Document Coversheet

Study Title: Phase II Study of Induction Platinum Doublet in Combination With Nivolumab Followed by Surgery or Concurrent Chemoradiation in Unresectable Stage IIIA-C Non-small Cell Lung Cancer (NSCLC)

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TITLE: Phase II study of induction platinum doublet in combination with nivolumab followed by surgery or concurrent chemoradiation in unresectable stage IIIA-C non-small cell lung cancer (NSCLC)

Short Title: Induction Chemo-Nivo in Unresectable Stage III NSCLC

PROTOCOL FACE PAGE FOR
MCC INTERVENTIONAL THERAPEUTIC PROTOCOL

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Coordinating Center: Markey Cancer Center, University of Kentucky
Participating Site: James Comprehensive Cancer Center, Ohio State University

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Commercially Available Agents: Cisplatin, Carboplatin, Gemcitabine, Paclitaxel and Pemetrexed (non-squamous only)

FDA IND Status: Trial is Non-exempt. IND # 165375 (01/20/2023)

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Revision 1 protocol / 27.February.2023 (PRMC response)
Revision 2 / 07.July.2023 (post-Medicare coverage analysis w/ BMS approval)
Revision 3 / 15.August.2023
Revision 4 / 06.September.2023
Amendment 1 / 21.December.2023
Amendment 2 / 22.February.2023
Amendment 3 / 11.April.2024

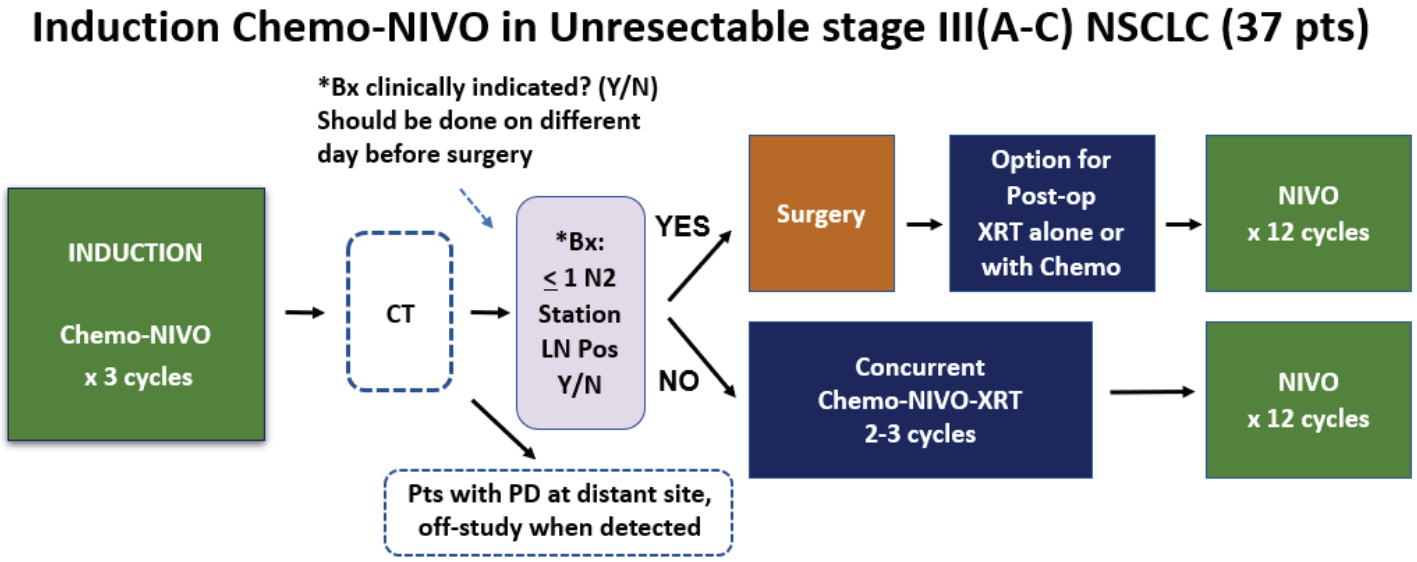
PROTOCOL HISTORY & ABBREVIATIONS

Protocol Development History – Original Version to Current Protocol Version, w/ major Summary of Changes noted	
November 2022	Lung CCART Review and Approval
FDA initial application and resubmissions in response 1/16/2023 – 1/20/2023	Protocol version dated 14DEC2022 (FDA0000) submitted to FDA. *Resolution*: FDA0001 sent LOA and label from IDS. FDA0002: revised protocol (11JAN2023), 8 items. We sent on 01/16/2023. On 1/16/2023, FDA stated revise/resubmit.
	FDA0003: On 1/17/2023, FDA sent question about stopping rule. On 1/18/2023 PI emailed FDA with a query about draft language. FDA0004: PI and Regs sent final memo and revised protocol (pvd 19JAN2023). *Resolution*: FDA deemed study as Non-exempt, 01/20/2023.
Original Protocol	Original protocol, version dated 19JAN2023.
01/30/2023	FRC Review of protocol (pvd 19JAN2023. *Resolution*: Approved (requests lab manual for correlatives)
02/24/2023	PRMC initial full review of original protocol version dated 19JAN2023 *Resolution*: Conditional Approval pending protocol edits (administrative review)
Revision 1 02/27/2023	PRMC Approval of Revision 1 protocol, pvd 27FEB2023.
03/23/2023	IRB Initial Review of Revision 1 protocol, pvd 27FEB2023. *Resolution*: IRB requested edits to IRB application and ICF, but not to protocol.
03/29/2023	UKHC CRSO completed Medicare coverage analysis.*Resolution*: Protocol edits requested.
6/13/2023	IRB Initial approval of Revision 1 protocol (pvd 27FEB2023).
05/05/2023 – 07/06/2023	UKHC CRSO reviewed the Revision 1 protocol (pvd 27FEB2023) for issues related to billing and research-related costs. We began edits in a new Revision 2 protocol draft (back/forth on draft revisions from 5/5 thru 6/15); on 06/22/23, we sent protocol draft (pvd 15JUN2023) to BMS for review; on 7/3 BMS requested minor edits to schema (note metastatic disease disqualifies pts from study); on 7/6, BMS approved the Revision 2 protocol pvd 07JUL2023.
Revision 2 07/07/2023	The Revision 1 protocol pvd 27FEB2023 was updated to reflect changes stemming from Medicare coverage analysis by UK CRSO as well as minor edits requested by the BMS sponsor. Edits comprise: a) removed docetaxel as standard agent per CRSO; b) updated study schema per BMS; c) updated research correlatives (5.1 and 11.0). new protocol is Revision 2, pvd 07JUL2023.
	07/06/2023: BMS sponsor approval of Revision 2 protocol, pvd 07JUL2023 07/19/2023: PRMC review of Revision 2 protocol, pvd 07JUL2023 *Resolution*: PRMC Approved 7/19/2023. 07/26/2023: IRB Submission of Revision 2 pvd 07JUL2023; *Resolution*: IRB Approval 04AUG2023
Revision 3 8/15/2023	The Revision 2 protocol (pvd 07JUL2023) has been updated to: a) deleted Treatment Plan B which allowed restaging biopsy to occur on same day as surgery (also deleted all language referring to plan A, plan B as now there is only one treatment plan); b) corrected Study Calendar to indicate patient-report FACT-TOI and created separate Appendix F.1 for this QOL instrument – these edits now map to Objective 1.2.5; c1) added language for 1.3.6 exploratory objective detailing the planned remote SX monitoring sub-study (Section 2.4.6); c2) Appendix F.2 clarified language for remote symptom monitoring where smartwatch will be provided to a subset of 6 Markey participants that own iOS devices; d) SCHEMA, revised title of elaborate figure (pg 4) – to reflect deletion of TX Plan B; e) corrected Table 14 radiation total doses to organs (pgs 57-58). New protocol is Revision 3, pvd 15AUG2023. PRMC approval, 8/25/2023; IRB approval, 8/22/2023.
Revision 4 9/06/2023	The Revision 3 protocol (pvd 15AUG2023) is being updated to: delete inclusion criterion re concurrent/prior malignancy; clarify collection timepoints for several exploratory correlatives (FcRn; PKs; Cytokines; EVs); update 2 other Eligib Criteria(3.1.12, 5mos post-last dose for contraception use; 3.2.16, nasal); clarify follow-up is q9wks for 2 yrs post TX initiation or death (Footnote A of Calendar); add NCT identifier. New protocol is Revision 4, pvd 06SEP2023.
9/22/2023	**Open to Accrual at Markey (on paper forms only developed for Cycle 1)**
Amendment 1 12/21/2023	The Revision 4 protocol (pvd 06SEP2023) is being updated to: add extra tube for FcRn collection (now collect 12mL, rather than 6mL; Section 5, Calendar footnotes, and Appendix G); language clarified on collection of PK samples (Section 5, Calendar footnotes, and Appendix G); new language added regarding processing and shipping of PKs and FcRn samples to Ohio State (Section 5, Calendar, and Appendix G); Multi-center language added (mostly Section 13) as Ohio State will move to open to accrual. New protocol is Amendment 1, pvd 21DEC2023.
Amendment 2 02/21/2024	The Amendment 1 protocol (pvd 21DEC2023) was revised as follows: correct the SCHEMA, noting post-induction biopsy is now as clinically indicated; add clarifying language to inclusion 3.1.1; add clarifying language to the synopsis of treatment sequence, section 6.0.
Amendment 3 04/11/2024	The Amendment 2 protocol (pvd 21FEB2024) was amended as follows: addition of separate day for collection of study required followup prior to biopsy after induction. Clarified windows for all study evalautions and clarified biopsies and nodal sampling at the time of surgery. Clarified which lymph nodes to biopsy after induction and which to biopsy at surgery.
	Placeholder for future amendment.

