropin will be required. [$]HIV, hepatitis B and C studies will be obtained at **Screening** only [&]Anti-heparin antibodies will be obtained at **Screening** only in patients who are currently or have recently (within 6 months) been on heparin, low-molecular weight heparin, fondaparinux or other similar agents.		

8.6.2 On-treatment laboratory assessments

Pre-C1D1 and on-treatment laboratory tests should be performed at the appropriate intervals as indicated in the trial **Schedule of Activities** (**Section 2.0**). Requirements are stated below in **Table 8.6.2-1**. Results must be reviewed by the Investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

There is a 2-day window to obtain on-treatment ($\mathbf{X_{K\text{-}B}}$ **and** $\mathbf{X_{K\text{-}T}}$) laboratory assessments.

Table 8.6.2-1: On-treatment laboratory assessments (X_{K-B} and X_{K-T})

Blood	Urine	Other
• CBC: WBC (total and differential including absolute neutrophil count), hemoglobin and hematocrit; platelet count • Renal function: blood urea nitrogen, bicarbonate, calcium, chloride, creatinine, glucose, phosphorus, potassium, magnesium and sodium • Liver function: albumin, total protein, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (direct only if total is elevated above ULN) • Lactate dehydrogenase (LDH) • Coagulation parameters: PT and aPTT • *Thyroid function studies: TSH, free T3 and free T4	• pH • Specific gravity • Glucose • Protein • Blood • Nitrites • WBCs • Microscopic battery (RBCs, WBCs, epithelial cells, casts) - only if significant positive findings on urinalysis	• *Fasting lipids (triglycerides, LDL, HDL, total cholesterol)
*Only on D1 of each cycle (X_{K-B}) only		

8.7 Efficacy Assessments

8.7.1 Tumor imaging and disease assessment

Tumor imaging is strongly preferred to be acquired by contrast-enhanced computed tomography (CT). Brain imaging will be obtained only at **Screening** in melanoma and NSCLC patients and need not be repeated unless prior CNS disease was identified, and previously treated CNS lesions were captured as non-target lesions. For CRC patients, brain imaging at screening is not required. For brain imaging a contrast enhanced MRI is ideal. If an MRI of the brain cannot be done (or is contraindicated) a contrast enhanced CT of the brain is acceptable.

Confirmation that the participant's imaging shows at least 1 lesion that is appropriate for selection as a target lesion per RECIST 1.1 is highly recommended prior to participation.

Participant eligibility will be determined using Investigator assessment based on RECIST 1.1. In addition, images (including via other modalities) that are obtained at an unscheduled time point to determine disease progression, as well as imaging obtained for other reasons, but which demonstrate radiologic progression, should also be used to determine progression.

When the Investigator identifies radiographic progression per RECIST 1.1, efforts should be made to verify radiologic PD. Treatment should continue until PD has been verified. Regardless of whether PD is verified, if the Investigator considers the participant has progressed, but elects to implement iRECIST, the Investigator will assess for confirmation of progression by iRECIST at subsequent time points.

8.7.2 Initial tumor imaging

Initial tumor imaging at Screening must be performed within 28 days prior to the **Cycle 1 Day 1** date. The site study team must review screening images to confirm the participant has measurable disease per RECIST 1.1.

8.7.3 Tumor imaging during the study

The first on-study imaging assessment should be performed at 8 weeks (56 days ± 7 days) from the date of start of treatment. Subsequent tumor imaging should be performed every 8 weeks (56 days ± 7 days) or more frequently if clinically indicated. In patients who complete therapy and enter Post-Treatment Surveillance, imaging will be performed as outlined in **Section 8.5.5**. Imaging timing should follow calendar days and should not be adjusted for delays in cycle starts. Imaging should continue to be performed until disease progression is identified by the Investigator.

All objective response should be ideally confirmed by a repeat imaging assessment where possible. Tumor imaging to confirm PR or CR should be performed at least 4 weeks after the first indication of a response is observed. Participants will then return to regular scheduled imaging every 8 weeks (56 days ± 7 days), starting with the next scheduled imaging time point. Participants who receive additional imaging for confirmation do not need to undergo the next scheduled tumor imaging if it is less than 4 weeks later; tumor imaging may resume at the subsequent scheduled imaging time point.

Per iRECIST (**Section 8.7.6**), disease progression should be confirmed 4 to 8 weeks after first radiologic evidence of PD in clinically stable participants. Participants who have unconfirmed disease progression may continue on treatment at the discretion of the investigator until progression is confirmed by the site provided, they have met the conditions detailed in **Section 8.7.6**. Participants who receive confirmatory imaging do not need to undergo the next scheduled tumor imaging if it is less than 4 weeks later; tumor imaging may resume at the subsequent scheduled imaging time point, if clinically stable. Participants who have confirmed disease progression by iRECIST (**Section 8.7.6**), as assessed by the site, will discontinue study treatment.

8.7.4 End of treatment and follow-up tumor imaging

In participants who discontinue study treatment, tumor imaging should be performed at the time of treatment discontinuation (±4-week window). If previous imaging was obtained within 4 weeks prior to the date of discontinuation, then imaging at treatment discontinuation is not mandatory. In participants who discontinue study treatment due to documented disease progression and the investigator elects not to implement iRECIST, this is the final required tumor imaging.

For participants who discontinue study treatment without documented disease progression, every effort should be made to continue monitoring their disease status by tumor imaging using the same imaging schedule used while on treatment (as outlined in **Section 8.5.5.**) to monitor disease status until the start of a new anti-cancer treatment, disease progression, pregnancy, death, withdrawal of consent, or the end of the study, whichever occurs first.

8.7.5 RECIST v1.1 disease assessment

Response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST v.1.1) Committee.[119] It is known that patients receiving immunotherapy have atypical response patterns that in some cases may lead to incorrect determination of the response status. In the case of a measurable lesion increase or detection of a previously occult tumor lesion, RECIST 1.1 would fail to recognize the potential pseudo-progression and long-term effectiveness of

immunotherapy. Since significant tumor growth and/or newly detectable tumor lesions will generally be classified as progressive disease (PD) based on RECIST 1.1, this could result in an erroneous termination of the treatment and unjustified patient exclusion from clinical studies.

Although RECIST 1.1 references a maximum of 5 target lesions in total and 2 per organ, the Sponsor-Investigator allows a maximum of 10 target lesions in total and 5 per organ, if clinically relevant to enable a broader sampling of tumor burden.

8.7.6 iRECIST disease assessment

To address this limitation of RECIST 1.1 in cases of pseudoprogression under immunotherapy, Wolchok et al. developed modified 'immune-related Response Criteria' (irRC) based on the WHO criteria for the first time in 2009.[120] Subsequently, bi-dimensional irRC were adapted to the uni-dimensional immune-related RECIST (irRECIST) criteria.[121,122] In both irRC and irRECIST, new measurable tumor lesions are to be added to the sum of the target lesions, while only a significant increase (irRC ≥25%; irRECIST ≥20%) results in determination of tumor progression (iPD = 'immune-related progressive disease'). One point of criticism with respect to these criteria, particularly irRC, was that non-measurable tumor lesions (i.e. non-target lesions) did not contribute to tumor progression. Moreover, in case of stable or minimal tumor decrease following pseudoprogression, iPD was confirmed according to irRC and irRECIST.