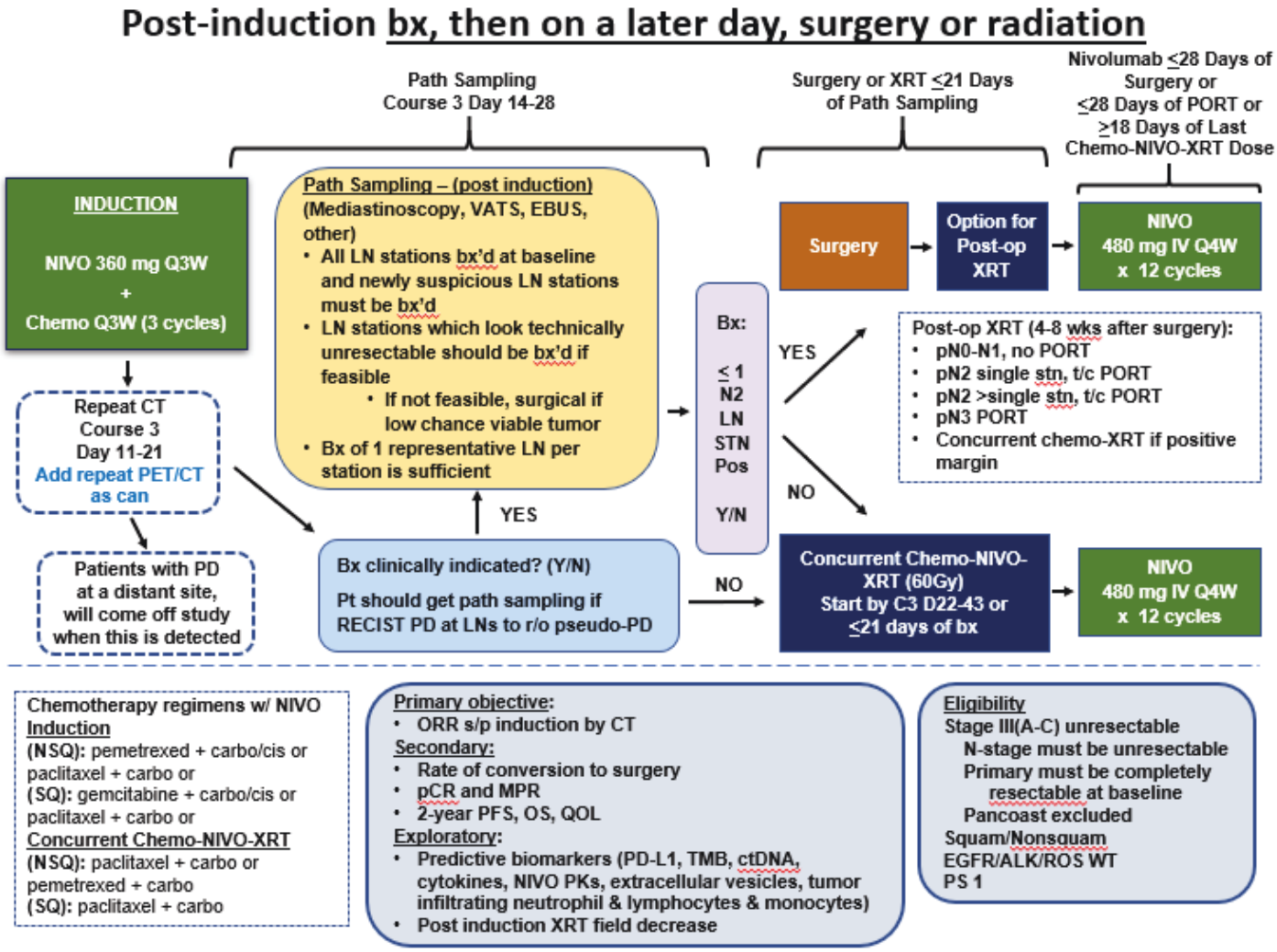
PROTOCOL ABBREVIATIONS

PROTOCOL ABBREVIATIONS	
FDA	U.S. Food and Drug Administration
IND	Investigational New Drug
MCC	Markey Cancer Center
PRMC	Protocol Review and Monitoring Committee
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
AE	Adverse event(s)
AUC	Area under the curve
CT	Computed Tomography
ctDNA	Circulating tumor Deoxyribonucleic acid
EFS	Event-free survival
LC	Lung cancer
mg	Milligram(s)
mL	Milliliter(s)
MPR	Major pathological response
NSCLC	Non-small cell lung cancer
ORR	Overall response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell(s)
pCR	Pathologic complete response
PFS	Progression-free survival
PKs	Pharmacokinetics
SAE	Serious adverse event(s)
XRT	Radiation

Basic Schema



Elaborated Schema



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1. OBJECTIVES

1.1 Primary Objective

Assess the overall response rate (ORR) based on RECIST v1.1 after induction platinum doublet + nivolumab

1.2 Secondary Objectives

1.2.1 Assess safety and feasibility of the combination induction treatment, as defined by toxicity profiles

1.2.2 Evaluate other parameters of disease response:

1.2.2.1 Rate of converting non-surgical stage III(A-C) to surgically resectable disease

1.2.2.2 Pathologic complete response (pCR)

1.2.2.3 Major pathological response (MPR) – MPR rate, defined as number of participants with ≤ 10% residual tumor in lung and lymph nodes

1.2.3 Evaluate rate and extent of post-induction radiation field decrease

1.2.4 Assess 2-year progression-free survival (PFS) and overall survival (OS)

1.2.5 Evaluate patient-reported Quality of Life as measured by FACT-TOI

1.2.6 Evaluate PDL1 status (mandatory, routinely performed as part of standard of care).
If tissue is available: also evaluate tumor mutational burden (TMB); optional. *Note*: TMB is routinely assessed as part of standard of care when tissue is available.

1.3 Exploratory / Correlative Studies

1.3.1 Measure serial circulating tumor DNA titers (ctDNA) to detect response to induction and early progression.

1.3.2 Measure cytokines via peripheral blood and pharmacokinetics (PKs) to detect response to induction and early progression.

1.3.3 Measure circulating T-cells and macrophages polarity to detect response to induction and early progression.

1.3.4 Measure extracellular vesicles to detect response to induction and early progression. These values will also be used to assess if they are a predictor of toxicity.

1.3.5 Assess pre- and post-induction tumor infiltrating neutrophil, lymphocyte and monocyte counts pathologically to assess predictors of response and efficacy assessed via available biopsy tissue (optional, if sufficient tissue is remaining diagnostic block).

1.3.6 Assess patient-reported symptoms collected daily via mobile device and app [PRO-CTCAE symptoms and mobile sensor data, e.g., heart rate] among a subset of 6 patients at Markey Cancer Center during induction (week 1 thru week 7)

2. BACKGROUND

2.1 Non-Small Cell Lung Cancer

Lung cancer is the largest cause of cancer specific death in both men and women in the United States.[1] Worldwide, 2.2 million cases of lung cancer were diagnosed with 1.8 million deaths in 2020.[2] Non-small cell lung cancer (NSCLC) accounts for 80-85% of all pulmonary neoplasms. In the US, with decreasing incidence of smoking through the decades, and improved early detection, there continues to be a decrease in the incidence of lung cancer in both men and women and a fall in the death rate from its peak by more than half. However, it is only recently, that the death rate has clearly been falling faster than the incidence rate pointing to improvements in therapy as a factor in fewer lung cancer deaths in the US.[3] The data averaged across the years 2011-2017 for all stages of lung cancer (NSCLC and SCLC combined) show only a 5-year OS (overall survival) of 21.7%. Though these data don't reflect more recent improvements in care, there is clearly an urgent need to improve these outcomes.[1]

2.2 Current Treatment: Unresectable NSCLC

Presently, patients with unresectable stage III(A-C) receive concurrent chemotherapy-radiation followed by durvalumab. Prior to the PACIFIC trial, the standard therapy was confined to concurrent chemotherapy with radiation without post-radiation consolidation. A number of trials had earlier shown concurrent chemotherapy radiation yielded superior OS, compared to sequential chemotherapy radiation.[4-7] Based on prior trials showing no improvement in OS with the addition of either induction chemotherapy or post-radiation consolidation chemotherapy, the standard of care prior to PACIFIC was concurrent chemotherapy-radiation alone.[8] Now, with the advent of immune checkpoint inhibitors (ICI), there has been recent improvement in OS in patients with unresectable stage III NSCLC.

ICIs improve OS or PFS (Progression-Free Survival) in all stages of NSCLC in which systemic agents have been approved as therapeutic options. With regional stage NSCLC in particular, prior to the PACIFIC regimen being approved, there had been an increase from 40% to 56% in two-year OS between 2001 and 2016.[3] The PACIFIC trial built on these improvements with the 2-year OS at 66% vs. 56% in the durvalumab arm vs. placebo arm, respectively. However, even with the improved OS with consolidation durvalumab, the estimated 5-year OS was still only 42.9%.[9] Thus, there remains a great ongoing need to improve the efficacy of our regimens and the consequent OS in patients with regional unresectable NSCLC.

There are a number of potential treatment strategies to consider to improve OS rates in stage III(A-C) unresectable NSCLC. Broadly, these can include **(1)** changing the systemic regimens by altering the ICI containing regimen, **(2)** its timing, and **(3)** the duration of treatment in addition to **(4)** altering the ablative therapies such as by adding surgery and further refining radiation. In the current protocol, we will be evaluating all of these strategies. In addition to potential implications for improved OS, the above potential alterations in treatment strategy may provide opportunities for de-escalation of surgery, radiation, and systemic therapy with resultant decreases in morbidity. They may also provide a platform for studying tumor biology with the chance to discover surrogate markers and identify sources of resistance.

2.2.1 Systemic Regimens

To address the first strategy, a change in the systemic regimen, there are data from both advanced and surgical stage nonsquamous and squamous NSCLC which guide thinking. In particular, chemotherapy plus an ICI appears to be superior to chemotherapy alone in both advanced and surgical NSCLC. This was first established in advanced disease. In previously untreated non-squamous advanced disease, in KEYNOTE-189, the chemo-ICI

combination was superior to chemotherapy alone, with response rates (RR) at 47.6% vs. 18.9% (P<0.001) in pembrolizumab/chemotherapy and chemotherapy alone respectively. With a median follow up of 23.1 months, OS was higher regardless of PDL-1 status (HRs were 0.52 for TPS <1%, 0.62 for TPS 1-49%, and 0.59 for TPS ≥ 50%).[10] There is evidence the OS benefit to chemotherapy plus an ICI is durable based data from KEYNOTE-21, a phase II study randomizing to carboplatin +/- pembrolizumab in nonsquamous NSCLC, a study upon which KEYNOTE-189 was based and which has longer follow-up. With a median time from randomization of 49.4 months, the PFS HR was 0.54 and the HR for OS was 0.71.[11] Indeed, at 3 years, 37% vs. 16% had not yet had PD and 50% and 37% respectively were alive.

Likewise, in first-line advanced squamous NSCLC, chemotherapy plus an ICI was superior as seen in the phase III KEYNOTE-407 trial. Patients were randomized to pembrolizumab/carboplatin/paclitaxel or nab-paclitaxel (PC) vs the same chemotherapy alone. OS was improved (15.9 and 11.3 months; HR 0.64, p=0.0017). PFS (6.4 and 4.8 months; HR 0.56; p<0.0001) and ORR (58% and 35%, p=0.0008) also favored the pembrolizumab arm.[12]

Combinations of immune therapies have also been compared to chemotherapy alone. In CheckMate-227, in patients with <1% PD-L1, with 54.8 months median follow-up, those randomized to nivolumab plus ipilimumab had superior OS compared to patients assigned chemotherapy (HR 0.76 for TPS ≥1% and 0.64 for TPS <1%. As with chemotherapy plus ICI, this benefit was sustained with four-year OS was 29% vs. 18% for TPS ≥1% and 24% vs. 10% TPS <1%.[13] Improved OS was seen in both squamous and nonsquamous NSCLC in this study. These data were built upon in CheckMate 9LA.

In CheckMate 9LA, the addition of 2 ICIs, nivolumab plus ipilimumab, to chemotherapy vs. chemotherapy alone also showed improved OS, PFS and RR in both squamous and nonsquamous and in patients with high and low PD-L1 expression levels in their tumors.[13] Patients were randomized to histology appropriate platinum doublet for 2 courses with ipilimumab plus nivolumab followed by the two ICIs until progression vs. histology appropriate platinum doublets for 4 courses with the option for pemetrexed maintenance. The OS HR was 0.69 and the median OS was 14.1 vs. 10.7 months for the experimental vs. control arm respectively.

The prospective data above represent chemotherapy +/- ICI and combination ICI vs. chemotherapy but there are no prospective data comparing ICI +/- chemotherapy. However, there are data from the advanced first-line NSCLC setting which suggest combination therapy offers as good or better OS benefit compared to single agent ICIs depending on PD-L1 expression status.

Thus, in comparing across studies in the PD-L1> 50% population, for pembrolizumab vs. chemotherapy in KEYNOTE-024, the HR for OS was 0.62 which is similar to that in KEYNOTE-189 (in the PD-L1> 50%) with a HR for OS of 0.59.[10,14] Interestingly, there was a substantial numerical RR superiority in KEYNOTE-189 vs. KEYNOTE-024 when comparing the investigational arm their respective chemotherapy alone controls. The RRs in KEYNOTE-024 were 44.8% (pembrolizumab) vs. 27.8% (Chemotherapy) to yield a 17.0% difference in response and the RRs in KEYNOTE-189 in the PD-L1 >50% cohort were 61.4% (chemotherapy + pembrolizumab) vs. 22.9% (chemotherapy) to yield a 38.5% difference in response. This advanced disease RR superior outcome in KEYNOTE-189 must be interpreted with caution but does encourage the hope that response would be higher with chemotherapy plus and ICI compared to an ICI in patients with PD-L1 ≥50% which in turn could have implications for de-escalating surgery in the neoadjuvant setting. In any case, in the advanced setting, for the population of patients with PDL1 ≥ 50%, either single agent pembrolizumab or combined chemotherapy plus pembrolizumab are a standard of care.[10,14,15] Other regimens listed in the NCCN guidelines in this PD-L1 ≥50% population include platinum doublets plus ipilimumab/nivolumab or platinum doublets plus atezolizumab and in nonsquamous, platinum doublets plus atezolizumab plus bevacizumab.

For advanced nonsquamous NSCLC patients with PDL1 of 1-50%, in an unplanned analysis in KEYNOTE-042, though there was not an HR estimate for OS, the authors concluded that the OS for single agent pembrolizumab appeared to be similar to chemotherapy, whereas in KEYNOTE-189, the HR was 0.62.[10,16] This suggests that chemotherapy plus pembrolizumab may offer superior OS compared to single agent pembrolizumab. In addition, there are retrospective data showing improved ORR, PFS and OS in chemotherapy-ICI vs ICI alone.[17] Indeed, immune combination therapy is a category I standard of care option for PDL1> 50%, and for PDL1 <1%, it is preferred over single agent ICI.[15]

Superior OS outcomes for combination chemotherapy plus ICI in advanced disease plausibly has implications for improved OS in early stage disease. The above nivolumab and pembrolizumab combination therapy studies show the potential for long term disease control of macroscopic disease in both squamous and non-squamous NSCLC regardless of PDL-1 status. This has implications for microscopic disease control in earlier stage disease; a key consideration given that micrometastatic disease is the greatest source of risk of recurrence and mortality in regional stage disease. In particular, it is plausible that if there is very effective treatment for macroscopic disease, that the same therapy would be effective for treating microscopic disease. Indeed, in PACIFIC, durvalumab likely improves OS principally on the basis of eradicating micrometastatic disease.

Based on early data from the above platinum doublet plus ICI trials in advanced NSCLC, a number of neoadjuvant studies entailing combined chemotherapy ICI were launched including phase II studies all of which reached their pathologic complete response endpoints, as was the case for the phase III study, CHECKMATE-816.[18-22] Indeed, the FDA approved neoadjuvant platinum doublet plus nivolumab given as 3 courses in resectable Ib (≥4 cm) to IIIA squamous and non-squamous NSCLC. NSCLC on March 4th 2022 when it met its two co-primary endpoints, complete pathologic response (PCR) and event-free survival (EFS) .[23] The pCR rate was a log higher with added nivolumab at 24% (95% CI: 18.0, 31.0) vs. 2.2% (95% CI: 0.6, 5.6) for chemotherapy alone. It was seen in patients with PD-L1 <1% as well as all cohorts with higher PD-L1 levels.[21] The median EFS results were 31.6 months and 20.8 months respectively (HR 0.63; p=0.0052). The addition of nivolumab yielded a numerically superior systemic therapy completion rate (94% vs. 85%) and similar surgery cancellation rates of 16% and 21% nivolumab vs. control arms respectively. Thus, since chemotherapy plus ICI is established in surgical stage and advance stage NSCLC, it is plausible it could have a role in stage III(A-C) unresectable, which are stages between surgical and advanced disease.

In this era, such a strategy would have to improve on the single agent consolidation durvalumab established in the PACIFIC trial. In advanced disease, combination chemotherapy ICI offers superior OS and as a neoadjuvant, improved pCR and EFS compared to chemotherapy alone. There are also intriguing data, as above, in advanced disease that chemotherapy plus ICI is superior to ICI alone, and indeed the default regimens for advanced disease are chemotherapy plus ICI.

Above we discussed the plausibility of chemotherapy-ICI being superior to single agent immunotherapy for all levels of PD-L1 expression was discussed. Therefore, it is reasonable to postulate that combination chemotherapy-ICI would improve on single agent ICI, durvalumab in setting of unresectable cancer. In fact, the idea of chemotherapy-ICI combination has already been extended to the non-metastatic setting, i.e. resectable cancer in CheckMate-816. These data in both advanced and in resectable stages of NSCLC point to the possibility that one can build on the improved OS demonstrated with consolidation durvalumab by the introduction of chemotherapy-ICI in unresectable stage IIIA-C NSCLC.

2.2.1.1 Timing of Systemic Therapy

Evidence from surgical stage disease offers perspective on how to sequence systemic therapy in nonsurgical stage III(A-C) NSCLC. Though the data from CHECKMATE-816 and the FDA approval validate the use of a platinum

doublet plus nivolumab in surgical disease, it does not prove that it needs to be given neoadjuvantly rather than adjuvantly. Indeed, either neoadjuvant or adjuvant chemotherapy (without an ICI) are indicated as per the NCCN based on meta-analyses showing both confer an absolute 5 year OS advantage of 5%.[24,25]

A key potential advantage to neoadjuvant systemic therapy in surgical patients is the potential to de-escalate surgery in patients who have a response. In CHECKMATE-816, there were numerically fewer pneumonectomies at 17% vs. 25% in the nivolumab vs. control arms and numerically more lobectomies at 77% vs. 61%, and similar R0 resections at 83% and 78%.[21] These outcomes will need to be verified prospectively but they are provocative and suggest the potential for improved long term morbidity with de-escalation of surgery with effective neoadjuvant systemic therapy.

Another potential advantage to neoadjuvant therapy is the ability to observe the efficacy of the systemic regimen which is not possible in patients who have no visible viable tumor when the tumor is either resected or treated with definitive chemotherapy- radiation therapy ahead of the systemic therapy. Such efficacy information from imaging or pathologic assessment of the tumor sample can provide critical prognostic and predictive information. Thus, there may be the potential to de-escalate post-ablative systemic therapy such as consolidation ICI in patients with profound response. For those who have recurrence, sensitivity to the initial regimen can inform the choice of a salvage regimen.

In addition, neoadjuvant ahead of surgery, provides the opportunity to match systemic therapy efficacy against investigational/translational outcomes since the systemic therapy efficacy can be observed by tracking response of the tumor. Key potential benefits from the study of such markers are evaluation of response, prediction of response, and the discovery of mechanisms of resistance. Efficacy is presently measured by repeat imaging and in neoadjuvantly treated patients, the level of pathologic response. It should be noted that pathologic response has not yet been prospectively verified as a marker of EFS and OS, but retrospective data are intriguing.[26] We await analyses of data linking MPR and pCR to EFS and OS from the CHECKMATE-816 study. The limits of imaging in evaluating the level of response were highlighted in CHECKMATE-816 in which pCR of 24% in the chemotherapy plus nivolumab arm was substantially underestimated by the repeat CT in which only 1% had a radiographic CR.

However, surgical evaluation of pCR is confined to those who get surgery. Thus, complements to imaging in evaluating efficacy are tenably of substantial interest not only for surgical in order to plan for de-escalation of surgery in deeply responding tumor in the future, but perhaps especially for those patients for whom surgery is not an option which includes both nonsurgical stage III(A-C) and patients with advanced stage NSCLC. The potential to identify predictors of pathologic response by matching efficacy to a marker was highlighted by the ctDNA data from CHECKMATE-816. Absence of ctDNA clearance was a strong predictor of failure to achieve pCR which 0% and 3% having pCR in the patients whose blood failed to clear in the chemotherapy plus nivolumab and chemotherapy arms respectively. This shows the potential utility of ctDNA and highlights the powerful opportunities for identifying surrogate markers through neoadjuvant therapy which would not be easily achievable when giving therapy after surgery.[21]

The potential advantages to neoadjuvant in surgical patients can plausibly apply to non-surgical patients. As with surgical stage disease, more effective systemic therapy will plausibly improve survival whether given before, during, or after radiation. However, as with neoadjuvant therapy in surgical patients, induction therapy has potential advantages over consolidation therapy. Though neither induction nor post radiation systemic therapy in earlier studies have shown an overall OS advantage in unresectable stage III (A-C) patients as compared to patients getting concurrent chemotherapy-radiation alone, improved systemic therapy offers a fresh opportunity to evaluate the potential role of systemic therapy given as induction in this population of patients. There are preclinical data supporting the use of an ICI before radiation. Thus, in a murine model, there was higher efficacy

when an ICI was given before radiation.[27] Then there are the potential clinical advantages observed in surgical stage disease which may have implications for non-surgical staged disease. Thus, as in the neoadjuvant setting, the completion of systemic therapy is more assured when given as induction when accounting for risks of toxicity from radiation. This is a key consideration given the high risk of distant micro-metastases in this population. Moreover, investigators can observe the efficacy of systemic therapy unobscured by radiation which permits linking antitumor activity to correlative molecular and cellular markers. In addition, as with neoadjuvant therapy in surgical patients, in responding nonsurgical patients, there is the opportunity to de-escalate the definitive treatment. In particular, the radiation field can be reduced which has implications for decreased morbidity and mortality.[28-31]

After induction, there are phase II data suggesting chemotherapy-ICI concurrent with definitive radiation may be well tolerated and further improve efficacy. In the phase II NICOLAS study, nivolumab was combined with platinum based therapy concurrent with radiation in unresectable stage IIIA/B. This was followed by a year of nivolumab as monotherapy. The 1-year PFS was 53.7%, the median OS was 38.8 months and the 2-year OS was 63.7%. Though the study did not reach its 1-yaer PFS endpoint, the PFS and OS were arithmetically higher than prior studies in the same population.[32] KEYNOTE-799, was a phase II of platinum doublets plus pembrolizumab concurrent with radiation (with the first cycle preceding radiation and the latter 2 during radiation) followed by a year of pembrolizumab monotherapy in unresectable stage III NSCLC. There was promising antitumor activity and manageable safety.[33] Together these 2 phase II studies continuing PD-1 inhibitors concurrent with chemotherapy and radiation therapy showed promising tolerability and efficacy. Such a concurrent chemotherapy-ICI regimen has addition relevance in the setting of an induction chemotherapy-ICI in which consolidation ICI monotherapy would be planned since it permits continuity of the ICI between induction and the 1-year consolidation.