To reduce inconsistency between different studies depending on which response assessment protocol was utilized, the official RECIST Working Group published the new iRECIST guideline in 2017 to assess response to immunotherapy in clinical trials. In iRECIST, the basic principles of defining tumor lesions as measurable or non-measurable and assessing tumor responses are unchanged from RECIST 1.1. **However, an additional confirmatory imaging study must be done to confirm or withdraw an 'unconfirmed' tumor progression after any initial increase in size.**

Given the novel nature of pixatimod and the uncertain nature of tumor kinetics in patients receiving nivolumab/pixatimod ± Cy, although RECIST will be primarily used to assess response, a key secondary endpoint is response by iRECIST as delineated in **Figure 8.7.6-1** and **Table 8.7.6-2** below.[123] iRECIST will be used by the Investigator to assess tumor progression, and make treatment decisions. This is further elaborated upon in **Appendix 4.**

Figure 8.7.6-1: iRECIST Response Evaluation

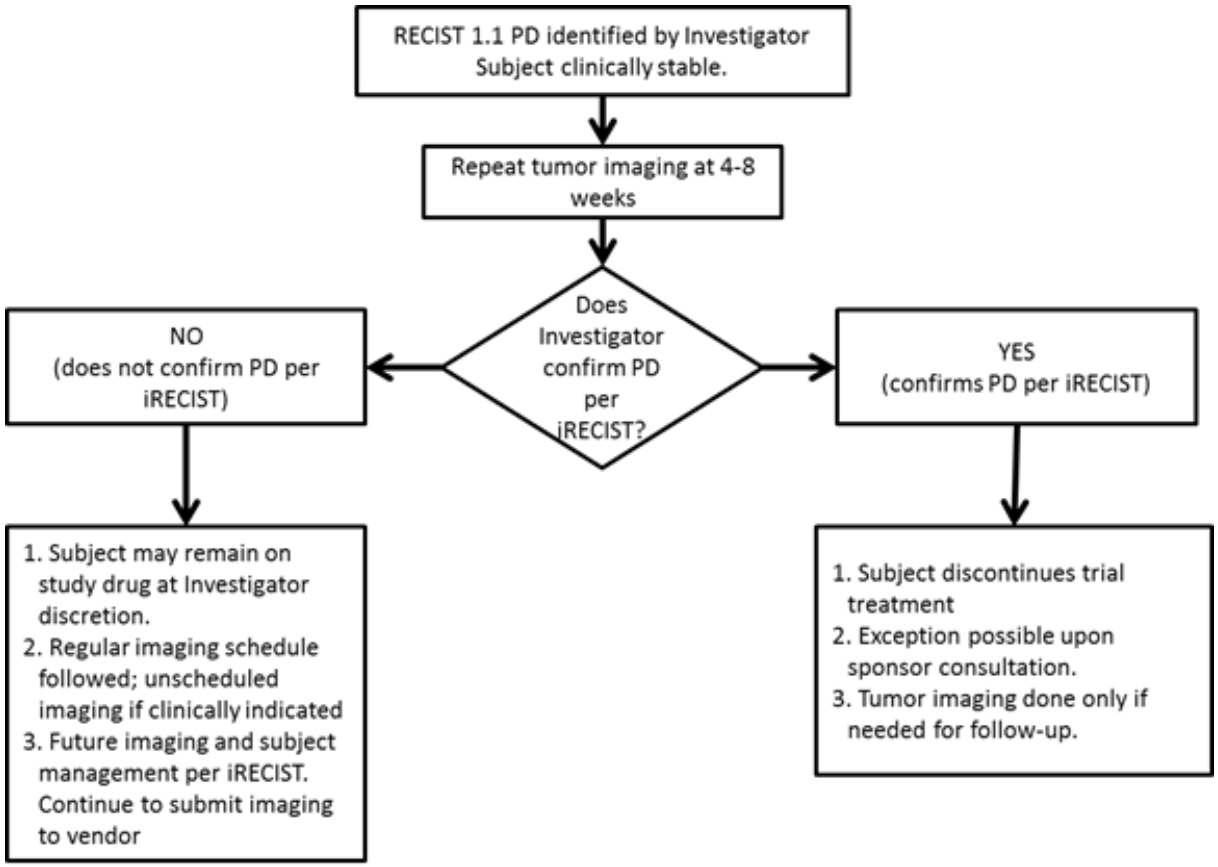

Table 8.7.6-2: Imaging and Treatment after First Radiologic Evidence of Progressive Disease

	Clinically Stable		Clinically Unstable	
	Imaging	**Treatment**	**Imaging**	**Treatment**
First radiologic evidence of PD by RECIST 1.1	Repeat imaging at 4 to 8 weeks to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory tumor imaging by site by iRECIST	Repeat imaging at 4 to 8 weeks to confirm PD per Investigator's discretion only	Discontinue treatment
Repeat tumor imaging confirms PD (iCPD) by iRECIST per Investigator assessment	No additional imaging required	Discontinue treatment (exception is possible upon consultation with Sponsor-Investigator)	No additional imaging required.	Not applicable
Repeat tumor imaging shows iUPD by iRECIST per Investigator assessment	Repeat imaging at 4 to 8 weeks to confirm PD. May occur at next regularly scheduled imaging visit	Continue study treatment at the Principal Investigator's discretion	Repeat imaging at 4 to 8 weeks to confirm PD per Investigator's discretion only	Discontinue treatment
Repeat tumor imaging shows iSD, iPR, or iCR by iRECIST per Investigator assessment.	Continue regularly scheduled imaging assessments	Continue study treatment at the Principal Investigator's discretion	Continue regularly scheduled imaging assessments.	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion. Next tumor imaging should occur according to the regular imaging schedule.

iCPD = iRECIST confirmed progressive disease; iCR = iRECIST complete response; iRECIST = modified Response Evaluation Criteria in Solid Tumors 1.1 for immune-based therapeutics; iSD = iRECIST stable disease; iUPD = iRECIST unconfirmed progressive disease; PD = progressive disease; RECIST 1.1 = Response Evaluation Criteria in Solid Tumors 1.1.

8.8 Adverse Events (AEs)

8.8.1 Definitions

8.8.1.1 Definition of AEs

An AE is an untoward or medical occurrence associated with the use of study drug (active or placebo drug, biologic, or device) in clinical investigation subjects, which does not necessarily have a causal relationship with the study drug. An AE can therefore be any unfavorable and unintended symptom, sign, disease or condition, or test abnormality whether or not considered related to study drug. AEs that do not meet the definition for serious AEs (SAEs) are considered non-SAEs.

AEs include:

- Changes described by the subject or signs observed by the Investigator or medical staff.
- Test abnormalities (i.e., laboratory tests, ECGs) that result in an alteration in medical care (diagnostic or therapeutic).

Disease Progression is not considered an AE in this study.

Abnormalities present at baseline are considered AEs only if they reoccur after resolution or they worsen during the study.

8.8.1.2 SAE and serious unexpected adverse reactions (SUSAR)

An SAE is any AE that fulfills one of the criteria outlined in **Table 8.8.1.2-1**.

Table 8.8.1.2-1: **Criteria for Determination of Serious Adverse Events (SAEs)**

Death	An AE that results in death. *In this study, deaths that are unequivocally due to Disease Progression are **not** to be reported as SAEs.
Life-threatening AE	An AE that places the subject, in the view of the Investigator, at immediate risk of death from the AE as it occurred (i.e., does not include an AE that had it occurred in a more severe form, might have caused death).
Required or prolonged inpatient hospitalization	An AE that results in an initial inpatient hospitalization or prolongs an existing hospitalization of the subject. If a subject is hospitalized as part of the clinical use of the study drug, a period of normal hospitalization will be outlined in the protocol or by the judgment of the Investigator. Hospitalizations longer than this period will be prolonged hospitalizations.
Persistent or significant disability/incapacity	An AE that results in a substantial disruption of a subject's ability to conduct normal life functions.
Congenital anomaly/birth defect	A congenital anomaly/birth defect that occurs in the offspring of a subject exposed to the study drug.
Important medical event	An AE that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed above.

Examples of such “important medical events” include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as with important medical events described above.

Events that meet SAE criteria must be recorded and reported regardless of expectedness or assessed association with study drug.

Note:

- Planned hospital admissions or surgical procedures for elective procedures or for an illness or disease that existed before the signing of the ICF or before the subject was enrolled in the study will not be captured as SAEs.
- If planned admissions or procedures occur at a time other than what was planned (i.e., due to an exacerbation in the preexisting illness or disease), they should be reported as SAEs.

8.8.1.3 Unexpected AEs

An unexpected AE is any AE that is not consistent in specificity or severity with the current IB for either pixatimod or nivolumab.

8.8.2 Evaluation of AEs and SAEs

The Investigator or designee is responsible for making an assessment as to the severity/grade (as defined in **Section 8.8.2.1**, below), causality/relationship (as defined in **Section 8.8.2.2**, below), and outcome of AEs and SAEs (as defined in **Section 8.8.2.3**, below). In addition, the Investigator or designee must report any actions taken as a result of an AE or SAE.