2.2.1.2 Duration of Systemic Therapy

Thus far, there has been consideration of potential advantages to introducing more effective systemic therapy, i.e., chemotherapy plus and ICI, and offering for offering such systemic therapy ahead of definitive therapy in unresectable stage III(A-C) patients. Earlier exploration for the potential benefit of adding systemic therapy before or after definitive concurrent chemotherapy radiation have shown no OS advantage to this approach. At present, data need to be developed to support more prolonged exposure of the patient to combined chemotherapy and ICI. There are clues as to the potential advantage to more prolonged exposure to an effective systemic agent. Thus, in advanced non-squamous NSCLC, continuation pemetrexed maintenance improves OS.[24] Prolonging exposure to systemic therapy by switching to another agent, durvalumab as consolidation therapy is established in stage III(A-C) unresectable NSCLC.[9] The study regimen proposed in this protocol will offer preliminary evidence for the safety and efficacy of prolonged exposure to chemotherapy plus nivolumab in those patients assigned post induction concurrent chemotherapy plus nivolumab concurrent with radiation. For patients in both this group and those assigned surgery, nivolumab will be offered as consolidation which is an analogue to the current standard of care of consolidation durvalumab.

2.2.2 **Ablative Therapy Options**

An additional way to potentially improve OS is by reducing the morbidity and improving the efficacy of the ablative therapy. In the above section (2) is mentioned the potential to reduce the radiation field with implications for decreased radiation toxicity and improved OS. An additional potential consequence of a response to induction is to convert pre-induction baseline unresectable cancers to resectable cancers thereby sparing these patients the ill-effects of radiation. There is evidence that neoadjuvant downstaging of lymph node disease in surgical IIIA patients predicts EFS or OS. In phase II study, in resectable IIIA N2 NSCLC, 75 of 90 enrolled patients completed neoadjuvant chemotherapy and had surgery. Patients who had downstaging at the time of surgery to N0-1 after

3 cycles of neoadjuvant cisplatin docetaxel had significantly improved EFS and OS (p=0.0001).[35] Of note, patients who did not have sufficient downstaging did not get surgery. In a 5-year follow-up paper, mediastinal lymph node response was confirmed as a predictor of OS in addition to clinical and pathologic response.[36]

In another study, 58 of 62 patients with stage III N2 NSCLC who received neoadjuvant cisplatin docetaxel, went to surgery. In a multivariate analysis, mediastinal downstaging to N0 yielded a HR 0.451; p=0.024 for better EFS.[37] Thus, downstaging with neoadjuvant chemotherapy is associated with improved survival outcomes and may have implications for rendering marginally surgical patients, surgical.[36] These findings suggest potential application to patients with more advanced stage than in these studies, i.e., those with unresectable stage IIIA-C. Given the demonstrated implications for mediastinal lymph node down-staging for improved OS, patients whose tumors were unresectable on the basis of advanced lymphadenopathy may not only have improved OS with radical downstaging of their nodal disease, but may also be rendered surgically resectable if lymph node sampling shows minimal to no remaining viable tumor in the lymph nodes. At surgery, the lymph nodes would be further evaluated as per standard surgical lymph node sampling protocols. This would provide guidance as to potential post-op radiation.

Such a switch to surgical cancer would be isolated to those patients whose tumors are fully resectable with a sufficient response. Surgery entails complete resection which in turn means all tissue attached to or involved by the tumor at baseline would need to be removed; thus, baseline unresectable tumors would remain unresectable. Thus, for those patients who had baseline resectable primary disease but were unresectable stage III(A-C) on the baseline of unresectable lymphadenopathy, there would be the potential for converting to resectability. Even in those patients who had post-operative radiation, with profound response, the volume of disease and the intensity of the radiation could be reduced thereby limiting the risk of long term radiation effects on the heart, lung and other tissue.

2.3 **Study Rationale and Hypothesis**

2.3.1 Rationale

Induction therapy prior to radiation is not a current standard of care. However, with the advent of the demonstrated powerful efficacy with no new safety signals for nivolumab added to neoadjuvant chemotherapy in stages I(≥4cm)-IIIA, now is the time to reconsider induction therapy prior to planned radiation in unresectable stage III(A-C).

More effective systemic therapy will plausibly improve OS whether given before, during, or after radiation. However, induction therapy has potential advantages similar to those in figure 1 which apply to neoadjuvant candidates whose tumors are resectable at baseline.

1) It provides the chance to observe the systemic therapy's efficacy, of great importance given the highest mortality risk in this population is micrometastases.
2) With earlier introduction of potentially highly effective systemic platinum doublet plus nivolumab, micrometastases will be plausibly more vulnerable since they will be smaller.
3) As mentioned above, there is the opportunity to de-escalate definitive therapy with implications for improved long term OS and well-being either by converting the patient to a surgical candidate or by shrinking the volume of normal tissue which is irradiated.
4) Not least is the powerful opportunity to match molecular and cellular surrogate markers against outcomes with the ability to monitor radiographic and pathologic response.

Though there are potential downsides to induction therapy as compared to post radiation consolidation therapy, there are data which encourage the expectation that risks to non-completion of definitive therapy, a principle concern surrounding neoadjuvant and induction therapy, are modest.[21] In the balance the precedents from the neoadjuvant setting are highly encouraging and the vital and urgent need to improve both OS and morbidity in stage III(A-C) remains very present. Indeed, the structure of an induction regimen presents a key opportunity to limit the acute and long term effects of ablative therapy, whether radiation or surgery. It is these two goals which impel this trial.

After induction, patients will either begin concurrent chemotherapy-nivolumab-radiation or go to surgery depending on the findings from post-induction lymph node biopsies. If the cancer is down-staged to a single lymph node station-N2, or N1 or N0 disease they would be surgical candidates. For those patients who are treated with definitive radiation after induction platinum doublet-nivolumab, the continuation of nivolumab together with standard chemotherapy concurrent with radiation is supported both by data from at least two phase II studies in which PD-1 inhibitors were given concurrently with chemotherapy and radiation in NSCLC.[38,39] Continuing nivolumab in an induction study in particular, has an additional potential advantage. It enables continuity of nivolumab treatment between the induction and the post-radiation consolidation single agent nivolumab. Although this study provisions a mandatory post-induction biopsy of lymph nodes in all patients who have not had distant progression or progression at the primary site (progression at lymph nodes does not exclude biopsy) to induction therapy, for patients who get surgery, there will be data additional to the post-induction biopsies at the time of surgery. These additional data from the surgical pathology assessment will also inform the multi-disciplinary team's judgement as to whether to treat with post-operative radiation which is optional. Surgical tumor specimens collected in surgical candidates will also enable formal assessment of pathologic response in the patients that proceed to surgery. After the ablative therapy is completed, whether with chemotherapy-nivolumab-radiation or surgery, all patients will receive a year of consolidation nivolumab monotherapy in line with the consolidation ICI which is the current standard of care (durvalumab) for this population of stage III patients with unresectable stage III NSCLC.

Finally, through this clinical trial, the structure of this study permits an assessment of pathologic response in initially unresectable patients. This will be done through the post-induction lymph node biopsies in all patients which will enable powerful pathologic response data to complement the radiographic assessments of response. It can also provision tissue to study the molecular and cellular effects of the induction regimen directly on the post-induction tumor tissue. Tissue availability will be more abundant in patients who are assigned post-induction surgery in which more extensive assessments of pathologic response and provision of tissue for exploratory molecular and cellular endpoints.

The induction study regimen of a platinum doublet with nivolumab in this trial is highly promising and will be thoroughly studied through this protocol. It is a revisiting of an old approach in this current era in which we now have much better systemic therapy, chemotherapy plus nivolumab, as an induction regimen and therefore a refreshed expectation of establishing induction as a treatment strategy. The current study has the express intent of rendering some initially unresectable tumors resectable in unresectable stage III NSCLC and of offering the chance to reduce the morbidity of radiation through a smaller radiation field in responding patients. The current study builds on a wealth of data supporting the proposition that both OS and morbidity can be improved through induction using combination platinum doublets and nivolumab. In addition, the regimen and treatment structure of the current study may also establish a new therapeutic template. This current study regimen could do so if it is ultimately established in future studies to improve OS and morbidity. Through this induction template, further strides in OS and morbidity through the introduction of future regimens can be made. This induction template also provides the opportunity to study future generations of systemic therapy in which their effects on the tumor

and the host can be assessed through pathologic response. As with the current regimen proposed in this trial, future regimens investigated through this induction template can also be characterized for molecular and cellular effects matched against the regimen's efficacy. Thus, this study has powerful implications for improved OS and morbidity not only for the promising induction combination of platinum doublet and nivolumab regimen in the current study, it also has the potential the establishment of an induction template, to accelerate the process of improved OS and morbidity through future studies in unresectable stage III(A-C).

2.3.2 Study Hypothesis

We hypothesize that induction platinum doublet plus nivolumab will improve the objective response rate assessed by post-induction CT scan in unresectable stage III(A-C) NSCLC.

This is the question which drives the statistics of this trial given the available historical data permitting an estimate of response to induction chemotherapy alone in this population and the ease with which it can be measured. Clues as to clinical response rates after 3 courses derive from large neoadjuvant modern platinum doublet (without an immune checkpoint inhibitor) which are stage Ib-IIIa studies with RR 35-53%.[21,36,37,40,41] Of note on the phase III neoadjuvant platinum doublet without and without added nivolumab study, the chemotherapy arm had 37.4% radiographic response and the platinum doublet plus nivolumab arm had 53.6%. Although pathologic response is likely a more accurate prognostic indicator, many of the patients on this study will not go to surgery and thus cannot have adequate assessments of their pathologic response which would require an examination of the resected primary tumor. PFS, another appealing endpoint given its implications for predicting OS, risks a long duration before it can be evaluated given the potential long delay of events with the potentially highly effective chemotherapy-nivolumab induction regimen. Radiographic response remains a standard primary endpoint in many advanced stage studies given its ability to assess efficacy rapidly and reproducibly and with similar considerations in mind, is the primary endpoint of this trial.

2.3.3 Rationale underlying Secondary Objectives

2.3.3.1 Rationale: Safety and feasibility (Objective 1.2.1)

Assess safety and feasibility of the combination induction treatment, as defined by toxicity profiles. The induction regimen has been characterized with some rigor in CheckMate-816 but its implications for patients who are to then receive concurrent chemotherapy-radiation are not well characterized. Moreover, it is imperative that risk of toxicity preventing definitive therapy, whether concurrent chemotherapy-radiation, surgery or some combination, be minimized. Thus, toxicity will be closely monitored and recorded during induction but also during the delivery of definitive therapy and when the patients are receiving consolidation nivolumab.

2.3.3.2 Rationale: Other parameters of disease response (Objective 1.2.2)

Rate of converting non-surgical stage III(A-C) to surgically resectable disease. We will examine the rate of conversion of baseline tumor unresectability, to surgical resectability with response to the chemotherapy-nivolumab induction regimen. This drives the decision to exclude patients whose primary tumors are not resectable at baseline. Thus, patients will be required to have resectable primary tumors at baseline thus limiting eligibility to those whose tumors would become resectable with response. The status of being unresectable at baseline will be driven instead by the lymph node stage instead. In particular, we will require the patients have lymph node involvement at baseline which is not resectable such as multi-station N2 disease or N3 lymph node involvement since the primary tumor will be required to be resectable. The reasoning for this is the unresectable lymph nodes which show evidence by repeat biopsy of harboring no tumor will not require resection unlike the primary tumor. By focusing on those patients who are potentially resectable with response to induction, we will

be enriching for this population of patients, thus increasing the number of patients we can evaluate for this key novel secondary endpoint of converting to resectability.

By confining the population of eligible patients to only those who are potentially resectable with response to the induction regimen, the trial will have a larger number of patients who can be observed for this endpoint. This in turn will serve the purpose of providing numbers of patients to allow for a tighter estimate such a rate of conversion to surgical resectability to inform a potential future trial.
The interest in exploring the rate of conversion to resectability is driven by the potential for limiting the degree of long-term morbidity from radiation by performing surgery as an alternative. There are other advantages to enriching for this population.

Obtaining a fully resected tumor also enables formal assessment of the level of pathologic response at the primary site. This is of interest given the substantial potential for becoming a powerful predictor of long-term post induction and post definitive therapy survival. In addition, with surgery, there will be more rigorous assessment of pathologic response at the lymph node which has implications for prognosis and will guide consideration of post-operative radiation. Such post-surgical specimens also permit assessments of the tumor specimen for cellular and molecular markers to match against the efficacy of the induction regimen. The baseline tissue can be assessed for predictors of response and the post induction resected and biopsied tissue can be used to match radiographic, serial blood samples (such as ctDNA) against response. In addition, cellular and molecular alterations in this tissue in response to the induction may be prognostic. Finally, with resection, there will be the chance to better match radiographic with pathologic response and in turn with investigational translational endpoints.

Pathologic complete response (pCR). There is emerging evidence that pCR and MPR are strong prognostic indicators. Over time, if this is further confirmed, it could guide post-surgical monitoring in patients whose tumors were resected and provide information to support future de-escalation trial designs.

Major pathological response (MPR) – MPR rate, defined as number of participants with ≤ 10% residual tumor in lung and lymph nodes serves a similar purpose as pCR.

2.3.3.3 Rationale: Radiation field decrease (Objective 1.2.3)

In responding nonsurgical patients, the radiation field can be reduced which has implications for decreased morbidity and mortality.[43-46] Thus, a formal comparison between pre and post radiation planning fields is included as an exploratory endpoint.

It is expected preplanning before induction therapy is given will be feasible in some of the patients and will be encouraged. Then patients who go on to get post-induction radiation will have repeat planning to which the two plans can be compared.

To assess this, preplanning target volumes (GTV, IGTV, CTV), PTV, and normal organs will need to be outlined on all appropriate CT slices prior to induction. As per standard of care, all planning CT scans must be performed in the treatment position using the same immobilization device for setup as is used at the linear accelerator. Optimal immobilization is critical for this protocol in order to ensure reproducibility of the daily setup.

Intravenous contrast during the planning CT is optional, provided that a recent diagnostic chest CT was performed with contrast to delineate the major blood vessels and involved mediastinal lymph nodes. If not, intravenous contrast must be given during the planning CT. Study sites should follow the SOC when planning for RT.

Acceptable methods of accounting for tumor motion include: design of the PTV to cover the excursion of the primary lung cancer and involved nodal CTV during free breathing (motion inclusive), or the more limited excursion during a voluntary or automatic breath hold (i.e., Elekta ABC device), abdominal compression, or a gating approach (e.g., Varian RPM system).

An FDG-PET/CTs are done at baseline as a standard of care. An additional post-induction PET/CT may be required. A treatment planning FDG-PET/CT scan (or FDG-PET alone) with the participant in the treatment position is encouraged for treatment planning. Where an FDG-PET/CT is obtained in the treatment position, the CT from this study may be used as the planning CT scan.

2.3.3.4 Rationale: 2-year PFS and OS (Objective 1.2.4)

These are standard fundamental endpoints representing key goals for our patients. Since chemotherapy-nivolumab induction is not standard of care in unresectable stage IIIA-C NSCLC, we will evaluate 2-year PFS among patients on this study as an additional preliminary indicator of efficacy. Patients will also be monitored for OS up to 2 years after enrollment with the option to further evaluate in follow up assessments years after.

2.3.3.5 Rationale: Patient-reported QOL (Objective 1.2.5)

Physician-rated AEs reflect only one aspect of tolerability, the extent to which a patient can tolerate a side effect for the potential anti-tumor benefit. Psychometrically validated patient-reported outcomes (PROs), combined with physician-rated AEs, provide robust data on the severity, frequency, and functional impact of side effects associated with treatment. Health-related quality of life refers to a multidimensional construct that includes symptoms, functional abilities, and perceived physical, mental, and social health. Health-related quality of life and symptom control are important, well-established endpoints when evaluating treatment. Whether a patient maintains health-related quality of life and experiences good symptom and treatment side effect control also have important implications for treatment itself. If bothersome symptoms and side effects from treatment and impairments in quality of life are not addressed, treatment can become intolerable, yielding non-adherence, dissatisfaction with care, treatment discontinuation, and ultimately, poorer survival. Few studies have included patient-reported assessments of health-related quality of life to evaluate surgery vs. concurrent systemic therapy-radiation in stage III unresectable non-small cell lung cancer. To achieve this aim, patient-reported health-related quality of life (FACT-TOI) will be collected at several timepoints, specifically pre-treatment baseline (before induction); Cycle 2 and Cycle 3 of induction; at restaging biopsy/before definitive treatment; and Cycle 1 and Cycle 3 of consolidation nivolumab. See **Appendix F.1** for details.

2.3.3.6 Rationale: PD-L1 and TMB (Objective 1.2.6)

Baseline tumor tissue needs to be adequate to enable analyses of PD-L1 and TMB. For PD-L1, any individual IHC test can be used as long as it has been internally validated for comparability for the categorical results against the FDA-approved clone[47] For TMB, next generation sequencing (NGS) should be used. Testing should be performed via a broad, panel-based approach. Given the rapid evolution of technologies, no single company, or institutional entity, or method is preferred or specified.

2.4 **Correlative Studies Background**

A number of correlative studies are planned as detailed below and in Table 5.1 and in the Study Calendar. All those entailing blood samples will be drawn together on the same days to ease the effort to compare and interpret

these assay results as prognostic and predictive approaches. For tumor-based assays, we will use pre-induction tumor samples, post-induction biopsy tissue and post-induction surgical resection tissue. Not all patients will have all samples available for analysis. Analysis will be performed on samples available for all patients. This will enable both exploration of markers which predict response to the induction regimen and assessment of alterations in post induction specimens for mechanisms of resistance to the induction and to discover or confirm markers predicting long-term survival. Baseline tumor specimens will be from either diagnostic tissue or from repeat biopsies which are encouraged for those who have limited diagnostic tissue. Post-induction tissue will be obtained both from biopsies done during restaging and additional potentially abundant tissue will be obtained in those patients who go to surgery.

1	Circulating tumor DNA (ctDNA) – 1.3.1
2	Pharmacokinetics (PKs) and Pharmacodynamics – 1.3.2
3	Cytokines (T-cells polarity and macrophages) - 1.3.3
4	Extracellular Vesicles – 1.3.4
5	Tumor infiltrating neutrophil, lymphocytes and monocytes counts – 1.3.5
6	Remote symptom monitoring (sub-study of 6 Markey participants) – 1.3.6

2.4.1 Ct-DNA

See **Appendix C** for details

Ct-DNA has the potential to improve prediction of pathologic response when added to imaging (CheckMate-816) and for tracking minimal residual disease.

To assess ctDNA as a predictor biomarker of response 7mL of whole blood will be collected in two Streck Cell-Free DNA Blood Collection tubes at the following four timepoints- pre-treatment, after cycle 1,after cycle 2 and just before surgery or definitive radiation.

2.4.2 Pharmacokinetic Studies

There is evidence that ICI response is associated with elevated systemic plasma clearance of ICI.[48] This rapid clearance, in turn, is associated with cancer cachexia. Thus, cachexia, an independent poor prognosis factor, may be reflected by ICI clearance.

1. Nivolumab concentration in plasma: pre-treatment and 30 min post administration (4mL each) of each dose. This will be at selected timepoints during induction and consolidation treatment.
2. Baseline and subsequent body weight, albumin, and LDH values which are values incidental to work up and planning for chemotherapy in these patients.
3. CT images for L3 to determine skeletal muscle index (SMI) as a measure of cachexia (these would be incidental to baseline staging scans.
4. Peripheral blood samples to quantify FcRn levels

2.4.2.1 PKs Nivo

Sample for checking nivolumab concentration, will be collected at 4 timepoints (pre-treatment, after cycle 1, after cycle 2 and after cycle 3 (visit just before surgery or definitive radiation). (See Appendix G for processing and shipping details)

2.4.2.2 FcRn

Collect 6 ml of peripheral blood in one Green top tube with heparin (to collect PBMC). Plan to sample at 1 timepoint,at baseline.

See **Appendix G** for processing and shipping details.

2.4.3 Cytokines & PBMCs / T-Cell Polarity & Macrophages

See **Appendix D** for details

We plan a series of in-depth correlative studies to help identify the mechanism of action of induction nivolumab combined with platinum doublet chemotherapy to identify biomarkers of response and explain the mechanism governing treatment response or failure. The, exploratory studies will be performed on peripheral blood tissues and biopsy tissue in a subset to subjects to examine 1) the correlation between immune biomarkers in the periphery and treatment outcome; 2) the correlation between immunologic factors in the tumor microenvironment and the treatment outcome. Peripheral blood will be obtained before treatment and after treatment for cytokine and PBMC analyses. Plasma from these samples will be stored for future use.

For immune studies four different panels of immune-cell markers (as in the appendix) will be studied by collecting peripheral blood into Vacutainer ™ CPT ™ Tube with Sodium Citrate (See Specimen collection table). Sample will be collected at 4 timepoints (pre-treatment, after cycle 1, after cycle 2 and after cycle 3 (i.e., visit just before surgery or definitive radiation).

2.4.4 Extracellular vesicles

See **Appendix H** for details

Titers of extracellular vesicles may predict tumor response and tumor recurrence.

15 cc are collected in tubes containing heparin to obtain plasma. Plasma will be stored frozen in Markey BPTP for batched EV isolation and dispense for analysis. Samples processing and shipping instructions can be found in Appendix H. Sample will be collected at 4 timepoints, specifically at pre-treatment, after cycle 1, after cycle 2 and after cycle 3 (i.e., visit just before surgery or definitive radiation).

2.4.5 Tumor infiltrating neutrophil, lymphocyte and monocyte counts

See **Appendix E** for details

This exploratory study assesses the T-cell subsets and monitors PD-1 expression along with activation and will correlate with therapy. Histologic sections of tumor (H&E stained, single and multiplex immunohistochemical or immunofluorescent stained) will be used to evaluate spatial relationships between immunologic factors in the tumor microenvironment. This will be done on baseline pre-induction tumor tissue when available and on post-induction tumor tissue (either from the restaging biopsies and from the resected tumor specimen for patients that undergo resection).

2.4.6 Remote symptom monitoring

See **Appendix F.2** for details

There are data showing improved quality of life and survival in patients whose symptoms are monitored. Towards this goal, symptoms of a subset of 6 study participants enrolled at Markey Cancer Center will be reviewed. The daily reported PRO-CTCAE™ symptoms (https://healthcaredelivery.cancer.gov/pro-ctcae/) and other sensor data will be collected remotely (patient-report) via a smartwatch app and mobile sensors. These symptoms will be reviewed at routine clinic visits (Week 4 and Week 7 during nivolumab induction). The symptom reporting period stretches from Course 1 Day 1 (initiation of induction) to the routine clinic visit during week 7 (also during induction).