8.8.1.4 AE severity/grade

For each recorded AE or SAE, the Investigator or designee must make an assessment of Grade using the Common Terminology Criteria for Adverse Events (CTCAE) version 5. Grade refers to the severity of the AE. Note that severity is not the same as “seriousness”. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.8.1.5 Causality and relationship to study drug(s)

For each AE or SAE, the Investigator will determine whether there is a reasonable possibility demonstrated by evidence that suggests a causal relationship between the study drug regimens – nivolumab and pixatimod (**Cohorts 2 and 3**) and nivolumab, pixatimod and low-dose cyclophosphamide (**Cohort 1**) - and the AE according to the categories provided in **Table 8.8.2.2-1**. Attribution of adverse events specifically to either pixatimod or nivolumab is challenging, therefore, the relationship to study drug should be based on attribution to the combination of the two drugs, not a single drug.

The cumulative experience with low-dose cyclophosphamide suggests that AE/SAE related to this agent and/or its administration are rare. However, investigators will make every effort to evaluate for AE/SAE that may be related to cyclophosphamide.

An AE with causal relationship not initially determined will require follow-up to assign causality which must be made by the Investigator prior to completion of the study. The Investigator may change his/her opinion of causality in light of follow-up information; if this occurs, the Investigator must amend the AE or SAE information accordingly.

Table 8.8.2.2-1: Classifications for Adverse Event (AE) Causality/Relationship

Classification	Definition
Unrelated	There is no suspicion of a causal relationship between exposure to the study drug regimen and the AE; another cause of the AE has been identified, no temporal association with study drug has been identified, or the study drug cannot be implicated.
Possibly related	There is some evidence supporting the possibility of a causal relationship between study drug regimen exposure and the AE; an alternative explanation (i.e., concomitant drug or concomitant disease) is inconclusive, the temporal association with study drug is reasonable, and the causal relationship cannot be excluded.
Probably related	An adverse event that has a timely relationship to the administration of the investigational drug regimen and follows a known pattern of response, but for which a potential alternative cause may be present.
Definitely related	There is strong evidence that there is a causal relationship between study drug regimen and the AE; the AE cannot be reasonably explained by an alternative explanation (i.e., concomitant drug or concomitant disease) and the temporal association with study drug is suggestive of a causal relationship.

8.8.1.6 Classification of AE outcome

Adverse event outcome describes the status of the AE at the last observation. The Investigator will document the outcome of each AE or SAE using the categories provided in **Table 8.8.2.3-1**.

Table 8.8.2.3-1: Classifications for Adverse Event (AE) Outcome

Classification	Definition
Fatal	Termination of life as a result of an AE.
Not recovered/not resolved	Subject has not recuperated, or the AE has not improved.
Recovering/resolving	Subject is recuperating or the AE is improving.
Recovered/resolved	Subject has recuperated, the AE resolved, or returned to baseline status / stabilized.
Recovered/resolved with sequelae	Adverse event has resolved, but the subject has been left with symptoms or pathology.
Unknown	Not known, not observed, not recorded, or refused.

8.8.1.7 Action taken regarding AE

The Investigator will provide the action taken regarding study drug in response to the AE. Classifications for each of the potential actions taken are provided in **Table 8.8.2.4-1**. More than one option may apply to a single AE/SAE. For example, study drug may be delayed, and the dose

reduced in response to an AE. Action related to nivolumab, pixatimod and/or low-dose cyclophosphamide will be assessed and recorded separately in the EDC.

Table 8.8.2.4-1: Classifications for Actions Taken Regarding an Adverse Event (AE)

Classification	Definition
Dose not changed	No change in administration of study drug
Dose reduced[1]	Reduction in the amount of study drug administered
Study drug interrupted	Temporary interruption (termination) in administration of the study drug
Study drug withdrawn	Administration of the study drug terminated (no further dosing)
Not applicable	Determination of a value is not relevant in the current context
Unknown	Not known, not observed, not recorded, or refused
[1]Refer to **Section 7.2.2. (nivolumab)**, **Section 7.2.3. (pixatimod)** and **Section 7.2.4. (Cy)** regarding dose reductions of nivolumab, pixatimod or Cy due to dose-limiting toxicities, respectively.	

8.8.3 Timeframe for AE/SAE Collection

The Investigator is required to record all AEs occurring during the clinical study (21 CFR 312.64[b] and ICH E6 [R1]) starting from the date of first dose of study treatment until 30 days after the last dose of nivolumab and pixatimod (± cyclophosphamide) on the AE page of the eCRF. 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. SAEs as defined in **Section 8.8.1.2.** must also be reported to the Sponsor-Investigator or representative within 24 hours of knowledge of their occurrence, in accordance with **Section 8.8.5**.

8.8.3.1 TRAE and treatment-related SAE (TRSAE)

TRAEs and treatment-related SAEs (TRSAEs) are defined as AEs or SAEs that started or worsened in severity on or after the date the study drugs were first administered.

TRAE/TRSAE information will be collected from the time of the subject's first receipt of nivolumab and pixatimod (± cyclophosphamide) until 30 days after the last dose of nivolumab and pixatimod (± cyclophosphamide).

8.8.3.2 SAEs

SAE information will be collected from the point the subject starts study treatment until 90 days after the last dose of nivolumab and pixatimod (± cyclophosphamide). If, at any time after the subject has completed participation in the study, the Investigator or study staff becomes aware of an SAE that they believe is possibly related or related to nivolumab and pixatimod (± cyclophosphamide, see Section **8.8.1.2**), then the event and any known details must be reported promptly to the Sponsor-Investigator. The reporting instructions described in **Section 8.8.5** must be followed.

8.8.4 Recording AEs/SAEs

All AEs and SAEs experienced by a subject will be recorded on the appropriate research record. Information including a concise description of the event; date and time of event onset and resolution; determination of seriousness, severity, corrective treatment, outcome, relationship to study drug; and action taken regarding the study drug will be recorded.

Vital signs, laboratory results, and other safety assessments as obtained as detailed in **Sections 8.4, 8.5,** and **8.6** will be recorded as AEs if they are determined to be clinically significant findings in the opinion of the Investigator. When possible, a diagnosis should be recorded as an AE, rather than symptoms or isolated laboratory abnormalities related to that diagnosis. A medical or surgical procedure is not an AE; rather the condition leading to the procedure should be recorded as the AE. If the condition is not known, the procedure must be recorded as an AE instead. Similarly, death is not an AE, but is rather the outcome of the AE(s) that resulted in death. If the AE(s) leading to death are not known, then death must be reported as an AE.

All SAEs experienced by the subject will be entered into the CTMA and reported to the Sponsor-Investigator or designee, in accordance with **Section 8.8.5**.

8.8.5 Reporting SAEs and Serious Unexpected AEs (SUAE)

8.8.5.1 SAE and SUAE reporting

All events meeting the definition of a serious adverse event, which occur after the date of first dose of study treatment and within 30 days after the last dose of study treatment, should be reported according to the CRS SAE checklist and SAE form. The initial SAE form should be sent to the following within 24 hours of the Principal Investigator becoming aware:

- Sponsor-Investigator
  Email: davard@upmc.edu;
- crssafetysubmissions@upmc.edu
- The Aculeus Therapeutics Drug Safety contact information is available for SAE reporting on a 24-hour basis and is reviewed during normal business hours. The contact information is as follows: **Email:** mdevlin@aculeustx.com
- Local Institutional Review Board when reporting requirements are met

In addition to completing appropriate patient demographic and suspect medication information, the report should include as applicable the following information that is available at the time of report within the CRS departmental SAE form:

  - CTCAE term(s) and grade(s)
  - Current status of study drug
  - Intervention(s) to address the AE (testing and result, treatment and response)
  - Hospitalization and/or discharge dates
  - Event relationship to study drug combination

8.8.5.2 Events of clinical interest (ECI)

Selected non-serious and serious adverse events are also known as **Events of Clinical Interest** (ECI) and must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Aculeus Therapeutics Drug Safety Contact (see **Section 8.8.5.1.**).