3. PATIENT ELIGIBILITY

3.1 Inclusion Criteria

3.1.1 Squamous and non-squamous non-small cell lung cancer that is at baseline, unresectable stage IIIA-IIIC (8th edition AJCC) and not previously treated. Patients will have a treatment plan for definitive chemotherapy-radiation. Measurable disease as defined by RECIST.

- For any eligible patient, the primary tumor must be resectable at baseline T1-T4 (resectable T4). Thus, unresectability is on the basis of the lymph nodes.

- Baseline histologic or cytologic confirmation and/or imaging determined nodal involvement is outlined below. Tissue confirmation of all mediastinal nodal involvement is not required, however at least one N2 or N3 lymph node needs to be positive. If only 1 N2 lymph node station is positive, patients can still be eligible if other mediastinal N2 lymph node stations or N3 nodal involvement can be declared by imaging when the nodes have distinct margins and the size of the shortest axis of one such lymph node is at least 1 node is ≥2.0 cm (MRI/CT scan). If a single N3 lymph node is biopsy positive, any T stage is permissible as long as the primary is resectable. No other pathologic or radiographic evidence of lymph node involvement is required if the N3 is biopsy positive.

- **IIIA** is eligible if the primary, T1-T2, is technically resectable at baseline and is unresectable on the basis of:
    - N2 with 2 or more ipsilateral lymph node stations involved
- **IIIB** is eligible if the primary is technically resectable at baseline but is unresectable on the basis of:
    - N2 with 2 or more ipsilateral lymph node stations involved (and T3 or T4 status)
      OR
    - N3 (including contralateral mediastinal, contralateral hilar, or supraclavicular) with T1- T2 status. Patients can have baseline bulky bilateral mediastinal lymph nodes and supraclavicular lymph nodes and be eligible.
- **IIIC** is eligible if the primary, T3-T4, is technically resectable at baseline but is unresectable on the basis of:
    - N3 (including contralateral mediastinal, contralateral hilar, or supraclavicular) Patients can have baseline bulky bilateral mediastinal lymph nodes and supraclavicular lymph nodes and be eligible

3.1.2 PD-L1 level needs to be measured with values 0-100% eligible.

3.1.3 EGFR/ALK/ROS1 Wild Type or unknown genetic alterations in these genes.

3.1.4 Age ≥18 years

3.1.5 ECOG Performance Status ≤ 1. See **Appendix A.**

3.1.6 Adequate organ and marrow function as defined below:

System)	Laboratory Value
Absolute neutrophil count (ANC)	≥ 1,500/mcL
Platelets	≥ 100, 000/mcL
Hemoglobin	≥ 9.0 g/dL
Measured or calculated creatinine clearance (Cockcroft-Gault formula, **Appendix B**)	≥ 45 mL/min
Total bilirubin	≤ 1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤ 3 × ULN

3.1.7 Adequate pulmonary reserve (e.g., FVC, FEV1, TLC, FRC, and DLCO) capable of tolerating the proposed lung resection according to the surgeon and must also be capable of tolerating definitive chemotherapy-radiation therapy with a minimum pulmonary function (PFT) as follows:

Has adequate PFT defined as a FEV1 >50% of predicted normal volume and the carbon monoxide lung diffusing capacity (DLCO) >40% of predicted normal value. Participants for whom DLCO measurements are not available will be deemed to have adequate oxygen transfer if pulse oximetry (O2 saturation) is determined to be ≥90% on room air.

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

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

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

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

3.1.12 The effects of systemic chemotherapy and nivolumab on the developing human fetus are unknown. For this reason and because systemic chemotherapy and nivolumab agents as well as other therapeutic agents used in this trial are known to be teratogenic, women who are pregnant or lactating are excluded from this trial. Additionally, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation, and for 5 months after completion of nivolumab administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 5 months after completion of nivolumab administration.

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

3.2 Exclusion Criteria

3.2.1 Patients who have participated in a study with an investigational agent or device within 2 weeks of enrollment

3.2.2 Any prior radiotherapy to the lung

3.2.3 Any prior treatment for the current NSCLC

3.2.4 Any prior therapy with anti-PD-1, anti-PD-L2, anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T-cell co-stimulation or immune checkpoint pathways

3.2.5 Any history of a severe hypersensitivity reaction to any monoclonal antibody

3.2.6 Any history of allergy to the study drug components

3.2.7 Patients cannot have a pancoast tumors whether T3 or T4 by 8th edition lung cancer stage classification.

3.2.8 Patients cannot have primary tumors involving the esophagus

3.2.9 Patients cannot have primary tumors which would remain unresectable even if an excellent response since this study obliges a complete resection of the primary tumor even if post-induction evaluation indicate potential pCR. An example would be tumors enveloping unresectable large blood vessels. However, since there is not an obligation to resect lymph nodes which on evaluation appear to have a pCR, such lymph nodes seen at baseline are not a barrier to study enrolment.

3.2.10 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Nivolumab or other agents used in study

3.2.11 Any active or history of autoimmune disease (including any history of inflammatory bowel disease), or history of syndrome that required systemic steroids or immunosuppressive medications (as defined in 3.2.12), except for subjects with vitiligo or resolved childhood asthma/atopy

3.2.12 Ongoing requirement for systemic corticosteroids greater than the equivalent of prednisone 10mg. However, inhalational steroids are allowed

3.2.13 Subjects with previous malignancies are excluded unless a complete remission was achieved at least 2 years prior to study entry and no additional therapy is required or anticipated to be required during the study period.

Exceptions to this exclusion are: non-melanoma skin cancers, in situ bladder, gastric, breast, colon or cervical cancers/dysplasia.

3.2.14 Subjects with a history of interstitial lung disease.

3.2.15 Patients requiring continuous supplemental oxygen are excluded.

3.2.16 Use of any live vaccines against infectious diseases (e.g., nasal influenza, varicella. etc.) within 4 weeks (28 days) of initiation of study therapy

3.2.17 Active systemic infection requiring therapy

3.2.18 Patients with uncontrolled intercurrent illness.

3.2.19 Patients with psychiatric illness/social situations that would limit compliance with study requirements.

3.3 Inclusion of Women and Minorities

For investigator-initiated trials (IITs) conducted by Markey Cancer Center, we adhere to NCI and NIH policy regarding trial accessibility. NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research.

4. INVESTIGATOR REQUIREMENTS AND REGISTRATION PROCEDURES

4.1 Trial Registration required by National Cancer Institute

The National Cancer Institute requires that interventional treatment trials be registered in clinicaltrials.gov and on NCI's CTRP prior to patient enrollment. The Investigator-Initiated Trials Office at Markey will provide assistance to the PI in completing these required registrations/ renewals.

4.2 Investigator and Research Associate Registration with MCC

All investigators must be qualified by education, training and experience to assume responsibility for the proper conduct of human subject research. Investigators are responsible for being able to provide evidence of such qualifications through up-to-date curriculum vitae and/or other relevant documentation and training per institutional, state and federal guidelines. All investigators conducting MCC trials will register with the MCC Clinical Research Office and complete all requisite training and registrations per MCC SOPs.

4.2.1 Delegation of Tasks Log (DTL)

All MCC studies require a Delegation Task Log which is maintained by the MCC Regulatory Unit of the Clinical Research Office.
The DTL for this study has training requirements as follows:
In order to be added to the DTL for a given study, each staff member must have appropriate training to conduct assigned duties including but not limited to protocol specific training. The DTL log will identify the protocol version on which each staff member was trained when being added to a study.

For External Sites: Site(s) must complete a protocol specific DTL using the DTL process outlined in their institutional SOPs and aligning with MCC SOPs and what is stated in this protocol. These DTLs are provided to the MCC MRU for the trial Master File.

The Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Principal Investigator and Statistician have access to the study data at all times through OnCore. All decisions regarding dose modifications require consultation with the Principal Investigator.

4.3 Overview and Informed Consent Guidelines

4.3.1 Overview of Enrollment Process

Eligible patients will be identified by the principal investigator and co-investigators of this study, eligible patients will be presented at multi-disciplinary tumor board. Potentially eligible patients will be screened in the University of Kentucky Markey Cancer Center clinics by the investigators and study personnel with oversight by the Principal Investigator (PI). Each potential candidate patient for the study will be reviewed by 2 thoracic surgeons, one from UK and the other from OSU, in order to determine if the patient has surgically resectable primary tumor. This can be done by phone at the earliest convenience of the surgeons. This two institution process will not begin until the study has opened to accrual at OSU.

The consenting professional will explain in detail the study to the patient and will review the informed consent with the patient (Section 4.3.2). Broadly, patients will be made aware of the protocol, its specific aims and objectives, and the potential risks and benefits the patient may incur. A copy of the signed informed consent form is provided to the patient. Upon obtaining consent, study staff will register potentially eligible patients in the study's OnCore database. During the screening and enrollment process, registering individuals (study staff and PI) will be required to complete a protocol-specific Eligibility Checklist for each patient. The PI or treating physician signing the Eligibility Checklist is confirming whether or not the patient is eligible to enroll in the trial. Upon confirmation of eligibility, the patient will be enrolled into the study as participant (i.e., on-study date is entered in OnCore).

4.3.2 Informed Consent

The goal of the informed consent *process* is to provide people with sufficient information so they can make informed choices about whether to begin or continue participation in clinical research. The process involves a dynamic and continuing exchange of information between the research team and the participant throughout the research experience. It includes discussion of the study's purpose, research procedures, risks and potential benefits, and the voluntary nature of participation.

The informed consent *document* provides a summary of the clinical study and the individual's rights as a research participant. The document acts as a starting point for the necessary exchange of information between the investigator and potential research participant. Also, research participants and their families may use the consent document as an information resource and reference throughout participation in the trial. The informed consent *document* is often considered the foundation of the informed consent process; it does not, however, represent the entirety of the process. Nor is the informed consent document a risk-management tool for the investigator and/or institution.

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained. The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with the protocol at the time of IRB review

4.4 Patient Registration

To register a patient, the following information should be reviewed by the Clinical Research Nurse (CRN) / Clinical Research Associate (CRA) with the study physician per MCC SOPs to confirm eligibility:

- Copy of required laboratory tests, pathology reports, Imaging reports
- Physician dictations, and/or referring physician records as available
- Signed patient consent form with HIPAA authorization form
- Other required screening procedures when applicable
- OnCore Eligibility Checklist

Once eligibility is confirmed, the CRN/CRA will complete subject registration in the OnCore database. To complete the registration process, the CRN/CRA will:

- assign a patient study number
- register the patient on the study in OnCore
- alert study team a new patient has been enrolled
- External Site: alert the MCC Mulit-Center Project Manager of patient enrollment

4.5 General Guidelines

Following registration, patients should begin protocol treatment within 21 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The study team should be notified of cancellations as soon as possible.