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

For the time period beginning at treatment initiation through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Checkmate product, must be reported within 24 hours to the Sponsor-Investigator and within 24 hours to Aculeus Therapeutics Drug Safety Contact.

ECI for this trial include:

- An overdose of Aculeus Therapeutics product

8.8.5.3 Pregnancies

Female subjects or the partners of male subjects who discover they are pregnant within a year of their last nivolumab and pixatimod (± cyclophosphamide) dose will be instructed to notify the Investigator immediately.

If the Investigator learns of a report of pregnancy at any time after signing the ICF, the Investigator must report the pregnancy to Aculeus Therapeutics Pharmaceuticals Drug Safety within 24 hours (following the same reporting process outlined in **Section 8.8.5.1)**.

The Investigator will inform the subject that the Sponsor-Investigator or its designee is required to gather information regarding the course and outcome of a pregnancy that has occurred after exposure to a study drug. The progress of the pregnancy must be followed until the outcome of the pregnancy is known (i.e., delivery, elective termination, or spontaneous abortion). If the pregnancy results in the birth of a child, additional follow-up information may be requested.

The Investigator will be asked to obtain follow-up information no later than 2 months after the gestational period to obtain maternal/fetal/neonatal outcome and any other relevant information. Follow-up information may be requested at additional time points. All study-related contacts involving a known pregnancy should include pregnancy status assessment until pregnancy outcome is known.

Please note that pregnancy in and of itself is not an AE or an SAE. Pregnancy should not be entered into the eCRF as an AE unless the Investigator suspects an interaction between the study drug and the contraceptive method. Additionally, all information received will be assessed for any AEs and SAEs and processed per study guidelines. If the subject is discontinued because of pregnancy, pregnancy will be documented as the reason for study discontinuation. Spontaneous abortions and stillbirths will be reported as SAEs.

8.8.6 IND safety report

The Sponsor-Investigator must notify FDA and all participating investigators (i.e., all investigators to whom the Sponsor-Investigator is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the Sponsor-Investigator determines that the information qualifies for reporting under **Sections 8.8.6.1. to 8.8.6.3.** below. In each IND safety report, the Sponsor-Investigator must identify all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction, and must analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information.

8.8.6.1 Serious and unexpected adverse reactions

The Sponsor-Investigator must report any suspected adverse reaction that is both serious and unexpected. The Sponsor-Investigator must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome);

- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);

- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

8.8.6.2 Findings from other studies

The Sponsor-Investigator must report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies (other than those reported under **Section 8.8.6.1.),** whether or not conducted under an IND, and whether or not conducted by the Sponsor-Investigator, that suggest a significant risk in humans exposed to the drug. Ordinarily, such a finding would result in a safety-related change in the protocol, informed consent, investigator brochure (excluding routine updates of these documents), or other aspects of the overall conduct of the clinical investigation.

8.8.6.3 Findings from animal and/or *in vitro* studies

The Sponsor-Investigator must report any findings from animal or *in vitro* testing, whether or not conducted by the Sponsor-Investigator, that suggest a significant risk in humans exposed to the drug, such as reports of mutagenicity, teratogenicity, or carcinogenicity, or reports of significant organ toxicity at or near the expected human exposure. Ordinarily, any such findings would result in a safety-related change in the protocol, informed consent, investigator brochure (excluding routine updates of these documents), or other aspects of the overall conduct of the clinical investigation.

8.8.6.4 Increase rate of occurrence of serious suspected adverse reactions

The Sponsor-Investigator must report any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.

8.8.6.5 Submission of IND safety reports

The Sponsor-Investigator must submit each IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. FDA will periodically issue guidance on how to provide the electronic submission (e.g., method of transmission, media, file formats, preparation and organization of files). The Sponsor-Investigator may submit foreign suspected adverse reactions on a Council for International Organizations of Medical Sciences (CIOMS) I Form instead of a FDA Form 3500A. Reports of overall findings or pooled analyses from published and unpublished in vitro, animal, epidemiological, or clinical studies must be submitted in a narrative format. Each notification to FDA must bear prominent identification of its contents, i.e., "IND Safety Report," and must be transmitted to the review division in the Center for Drug Evaluation and Research or in the Center for Biologics Evaluation and Research that has responsibility for review of the IND. Upon request from FDA, the Sponsor-Investigator must submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

8.8.6.5.1 Unexpected fatal or life-threatening suspected adverse reaction reports

The Sponsor-Investigator must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

8.8.6.5.2 Reporting format or frequency

FDA may require the Sponsor to submit IND safety reports in a format or at a frequency different than that required under Sponsor-Investigator paragraph. The Sponsor may also propose and adopt a different reporting format or frequency if the change is agreed to in advance by the director of the FDA review division that has responsibility for review of the IND.

8.8.6.5.3 Investigations of marketed drugs

A Sponsor of a clinical study of a drug marketed or approved in the United States that is conducted under an IND is required to submit IND safety reports for suspected adverse reactions that are observed in the clinical study, at domestic or foreign study sites. The Sponsor must also submit safety information from the clinical study as prescribed by the post marketing safety reporting requirements.

8.8.6.5.4 Reporting study endpoints

Study endpoints (e.g., mortality or major morbidity) must be reported to FDA by the Sponsor-Investigator as described in the protocol and ordinarily would not be reported under **Section 9.6**. However, if a serious and unexpected adverse event occurs for which there is evidence suggesting a causal relationship between the drug and the event (e.g., death from anaphylaxis), the event must be reported under Serious and unexpected suspected adverse reaction as a serious and unexpected suspected adverse reaction even if it is a component of the study endpoint (e.g., all-cause mortality).

8.8.7 Follow-Up

- The sponsor must promptly investigate all safety information it receives.
- Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such, i.e., "Follow-up IND Safety Report."
- If the results of a sponsor's investigation show that an adverse event not initially determined to be reportable under section IND safety reports of this section is so reportable, the sponsor must report such suspected adverse reaction in an IND safety report as soon as possible, but in no case later than 15 calendar days after the determination is made.

8.8.8 Disclaimer

A safety report or other information submitted by a sponsor under this part (and any release by FDA of that report or information) does not necessarily reflect a conclusion by the sponsor or FDA that the report or information constitutes an admission that the drug caused or contributed to an adverse event. A sponsor need not admit, and may deny, that the report or information submitted by the sponsor constitutes an admission that the drug caused or contributed to an adverse event.

The principal investigator must promptly review all information relevant to the safety of the drug obtained or otherwise received from foreign or domestic sources, including information derived from any clinical or epidemiological investigations, animal or in vitro studies, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities and reports of foreign commercial marketing experience for drugs that are not marketed in the United States. The study sponsor must notify all participating investigators of potential serious risks, from clinical trials or any other source, as soon as possible.

8.8.9 Reporting AEs to the responsible Institutional Review Board (IRB)

In accordance with applicable policies of the University of Pittsburgh Institutional Review Board (IRB), the Sponsor-Investigator will report, to the IRB, any observed or volunteered adverse event that is determined to be 1) associated with the investigational drug or study treatment(s); 2) serious; and 3) unexpected. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the sponsor-investigator’s receipt of the respective information. Adverse events which are 1) associated with the investigational drug or study treatment(s); 2) fatal or life-threatening; and 3) unexpected will be reported to the IRB within 24 hours of the Sponsor-Investigator’s receipt of the respective information.

Follow-up information to a reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the Sponsor-Investigator’s follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the Sponsor-Investigator will report the adverse event to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

8.8.10 Follow-up of AEs and SAEs

All AEs and SAEs documented at a previous visit that are designated as either recovering/resolving or not recovered/resolved, will be reviewed by the Investigator at subsequent visits.

All AEs will be followed until resolution of AE, completion of the subject's participation, or study termination, whichever occurs first.

Serious AEs and AEs resulting in discontinuation will be followed until one of the following occurs:

- The event resolves.
- The event stabilizes.
- The event returns to a baseline value, if a baseline value is available.
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct.
- The Investigator agrees that follow-up is no longer necessary.

Follow-up reports from the Investigator must be provided as indicated using the SAE report form within **24 hours** of the Investigator's first knowledge of the new information. Additional information (i.e., hospital records, laboratory, or other diagnostic test results) should be provided if requested and/or indicated.