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Research Objective & Timepoint	***Specimen(s) to be collected at each of the timepoints***	***Send Specimens To:***
Archival Tumor Tissue (where archival tumor tissue is available)		
1.3.5 Tumor-infiltrating neutrophils, lymphocytes and monocytes (Brainson)	If archival tissue is available after SOC diagnostic evaluation (via biopsy), then the extra biopsied tumor tissue will be analyzed using Halo AI, IHC, IF and other spatial analysis platforms. If patient has sufficient baseline tissue remaining in diagnostic block, submit 1 FFPE block (preferred) or 1-10 unstained slides cut at 4 um on plus charged slides. Block will be returned. (Optional) External sites: please include a pathology report and as available, send archival tissue to Brainson lab at MCC.	MCC's BPTP SRF; **NOTE**: external sites will ship tissue samples to MCC's BPTP SRF
TIMEPOINT #1: Pre-treatment (within 2 weeks before C1D1)		
1.3.1 CtDNA (Zhang)	A total of 15 mL of whole blood collected in two Streck Cell-Free DNA Blood Collection tubes (collect prior to dosing). *NOTE*: 15mL collected via two tubes → - draw approximately 8mL into the first cfDNA tube - -draw 7mL into the second tube.	MCC BPTP; Deliver or Ship samples to MCC's BPTP
1.3.3 Cytokines (Woodward)	Four 8mL tubes (BD Vacutainer CPT tube w/ sodium citrate glass tube with blue rubber stopper with black stripes). (collect all four tubes prior to dosing). *NOTE*: Draw a total of 32mL at this timepoint.	MCC's FCIM; Deliver or Ship samples to MCC's BPTP
1.3.4 EVs (St. Clair)	15 mL peripheral blood in heparin tube to obtain plasma. (collect prior to dosing).	MCC BPTP; Deliver or Ship samples to MCC's BPTP
1.3.2 Nivo PKs (Phelps)	Prior to dosing, draw one 4mL peripheral blood in red top Vacutainer tubes (with clot activator). At 30-mins post-administration, draw 4mL into a second tube. *NOTE*: A total of 8mL is collected at this timepoint.	MCC BPTP; Ship samples to OSU
1.3.2 FcRn (Phelps)	A total of 12mL of peripheral blood (collect two 6mL green-top tubes with heparin to assess PBMC for neonatal Fc Receptor). (collect prior to dosing).	MCC BPTP; Ship samples overnight to OSU
TIMEPOINT #2: C2D1 visit (after Cycle 1)		
1.3.1 CtDNA (Zhang)	A total of 15 mL of whole blood collected in two Streck Cell-Free DNA Blood Collection tubes (collect prior to dosing). *NOTE*: 15mL collected via two tubes → - draw approximately 8mL into the first cfDNA tube - -draw 7mL into the second tube.	MCC's BPTP; Deliver or Ship samples to MCC's BPTP

1.3.3 Cytokines (Woodward)	Four 8mL tubes (BD Vacutainer CPT tube w/ sodium citrate glass tube with blue rubber stopper with black stripes). (collect the four tubes prior to dosing). *NOTE*: Draw a total of 32mL at this timepoint.	MCC's FCIM; Deliver or Ship samples to MCC's BPTP
1.3.4 EVs (St. Clair)	15 mL peripheral blood in heparin tube to obtain plasma. (collect prior to dosing).	MCC's BPTP; Deliver or Ship samples to MCC's BPTP
1.3.2 Nivo PKs (Phelps)	Prior to dosing: draw one 4mL peripheral blood in red top Vacutainer tubes (with clot activator). At 30-mins post-administration: draw 4mL in a second tube. *NOTE*: A total of 8mL is collected at this timepoint.	MCC's BPTP; Ship samples to OSU
TIMEPOINT #3: C3D1 visit (after Cycle 2)		
1.3.1 CtDNA (Zhang)	A total of 15 mL of whole blood collected in two Streck Cell-Free DNA Blood Collection tubes (collect prior to dosing). *NOTE*: 15mL collected via two tubes → - draw approximately 8mL into the first cfDNA tube - -draw 7mL into the second tube.	MCC's BPTP; Deliver or Ship samples to MCC's BPTP
1.3.3 Cytokines (Woodward)	Four 8mL tubes (BD Vacutainer CPT tube w/ sodium citrate glass tube with blue rubber stopper with black stripes). (collect the four tubes prior to dosing). *NOTE*: Draw a total of 32mL at this timepoint.	MCC's FCIM; Deliver or Ship samples to MCC's BPTP
1.3.4 EVs (St. Clair)	15 mL peripheral blood in heparin tube to obtain plasma (collect prior to dosing).	MCC's BPTP; Deliver or Ship samples to MCC's BPTP
1.3.2 Nivo PKs (Phelps)	Prior to dosing, draw one 4mL peripheral blood in red top Vacutainer tubes (with clot activator). At 30-mins post-administration, draw 4mL in a second tube. *NOTE*: A total of 8mL is collected at this timepoint.	MCC's BPTP; Ship samples to OSU
TIMEPOINT #4: Restaging Biopsy after Chemo-Nivo Induction (Cycle 3 between Days 14-28)		
1.3.5 Tumor-infiltrating neutrophils, lymphocytes and monocytes (Brainson; optional sample, will be collected if sufficient tissue is available)	Re-staging biopsy of lymph nodes to determine surgical eligibility will be conducted as clinically indicated. After this restaging biopsy if sufficient tissue is available, it will be analyzed using Halo AI, IHC, IF and other spatial analysis platforms. If patient has sufficient tissue remaining in block, submit 1 FFPE block (preferred) or 1-10 unstained slides cut at 4 um on plus charged slides. Blocks will be returned. Any tumor, tumor bed or lymph node specimens should be submitted (Optional). External Sites: please include a pathology report and as available, send tissue to Brainson lab at MCC.	MCC's BPTP; Deliver or Ship samples to MCC's BPTP

TIMEPOINT #5: Visit after Cycle 3 is completed (just before surgery or chem-xrt)		
1.3.1 CtDNA (Zhang)	A total of 15 mL of whole blood collected in two Streck Cell-Free DNA Blood Collection tubes. *NOTE*: 15mL collected via two tubes → - draw approximately 8mL into the first cfDNA tube - -draw 7mL into the second tube.	MCC's BPTP; Deliver or Ship samples to MCC's BPTP
1.3.3 Cytokines (Woodward)	Four 8mL tubes (BD Vacutainer CPT tube w/ sodium citrate glass tube with blue rubber stopper with black stripes). *NOTE*: Draw a total of 32mL at this timepoint.	MCC's FCIM; Deliver or Ship samples to MCC's BPTP
1.3.4 EVs (St. Clair)	15 mL peripheral blood in heparin tube to obtain plasma.	MCC's BPTP; Deliver or Ship samples to MCC's BPTP
1.3.2 Nivo PKs (Phelps)	Draw one 4mL peripheral blood in red top Vacutainer tube (with clot activator). *NOTE*: A total of 4mL is collected at this timepoint.	MCC's BPTP; Ship samples to OSU
TIMEPOINT #6: Surgical Resection		
1.3.5 tumor-infiltrating neutrophils, lymphocytes and monocytes (Brainson)	Post-Surgical Lung tissue sample will be evaluated for pathologic treatment response as per standard of care. - Mandatory tissue sample collection for any patient who undergoes surgical resection where sufficient tissue is remaining - NOTE: if a patient does not go to surgery, then the tissue from the restaging biopsy (post-induction) will be analyzed (see Timepoint #4 above; where sufficient extra tissue is available) After surgical resection: *<u>if sufficient post-surgical lung tissue is available</u>*, it will be analyzed using Halo AI, IHC, IF and other spatial analysis platforms. If patient has sufficient tissue remaining in block, submit 1 FFPE block (preferred) or 1-10 unstained slides cut at 4 um on plus charged slides. Blocks will be returned. Any tumor, tumor bed or lymph node specimens should be submitted (Optional). External Sites: please include a pathology report and as available, send tissue to Brainson lab at MCC.	MCC's BPTP SRF; external sites will ship samples to MCC's BPTP SRF

5.2 Specimen Procurement Kits and Shipment Scheduling

Objective 1.3.1: **Zhang – ctDNA**

- For samples collected at Markey Cancer Center, samples will be transported to FCIM for processing. After collection, all STRECK tubes will be processed to isolate cell-free DNA by MCC's FCIM (or the oncogenonics) core will process this.
- Outside facilities will process and ship per Appendix C

Objective 1.3.2a **Phelps at OSU – Nivolumab PKs (plasma concentrations)**

PKs: Collect peripheral blood – pre-treatment and 30-min post-administration (4 mL each) of each dose in Vacutainer red top [plastic (has a clot activator)]. Samples will be collected at 4 timepoints, as shown above and in study calendar

- Specimen collected at Markey Cancer Center will be transported to BPTP for processing.
- Outside facilities will process and ship per Appendix G

Objective 1.3.2b **Phelps at OSU – FcRn (neonatal Fc Receptor)**

- Adherent labels will be placed on each tube and using an ethanol-resistant permanent marker write the following information: the study number (NCT06003075) the subject's ID, the date and time of collection, and the study time point (Baseline).
- Collect a total of 12mL (2 tubes x 6mL) of peripheral blood in Green Top tubes with sodium heparin (to collect PBMC). Collect at baseline (pre-treatment) timepoint; draw blood prior to dosing.
- After collection, samples will be gently inverted several times.
- Samples should be shipped the same day as collection and can be stored at ambient temperature until shipped.
- The samples will be shipped overnight per Appendix G.

Objective 1.3.3: **Woodward – Isolation of PBMCs** and Plasma for cytokines, T-cells and macrophages polarity.

- For samples collected at Markey Cancer Center, samples will be transported to FCIM for processing. The PBMC and plasma processed will be transported to the Biospecimen core facility at Markey Cancer Center, where they will be cataloged and stored until analysis.
- Outside facilities will process and ship per Appendix D

Objective 1.3.4 **St. Clair - Extracellular vesicles**

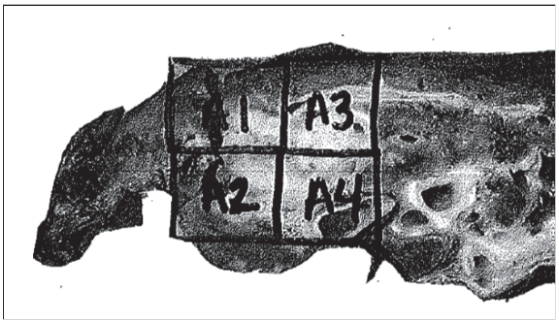
15 mL peripheral blood are collected in tubes containing heparin to obtain plasma. Plasma will be frozen and stored until batched EV isolation and dispense for analysis.

- Outside facilities will process and ship per Appendix H.