Rules for AE/SAE follow up apply to all subjects, including those who withdraw consent prior to study completion (to the extent allowed). The Investigator will ensure that follow up includes further investigations to elucidate the nature and/or causality of the AE/SAE. These investigations must be consistent with appropriate medical management and subject consent.

Investigators are not obligated to actively seek AEs or SAEs in former study subjects that occur pursuant to the follow-up period. However, if the Investigator or designee learns of any AE or SAE at any time after a subject has been discharged from the study and the event is considered as reasonably related to the study drug, the Sponsor-Investigator will notify Aculeus Therapeutics.

8.9 Tumor Tissue Collection and Correlative Studies

The total amount of blood/tissue to be drawn/collected over the course of the trial (from pre-trial to post-trial visits), including approximate blood/tissue volumes drawn/collected by visit and by sample type per subject can be found in the **Laboratory Manual** and is reproduced below.

Planned correlative analyses will include flow cytometric analyses of tumor samples, TIL and peripheral blood mononuclear cells (PBMC), IHC analyses of pre-/post- treatment tumor samples, tumor whole exome/RNA sequencing. Single-cell RNA sequencing will be performed on a subset of treated patients. Studies will be performed under the direction of the Principal Investigator along with co-Investigator Dr. Hassane Zarour in Dr. Zarour's lab.

<u>Tumor tissue</u>
Patients must undergo protocol mandated research biopsies during **Screening** and on **Cycle 2 Day 1.** Biopsies may be on any suitable previously non-radiated representative lesion. Biopsy site must be sized suitably to permit investigator to obtain minimum quantity of tissue as specified in

the **Schedule of Activities** (**Section 2.0**), which is 6 core biopsies of minimum 16gauge (or greater). Tumor tissue will be processed as described in the **Laboratory Manual**.

Primary tumor (if available)
FFPE tissue block(s) or 10 unstained slides (air dried) from patients' originally resected primary tumor (melanoma, NSCLC or CRC) will be requested as well.

Blood biospecimens
Blood biospecimens will be collected at baseline, and per the schedule outlined in the trial **Schedule of Activities** (**Section 2.0**).

At **EACH time point** please submit the following:

- Six (6) 10mL sodium heparin tubes (Fisher Scientific catalog number 02-685-114A)
- Two (2) 5mL SST tubes (Fisher Scientific catalog number 02-683-145)
- One (1) 10mL Streck cf DNA tube
- One (1) 4mL K2 EDTA tube (**SCREENING ONLY**) (Fisher Scientific catalog number 02-689-4)

Each tube must be clearly labeled to include:

- Protocol number: **HCC 20-266**
- Patient number: UOP-XXX
- Patient initials:
- Originating institution/investigator name:
- Date and time drawn:
- Collection time point:

Stool specimens
Stool biospecimens will be collected at baseline, and per the schedule outlined in the trial **Schedule of Activities** (**Section 2.0**).

Dietary questionnaire
DHQ-3 questionnaire may be administered electronically or on paper. Electronic administration is preferred. Data will be entered into DHQ-3 online per instructions as outlined in **Appendix 1**.

8.9 Pharmacokinetics Sampling

8.9.1 Pixatimod (PG545) Pharmacokinetics

Pixatimod pharmacokinetic sampling will be collected from 10 subjects in Cohort 1, and a total of 10 subjects from Cohorts 2 and 3. Samples will be collected at the following time-points relative to the pixatimod infusion (Cycle 1 Day 1):

Pre-dosing
Post-dosing:

- 30 minutes post-infusion (± 5 minutes)
- 2 hours post-infusion (± 15 minutes)
- 4 hours post-infusion (± 15 minutes)
- 6 hours post-infusion (± 30 minutes)

- 24 hours post-infusion (± 60 minutes)
- 72 hours post-infusion (± 24 hours)
- 168 hours post-infusion (± 24 hours) (i.e. prior to dosing on Cycle 1 Day 8).

Post-dose samples cannot be taken from the same vein as used for dosing. At each of the sampling periods, samples will be collected into 2.7 mL sodium citrate tubes. At each of the collection time points each subject will have 1 x 2.7 mL collection, with the exception of the Cycle 1 Day 1 pre-dose sample where 2 x 2.7 mL collections will be required. Pharmacokinetic analysis will be performed using an LC-MS/MS method validated for pixatimod in human plasma. Samples will be clearly labelled, frozen, and forwarded to the contracted laboratory for analysis. The laboratory will measure pixatimod concentrations on each sample. The exact date and time of infusion state and finish, and the exact time of each sample, will be recorded on a supplementary page of the CRF.

The individual pixatimod pharmacokinetic profile and parameters of the AUC (area under the curve), Cmax (maximum concentration), Tmax (time to maximum concentration), and t1/2, clearance (Cl) and volume of distribution (Vd) will be estimated provided the 'fit' of the time versus concentration curves permit such analysis. Summary statistics will be used to describe pharmacokinetic results.

8.9.2 Cyclophosphamide Pharmacokinetics

Cyclophosphamide pharmacokinetic sampling will be collected from 10 subjects in **Cohort 1** only. Samples will be collected at the following time-points relative to the pixatimod infusion (Cycle 1 Day 1):

Pre-dosing
Post-dosing:

- 10 minutes (± 5 minute)
- 30 minutes post-dose (± 5 minutes)
- 1 hour post-dose (± 10 minutes)
- 2 hours post-dose (± 15 minutes)
- 4 hours post-dose (± 15 minutes)
- 6 hours post-dose (± 30 minutes)
- 24 hours post-dose (± 1 hour)

At each of the sampling periods, samples will be collected into 2.0 mL sodium heparin tubes. At each of the collection time points each subject will have 1 x 2.0 mL collection, with the exception of the Cycle 1 Day 1 pre-dose sample where 2 x 2.0 mL collections will be required. Pharmacokinetic analysis will be performed using an LC-MS/MS method validated for cyclophosphamide in human plasma. Samples will be clearly labelled, frozen, and forwarded to the contracted laboratory for analysis. The laboratory will measure cyclophosphamide concentrations on each sample. The exact date and time of infusion state and finish, and the exact time of each sample, will be recorded on a supplementary page of the CRF.

The individual cyclophosphamide pharmacokinetic profile and parameters of the AUC (area under the curve), C_{max} (maximum concentration), T_{max} (time to maximum concentration), and $t_{1/2}$, clearance (Cl) and volume of distribution (V_d) will be estimated provided the 'fit' of the time versus

concentration curves permit such analysis. Summary statistics will be used to describe pharmacokinetic results.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design

This is a phase II study of nivolumab in combination with pixatimod in 3 separate cohorts:

- Cohort 1: nivolumab, pixatimod and low-dose cyclophosphamide in MSS mCRC
- Cohort 2: nivolumab and pixatimod in PD-1 relapsed/refractory melanoma
- Cohort 3: nivolumab and pixatimod in PD-1 relapsed/refractory NSCLC.

The goal of this study is to assess response of PD-1 R/R melanoma/NSCLC to nivolumab and pixatimod; and MSS mCRC to nivolumab, pixatimod and low-dose cyclophosphamide. Simon's two-stage design will be used for each cohort separately.

9.2 Safety Monitoring

Anti-PD-1 therapy including nivolumab has been tested in PD-1 R/R melanoma, NSCLC and PD-1 naive MSS mCRC in multiple studies.

The safety profile observed thus far suggests that the combination of nivolumab and pixatimod is unlikely to pose a high risk of toxicity in the setting of PD-1 R/R melanoma and PD-1 R/R NSCLC. Hence, in this study, we will monitor dose limiting toxicities (DLTs, defined below at the end of this section) for 4 weeks for the 1st 3 patients accrued in **Cohorts 2 and 3**. If we see at least 1 DLT out of the 3 patients, we will hold the accrual in this cohort, and the study committee and the Principal Investigator (Sponsor-Investigator) will decide whether to modify or discontinue the study.

The combination of nivolumab and pixatimod has been studied in MSS mCRC in a small phase IB study wherein no untoward safety signals were observed. PD-1 inhibitor pembrolizumab and low-dose cyclophosphamide (50 mg twice daily, 1-week-on, 1-week-off) has been explored in two studies in patients with advanced osteosarcoma and soft tissue sarcomas with a low incidence of grade 3/4 adverse events.[95,96] Based on the above, it is unlikely that the combination of nivolumab, pixatimod and low-dose cyclophosphamide has overlapping toxicities, nor is there a plausible basis for pharmacodynamic or pharmacokinetic interactions leading to a DLT. As such, it is not felt that this combination requires a formal phase I study. However, the combination of nivolumab, pixatimod and low-dose cyclophosphamide has hitherto not been studied in advanced cancer patients in general and MSS mCRC patients in particular. To mitigate toxicity, in **Cohort 1**, we will utilize a safety run-in and monitor toxicities continuously using the following method and decision rule.

In the safety run-in (**Cohort 1 only**), 6 patients will be enrolled at a rate of no more than 1 patient per month. Toxicities will be monitored as delineated below. At the end of the safety run-in period (defined as 1 month following the enrollment of the 6^{th} patient in **Cohort 1**), the formal DLT rate will be calculated. If the DLT rate is ≤25%, the study will continue to proceed. Should the DLT rate exceed 25% (i.e. at least 2 DLTs in the first 6 enrolled patients), the study will be stopped.

To further mitigate toxicity, we will use a Bayesian monitoring scheme to continuously monitor the DLT rate of the study combination at 25%. A non-informative prior of Beta(1, 1) for the DLT rate will be used. We will hold the accrual if the posterior probability Pr (DLT rate>25%) ≥ 0.7 and the study committee and the PI will decide whether to modify or discontinue the study. The stopping boundary for toxicity is given in the following table.

Number of DLTs≥	in Number of Patients =
4	7-9
5	10-12
6	13-15
7	16-18
8	19-22
9	23-25
10	26-27

We simulate the operating characteristics under various assumed true toxicity rate (see the following table):

Scenario	Prob.Of.Tox	Prob.Early.Stop
1	0.05	0.001
2	0.15	0.05
3	0.25	0.30
4	0.35	0.67
5	0.45	0.92

A DLT is defined as any adverse event(s) (AEs) considered related to nivolumab and pixatimod that occurs during the first 28 days of therapy in **Cohorts 2** and **3**; and related to nivolumab and pixatimod and cyclophosphamide that occurs during the safety run-in period (defined as 1 month following the enrollment of the 6th patient) in **Cohort 1**. During DLT monitoring period, no further accrual will be permitted. Any patient who has started the study treatment will be evaluable for safety.

The following events will be considered DLTs if deemed related to study therapy:

- Hematologic
    - Grade 4 neutropenia
    - Febrile neutropenia, defined as absolute neutrophil count (ANC) ≤1000/mm3 with a temperature of ≥ 38.3 degrees °C
    - Grade ≥3 neutropenic infection
    - Grade ≥3 thrombocytopenia with bleeding
    - Grade 4 thrombocytopenia
- Non-hematologic
    - Grade >3 fatigue lasting ≥1 week
    - Grade ≥3 nausea, vomiting or diarrhea despite maximal medical intervention
    - Grade ≥3 toxicities (non-laboratory)

  - Grade >3 electrolyte abnormality that last >72 hours, unless the patient has clinical symptoms, in which case all grade >3 electrolyte abnormalities regardless of duration should count as a DLT.
  - Grade ≥4 hepatic toxicity [excluding isolated grade ≥3 gamma-glutamyltransferase (GGT) abnormalities] according to Hy's law.[124]
- Any death (grade 5 event) not clearly due to the underlying disease and/or extraneous cause(s).

- Other (non-AST/non-ALT) non-hematologic Grade ≥3 laboratory value if the abnormality leads to overnight hospitalization.

An AE believed to be caused by tumor flare or pseudoprogression is not considered a DLT. Any such cases that would otherwise meet DLT criteria must be discussed immediately with the Principal Investigator. If the Principal Investigator agrees that tumor flare is a likely explanation for the AE, treatment with nivolumab/pixatimod may continue so long as the subject is closely monitored by the Principal Investigator or study staff while on study.

9.3 Efficacy Decision Rule

We hypothesize that the nivolumab/pixatimod combination in PD-1 relapsed/refractory (R/R) cutaneous melanoma and NSCLC patients will be associated with anti-tumor effects. We also hypothesize that the nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC patients will be associated with anti-tumor effects.

We further hypothesize that nivolumab/pixatimod/cyclophosphamide combination in MSS mCRC patients and nivolumab/pixatimod combination in PD-1 R/R melanoma or NSCLC will be associated with increased $CD8^+$ T cells *intra-tumorally* and $PD\text{-}1^+Ki67^+$ $CD8^+$ T cells *peripherally* in responders *on treatment* compared to *baseline*; and that response will be associated with evidence of antigen-specific immunity responses intra-tumorally and peripherally.

In the 1st stage of **Cohort 1** (**MSS mCRC**), we will enroll 13 patients. If ≥1 response(s) are seen, 14 additional patients will be enrolled in the 2nd stage. The nivolumab/pixatimod/cyclophosphamide combination will be considered worthy of further evaluation if ≥4 responses (ORR 15%) are seen across both stages in this cohort. This study design, using Simon's 2-stage Minimax design, provides 80% power with 5% type I error to detect a response rate of 20% against a null hypothesis of 5%.

For **Cohorts 2** (**PD-1 R/R melanoma**) and **3** (**PD-1 R/R NSCLC**), we will use the same design, but separately, as follows. In the 1st stage, will enroll 9 patients. If ≥1 response(s) are seen, 8 additional patients will be enrolled in the 2nd stage. The combination will be considered worthy of further evaluation if ≥3 responses (ORR 18%) are seen across both stages. This study design, using Simon's optimal 2-stage design, provides 81% power with 5% type I error to detect a response rate of 25% against a null hypothesis of 5%.

9.4 Efficacy Analysis

Definition of response-evaluable patient

Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6)

early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). All patients who **met the eligibility criteria**; received **at least 1 dose each** of nivolumab and pixatimod (**cohorts 2 and 3**); and **at least 1 dose each** of nivolumab, pixatimod and low-dose Cy (**cohort 1**); and underwent restaging imaging are evaluable for response.

Definition of survival endpoints
PFS is defined as the time from initiation of treatment till cancer relapse or death. All suspected relapses should be biopsied to confirm relapse. In the event where this is difficult and/or dangerous, imaging may be used as a surrogate.

OS is defined as the time from initiation of treatment till death. Where possible, investigators should endeavor to confirm if death is related to disease or other concomitant illness. In the event where this is impossible to confirm conclusively, it will be assumed that death is related to disease progression.

Kaplan-Meier estimates of PFS and OS will be provided. The corresponding median survival time (with 95% confidence intervals) will be determined, along with survival estimates at selected time points (e.g. 6 months, 1 years, and 2 years).

9.5 Safety Analysis

Definition of safety-evaluable patient
Any patient who has received at least 1 dose each of nivolumab and pixatimod (**cohorts 2 and 3**); and at least 1 dose each of nivolumab, pixatimod and cyclophosphamide (**cohort 1**) is evaluable for safety.

As per NCI CTCAE Version 5.0, the term toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. The maximum grade for each type of toxicity will be recorded for each patient, and frequency tables will be reviewed to determine toxicity patterns.

As these agents singly and in combination have previously been studied in the diseases in question and the relevant biologically active doses determined, no “dose-escalation” will be performed in this study. However, to accurately capture toxicities for the use of this agent in this space, we will be monitoring toxicities closely. The detailed data and safety monitoring plan is in **Section 9.7**.

9.6 Biomarker Analysis

To search for potential prognostic biomarkers (and toxicity marker) for the regimen, logistic regression will be used to assess the association between each marker and objective radiographic response.

The Cox proportional hazards model will be used to assess the association of each marker and survival endpoints (i.e. PFS and OS).

9.7 Data Safety and Monitoring Plan

All enrolled patients will be reviewed weekly to discuss AEs, in particular during DLT period.

Principle Investigator/Sub-investigators, regulatory, CRS management, clinical research coordinators, clinical research associates, data managers, and clinic staff meet monthly in disease center Data Safety Monitoring Boards (DSMB) to review and discuss study data to include, but not limited to, the following:

- SAEs
- Subject safety issues
- Recruitment issues
- Accrual
- Protocol deviations
- Unanticipated problems
- Breaches of confidentiality

Minutes from the disease center DSMB meetings are sent to those who are unable to participate during the scheduled meeting time.

All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 5.0. All study treatment associated adverse events that are serious, at least possibly related and unexpected will be reported to the IRB. Any modifications necessary to ensure subject safety and decisions to continue or close the trial to accrual are also discussed during these meetings. If any literature becomes available which changes the risk/benefit ratio or suggests that conducting the trial is no longer ethical, the IRB will be notified in the form of an Unanticipated Problem submission and the study may be terminated.

All study data reviewed and discussed during these meetings will be kept confidential. Any breach in subject confidentiality will be reported to the IRB in the form of an Unanticipated Problem submission. The summaries of these meetings are forwarded to the UPCI DSMC which also meets monthly following a designated format.

For all research protocols, there will be a commitment to comply with the IRB's policies for reporting unanticipated problems involving risk to subjects or others (including adverse events). DSMC progress reports, to include a summary of all serious adverse events and modifications, and approval will be submitted to the IRB at the time of renewal.

Protocols with subjects in long-term (survival) follow-up or protocols in data analysis only, will be reviewed twice a year rather than monthly by the disease center DSMB.

Both the UPMC Hillman Cancer Center DSMC as well as the individual disease center DSMB have the authority to suspend accrual or further investigate treatment on any trial based on information discussed at these meetings.

All records related to this research study will be stored in a double locked environment. Only the researchers affiliated with the research study and their staff will have access to the research records.

10. LABELING, PACKAGING, STORAGE, AND RETURN OF CLINICAL SUPPLIES

10.1 Investigational Product: Pixatimod (PG545)

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

Clinical supplies provided by Aculeus Therapeutics are summarized in **Table 10.1-1**.

Table 10.1-1: Product Description

Product Name	Dosage Form
Pixatimod (PG545)	Pixatimod is a white to off-white powder with no odor. It is manufactured using maltotetraose derived from hydrolysis of starch. The compound also features a cholestanol moiety conjugated to the synthetic, sulfated tetrasaccharide component. This lipophilic moiety is considered a critical structural feature of the molecule which leads to novel immunomodulatory activity and enhanced pharmacodynamic and pharmacokinetic properties compared to similarly designed compounds (known as heparan sulfate mimetics) lacking the aglycone. Pixatimod is presented in an anomerically pure form. Pixatimod drug product is presented as a frozen aqueous solution for infusion containing 60 mg or 100 mg pixatimod per vial (25 mg/mL). Prior to administration the appropriate dose of pixatimod is diluted in 250 mL 0.9% saline infusion solution and infused over one hour.

10.1.1 Packaging and Labeling Information: Pixatimod (PG545)

The pixatimod drug product vials will be labeled with the following information:

- Hillman Cancer Center (HCC) protocol number
- The batch number of the drug
- The drug name, concentration, and nominal volume per vial
- The recommended storage conditions of the secondary container
- Cautionary statement to keep away from children
- The route of administration
- Cautionary statement indicating that the drug is for investigational use only
- The name and address of Aculeus Therapeutics (Aculeus Therapeutics Pty Ltd; Bio 21 Incubator, 30 Flemington Rd, Parkville VIC 3052, Australia).

The pixatimod kit carton will be labeled with the following information:

- Hillman Cancer Center (HCC) protocol number
- Number of vials per kit
- The batch number of the drug
- The drug name, concentration, and nominal volume per vial
- The recommended storage conditions of the drug
- Cautionary statement to keep away from children
- The route of administration
- Cautionary statement indicating that the drug is for investigational use only;
- The name and address of Aculeus Therapeutics (Aculeus Therapeutics Pty Ltd; Bio 21 Incubator, 30 Flemington Rd, Parkville VIC 3052, Australia)

10.1.2 Handling, Storage and Accountability: Pixatimod (PG545)

All pixatimod drug product vials will be transported, received, stored, and handled in accordance with the container or drug product kit/vial label, the instructions supplied to the site and its designated pharmacy personnel, the site's standard operating procedures (SOPs), and applicable regulations.

Appropriate storage and transportation conditions will be maintained for the pixatimod drug product vials from the point of manufacture up to delivery of pixatimod. All shipments of pixatimod drug product vials will include a temperature monitoring device that records required storage conditions for the vials, at regular intervals for the entire time the shipment is in transit.

Upon receipt by the site, the designated site personnel will examine the shipment and temperature monitoring devices to verify the pixatimod drug product vials were received in acceptable condition. If not received in acceptable condition, the site must notify Aculeus Therapeutics and the site should quarantine the drug until a decision has been made by Aculeus Therapeutics. Once inspected, vials should be stored at the specified temperature (-20°C) in a locked area accessible only to designated site personnel until dispensing. Once dispensed, pixatimod drug product vials will be stored in a limited access area under appropriate environmental conditions.

The designated site personnel will be responsible for maintaining accurate records of the quantity and dates of all study drug supplies received, dispensed, and returned, in accordance with applicable regulations and the site's SOPs. The quantity of study drug lost, destroyed, etc. must also be accounted for and documented.

All original vials, whether empty or containing pixatimod will be kept at the site and destroyed according to the site's drug destruction standard operating procedures. Used pixatimod vials will not be dispensed again (even to the same subject) nor will they be relabeled or reassigned for use by other subjects. Contents of the pixatimod drug product vials will not be combined. At the termination of the study, a final drug accountability review and reconciliation must be completed, and any discrepancies must be investigated and their resolution documented.

All pixatimod drug product vials will be destroyed onsite as per institutional standard operating procedures, after site close out has been completed.

10.1.3 Dispensing: Pixatimod (PG545)

The pixatimod drug product vials will be dispensed and pixatimod drug product will be administered according to applicable site SOPs. Details regarding the preparation and administration of the study drug will be outlined in a study-specific pharmacy manual. Only eligible subjects participating in the study will receive pixatimod. Only authorized and qualified site staff may supply or administer pixatimod.

10.2 Commercial Product: Nivolumab

Pharmaceutical and therapeutic background of nivolumab is detailed in **Section 3.2.** Preclinical and clinical trial data pertaining to nivolumab is summarized in **Nivolumab IB**. For the purposes of this trial, commercial supply of nivolumab will be used. Nivolumab dose, schedule and administration is detailed in **Section 3.8.3**.

10.3 Commercial Product: Cyclophosphamide

Pharmaceutical and therapeutic background of cyclophosphamide is detailed in **Section 3.4.** Preclinical and clinical trial data pertaining to cyclophosphamide is summarized in **Section 3.4**. For the purposes of this trial, commercial supply of cyclophosphamide will be used. Cyclophosphamide dose, schedule and administration is detailed in **Section 3.8.6**.

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12. APPENDICES

12.1 Appendix 1: ECOG Performance Status[125]

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
*As published in Oken MM et al, Am J Clin Oncol 1982.[125]	

12.2 Appendix 2: Common Terminology Criteria for Adverse Events V5.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

12.3 Appendix 3: Contraceptive Guidance and Pregnancy Testing

Woman of Childbearing Potential (WOCBP)

- A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)
- Women in the following categories are not considered WOCBP:
  - Premenarchal
  - Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy
    - Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
  - Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
    - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

Male Participants:
Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol defined time frame in **Section 6.1**.

- Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent.
- Use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in Table A3-1 when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.
  - Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

Female Participants:

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in **Table A3-1** during the protocol-defined time frame in **Section 6.1**.

Table A3-1 Highly Effective Contraception Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
● Combined (estrogen- and progestogen- containing) hormonal contraception [b, c] ○ Oral ○ Intravaginal ○ Transdermal ○ Injectable
● Progestogen-only hormonal contraception [b, c] ○ Oral ○ Injectable
Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
● Progestogen- only contraceptive implant [b, c] ● Intrauterine hormone-releasing system (IUS) [b] ● Intrauterine device (IUD) ● Bilateral tubal occlusion
● **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
● **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies. a) Typical use failure rates are lower than perfect-use failure rates (i.e. when used consistently and correctly). b) If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable hormonal contraceptives are limited to those which inhibit ovulation.

Pregnancy Testing

WOCBP should only be included after a negative highly sensitive urine or serum pregnancy test. Pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected.

12.4 Appendix 4: Description of the iRECIST Process for Assessment of Disease Progression

Assessment at Screening and Prior to RECIST 1.1 Progression

Until radiographic progression based on RECIST 1.1, there is no distinct iRECIST assessment.

Assessment and Decision at RECIST 1.1 Progression

In participants who show evidence of radiological PD by RECIST 1.1 the Investigator will decide whether to continue a participant on study treatment until repeat imaging is obtained using iRECIST for participant management (see **Section 8.7.6, Figure 8.7.6-1 and Table 8.7.6-2**). This decision by the Investigator should be based on the participant's overall clinical condition.
Clinical stability is defined as the following:

- Absence of symptoms and signs indicating clinically significant progression of disease
- No decline in ECOG performance status
- No requirements for intensified management, including increased analgesia, or other palliative care

Any participant deemed clinically unstable should be discontinued from study treatment at site-assessed first radiologic evidence of PD and is not required to have repeat tumor imaging for confirmation of PD by iRECIST.

If the Investigator decides to continue treatment, the participant may continue to receive study treatment and the tumor assessment should be repeated 4 to 8 weeks later to confirm PD by iRECIST, per Investigator assessment.

Tumor flare may manifest as any factor causing radiographic progression per RECIST 1.1, including:

- Increase in the sum of diameters of target lesion(s) identified at baseline to ≥ 20% and ≥ 5 mm from nadir
    - Please note: the iRECIST publication uses the terminology "sum of measurements", but "sum of diameters" will be used in this protocol, consistent with the original RECIST 1.1 terminology.
- Unequivocal progression of non-target lesion(s) identified at baseline
- Development of new lesion(s)

iRECIST defines new response categories, including iUPD (unconfirmed progressive disease) and iCPD (confirmed progressive disease). For purposes of iRECIST assessment, the first visit showing progression according to RECIST 1.1 will be assigned a visit (overall) response of iUPD, regardless of which factors caused the progression.

At this visit, target and non-target lesions identified at baseline by RECIST 1.1 will be assessed as usual.

New lesions will be classified as measurable or non-measurable, using the same size thresholds and rules as for baseline lesion assessment in RECIST 1.1. From measurable new lesions, up to 5 lesions total (up to 2 per organ), may be selected as New Lesions – Target. The sum of diameters of these lesions will be calculated and kept distinct from the sum of diameters for target lesions at baseline. All other new lesions will be followed qualitatively as New Lesions – Non-target.

Assessment at the Confirmatory Imaging

On the confirmatory imaging, the participant will be classified as progression confirmed (with an overall response of iCPD), or as showing persistent unconfirmed progression (with an overall response of iUPD), or as showing disease stability or response (iSD/iPR/iCR).

Confirmation of Progression

Progression is considered confirmed, and the overall response will be iCPD, if ANY of the following occurs:

- Any of the factors that were the basis for the initial iUPD show worsening
    - For target lesions, worsening is a further increase in the sum of diameters of ≥ 5 mm, compared to any prior iUPD time point
    - For non-target lesions, worsening is any significant growth in lesions overall, compared to a prior iUPD time point; this does not have to meet the "unequivocal" standard of RECIST 1.1
    - For new lesions, worsening is any of these:
        - An increase in the new lesion sum of diameters by ≥ 5 mm from a prior iUPD time point
        - Visible growth of new non-target lesions
        - The appearance of additional new lesions
- Any new factor appears that would have triggered PD by RECIST 1.1

Persistent iUPD

Progression is considered not confirmed, and the overall response remains iUPD, if:

- None of the progression-confirming factors identified above occurs AND
- The target lesion sum of diameters (initial target lesions) remains above the initial PD threshold (by RECIST 1.1)

Additional imaging for confirmation should be scheduled 4 to 8 weeks from the scan on which iUPD is seen. This may correspond to the next visit in the original visit schedule. The assessment of the subsequent confirmation scan proceeds in an identical manner, with possible outcomes of iCPD, iUPD, and iSD/iPR/iCR.

Resolution of iUPD

Progression is considered not confirmed, and the overall response becomes iSD/iPR/iCR, if:

- None of the progression-confirming factors identified above occurs, AND
- The target lesion sum of diameters (initial target lesions) is not above the initial PD threshold.

The response is classified as iSD or iPR (depending on the sum of diameters of the target lesions), or iCR if all lesions resolve.

In this case, the initial iUPD is considered to be pseudo-progression, and the level of suspicion for progression is "reset". This means that the next visit that shows radiographic progression, whenever it occurs, is again classified as iUPD by iRECIST, and the confirmation process is repeated before a response of iCPD can be assigned.

Management Following the Confirmatory Imaging

If repeat imaging does not confirm PD per iRECIST, as assessed by the Investigator, and the participant continues to be clinically stable, study treatment may continue and follow the regular imaging schedule. If PD is confirmed, participants will be discontinued from study treatment.

NOTE: If a participant has confirmed radiographic progression (iCPD) as defined above, but the participant is achieving a clinically meaningful benefit, an exception to continue study treatment may be considered. In this case, if study treatment is continued, tumor imaging should continue to be performed following the intervals as outlined in Section 6.

Detection of Progression at Visits After Pseudo-progression Resolves

After resolution of pseudo-progression (ie, achievement of iSD/iPR/iCR), iUPD is indicated by any of the following events:

- Target lesions
  - Sum of diameters reaches the PD threshold (≥ 20% and ≥ 5 mm increase from nadir) either for the first time, or after resolution of previous pseudo-progression. The nadir is always the smallest sum of diameters seen during the entire trial, either before or after an instance of pseudo-progression.
- Non-target lesions
  - If non-target lesions have never shown unequivocal progression, their doing so for the first time results in iUPD.
  - If non-target lesions had shown previous unequivocal progression, and this progression has not resolved, iUPD results from any significant further growth of non-target lesions, taken as a whole.
- New lesions
  - New lesions appear for the first time
  - Additional new lesions appear

- Previously identified new target lesions show an increase of ≥ 5 mm in the new lesion sum of diameters, from the nadir value of that sum
- Previously identified non-target lesions show any significant growth

If any of the events above occur, the overall response for that visit is iUPD, and the iUPD evaluation process (see Assessment at the Confirmatory Imaging above) is repeated. Progression must be confirmed before iCPD can occur.

The decision process is identical to the iUPD confirmation process for the initial PD, except in one respect. If new lesions occurred at a prior instance of iUPD, and at the confirmatory scan the burden of new lesions has increased from its smallest value (for new target lesions, their sum of diameters is ≥5 mm increased from its nadir), then iUPD cannot resolve to iSD or iPR. It will remain iUPD until either a decrease in the new lesion burden allows resolution to iSD or iPR, or until a confirmatory factor causes iCPD.

Additional details about iRECIST are provided in the iRECIST publication.[123]

12.5 Appendix 5: Dietary History Questionnaire (DHQ-3) Administration

Dietary history questionnaire will be administered either on paper or electronically and data entered and stored securely electronically. Instrument will be administered by PI or designee.

Detailed information for study staff:

1. Please obtain patient's username and password from study PI.
2. Please provide instructions (see below) to patients.
3. Patients are to be instructed to provide detailed dietary information either during visit (preferred) or at home.
4. Please instruct the patients to report intake as averaged over the preceding 4 weeks.

Detailed information for patients:

- Thank you for participating in this study. We are interested in evaluating your dietary history. To do this we are using a validated questionnaire termed the "Diet History Questionnaire (DHQ-3)".
- This questionnaire takes approximately 30 minutes to complete. You can do this either while receiving your therapy or at home.
- After completing the questionnaire, you will receive a Respondent Nutrition Report. This report shows estimated daily nutrient and food group intakes based on questionnaire responses. Recommended values are only available for some nutrients and food groups.
- Please feel free to discuss this with your study doctor.

Dietary study login URL:
Your username (case sensitive): _____
Your study password (case sensitive): _____