5.3 Lung Tissue: Pathology Processing and Evaluation of Specimens

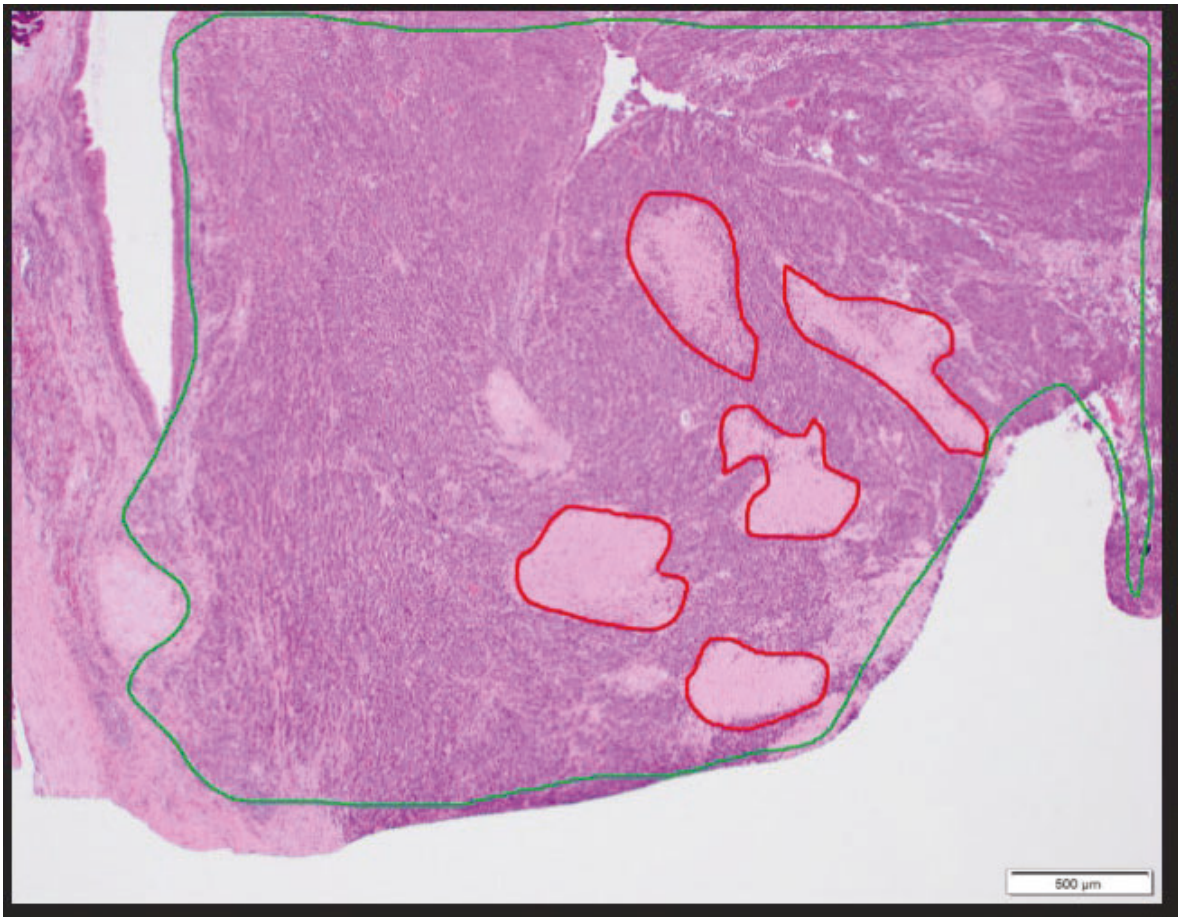
- FFPE Tissue for Central Pathology Review
  - Original biopsy material and post therapy biopsy and resection specimen (for those who go to surgery) should be submitted for central pathology review
  - Any tumor, tumor bed or lymph node specimens should be submitted for review
  - Diagnostic Pathology Report must be sent with slides

- Post-Surgical Lung tissue submission
  - <3 cm: tumor/ tumor bed submitted entirely for processing
  - >3 cm: Complete cross section of largest tumor area mapped and submitted for processing, plus one section per additional tumor slice
  - Include gross assessment of tumor necrosis in pathology report



- Lymph node sampling:
  - If < 1cm bisect along long axis
  - If > 1 cm, serially section and submit entirely
- Any photograph/sketches noting the measurements of the primary tumor bed or showing where sections were taken from should be included

- Tumor viability assessment
  - Formal assessment for level of pathologic response as per Hellmann.[26]
  - Slides will be scanned using Aperio AT2 whole scanning system (Leica Biosystems Inc., Buffalo Grove, IL, USA)
  - Tumor necrosis will be assess using Halo image analysis software (Indica Labs, Albuquerque, NM, USA)
- One full slice of the largest tumor bed area will be submitted and evaluated. The slice will be photographed and the sections mapped at the time of grossing the specimen. The study pathologist will evaluate whole slide images and annotate them for tumor area and necrosis. The viable tumor area will be measured using image analysis software. Tumor viability will be averaged across the entire tumor slice.



Slide of an annotated lung tumor (green is tumor bed, red is necrosis)

6. TREATMENT PLAN

Prior to any study-required tests, subjects must first provide written informed consent to participate in this study. Section 11 details windows for completion of baseline scans and labs to verify eligibility.

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 10. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Overview of the Sequence of Treatments

Synopsis

At baseline, a work up to establish stage and eligibility will be completed as in the study calendar in section 11. Presented here are comments on the baseline lymph node biopsy sampling.

Baseline histologic or cytologic confirmation of nodal involvement is outlined below. Tissue confirmation of all mediastinal nodal involvement is not required, however at least one N2 or N3 lymph node needs to be positive. If only 1 N2 node station is biopsy positive, patients can still be eligible if other mediastinal N2 lymph node stations or N3 nodal involvement can be declared by imaging when the nodes have distinct margins and the size of the shortest axis of one such lymph node is at least 1 node is ≥2.0 cm (MRI/CT scan). Note, a single N3 lymph node being biopsy positive is sufficient for eligibility and thus there is no requirement for further lymph nodes sampling or for other lymph nodes to be declared by imaging. Tissue confirmation of ALL mediastinal nodal involvement is not required. Thus, patients can be eligible even if biopsied lymph nodes are negative if at least 1 N2 lymph nodes is biopsy positive but at least 1 other different N2 station lymph node or any N3 lymph nodes which were not biopsied are deemed to harbor metastatic disease. Mediastinal or other (contralateral hilar, supraclavicular) N3 nodal involvement can be declared by imaging when the nodes have distinct margins and the size of the shortest axis of at least 1 node is ≥2.0 cm (MRI/CT scan).

All patients must have the following baseline biopsy sampling:

(a) Tissue needs to be sufficient for PDL1 testing. Obtaining ≥ 5 core biopsies specimens is preferable but not required. Baseline repeat biopsy is encouraged but not required if the initial baseline is an aspirate.
(b) Baseline pathologic staging should be sufficient to demonstrate at least 1 N2 or N3 lymph node harboring tumor. It is desirable but not required that at least one contralateral and two ipsilateral mediastinal stations (at least stations 2 or 4 and 7) be biopsied. If a patient has imaging evidence of involved lymph node stations beyond 2 or 4 and 7, a biopsy of these is encouraged but not required.
(c) Sampling of supraclavicular lymph nodes positive on PET/CT is encouraged but not required if the patient is otherwise eligible.

Patients receive induction/neoadjuvant pathology determined platinum doublet plus nivolumab over 3 cycles. This is followed by a CT with IV contrast. If there is an IV contrast allergy, offering sufficient proper prophylaxis to enable the IV contrast is strongly encouraged at baseline and follow-up CTs. The result of this CT will be used to assess radiographic RECIST response, which is the primary endpoint of the study. Also, at

this time, repeat PET/CT should be done as feasible. The CT will also serve to determine the next diagnostic/therapeutic step. If it shows distant metastatic disease, the patient will be off of study. Otherwise, the next therapeutic step will be either surgery or definitive radiation concurrent with chemotherapy and nivolumab.

If there is PD at the primary site by repeat CT (with IV contrast) or if it is otherwise determined to be unresectable on the basis of the CT alone, the patient will not need a pathology restaging assessment. Increased lymphadenopathy is not automatically considered PD given the risk that this represents pseudo-progression and these patients should have a post-induction repeat biopsy unless in the judgement of the multi-disciplinary team, the lymphadenopathy represents PD, such as if it becomes matted and/or appears to be invading local structures.

In the event the patient is not deemed a surgical candidate on the basis of repeat imaging, a repeat biopsy with an effort to get core pathologic samples is strongly encouraged. Unresectable patients will be treated with concurrent chemotherapy radiation on study. However, if there is not CT determined PD at the primary site, the patient will have pathology restaging even if some of the lymph nodes showed radiographic RECIST determined evidence of PD. This is the default approach given the substantial potential likelihood these lymph nodes represent pseudo-progression. Patients determined to be a candidate for restaging will then have a biopsy procedure of a kind determined by the team to best enable adequate sampling of the lymph nodes, whether EBUS, mediastinoscopy, or other.

Post-induction tumor sampling:

Details on post-induction tumor sampling are in section 6.2.

If the repeat pathology sampling shows the patient has at most N2 disease with at most 1 lymph node station positive (may have more than 1 lymph node positive in that station), then the patient will get surgery. Lymph node sampling will be done in accordance with NCCN guidelines (with additional criteria as in section 6.2).

After surgery, the multidisciplinary team will decide on whether to offer post-operative radiation with the following guidance:

Post-op XRT + nivolumab (4-8 weeks after surgery):

- pN0-N1, no PORT
- pN2 single station, t/c PORT
- pN2 >single station, PORT
- Concurrent chemo-nivolumab-XRT if positive margin

Once all definitive therapy is completed, whether radiation or surgery, the patients will have a repeat CT scan. If there is no evidence of disease, the patients will then receive 1 year of consolidation nivolumab. The patients will then be followed off of therapy.

6.1 Induction Regimens:

Concomitant use of anti-emetics should follow institutional guidelines for highly or moderately emetogenic chemotherapeutic regimens based on classification of the chemotherapy's emetic potential. Additional guidance can be found at www.nccn.org.

G-CSF, if given, should be administered as per local hospital guidelines and/or The American Society of Clinical Oncology guidelines for use of CSFs.

6.1.1 Nonsquamous: Nivolumab with Cisplatin/Pemetrexed

Cisplatin/Pemetrexed/Nivolumab					
Agent	**Precautions;**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Nivolumab	After administration of nivolumab, monitor for hypersensitivity reactions (refer to section 7.1) **Give nivolumab prior to chemotherapy**	360 mg (diluted in 100mL 0.9% sodium chloride)	IV over 30 minutes with 0.2 micron filter	Day 1, Q3 weeks	21 days (3 weeks)
Pemetrexed	**Give pemetrexed prior to cisplatin**	500 mg/m2 (diluted in 100mL 0.9% sodium chloride)	IV over 10 minutes	Day 1, Q3 weeks	21 days (3 weeks)
Cisplatin	**Note: substitution of carboplatin for cisplatin is permitted for participants who cannot tolerate cisplatin at discretion of treating physician**	70 mg/m2 (diluted in 500 mL 0.9% sodium chloride)	IV over 2 hours or per institutional standard	Day 1, Q3 weeks	21 days (3 weeks)

6.1.2 Nonsquamous: Nivolumab with Carboplatin/Pemetrexed

Carboplatin/Pemetrexed/Nivolumab					
Agent	**Premedications; Precautions; Postmedications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Nivolumab	After administration of nivolumab, monitor for hypersensitivity reactions (refer to section 7.1) **Give nivolumab prior to chemotherapy**	360 mg (diluted in 100mL 0.9% sodium chloride)	IV over 30 minutes with 0.22 micron filter	Day 1, Q3 weeks	21 days (3 weeks)
Pemetrexed	**Give pemetrexed prior to carboplatin**	500 mg/m2 (diluted in 100mL 0.9% sodium chloride)	IV over 10 minutes	Day 1, Q3 weeks	21 days (3 weeks)
Carboplatin	**Note: substitution of carboplatin for cisplatin is permitted for participants who cannot tolerate cisplatin at discretion of treating physician**	AUC 5 (diluted in 250mL 0.9% sodium chloride)	IV over 30 minutes	Day 1, Q3 weeks	21 days (3 weeks)

6.1.3 Squamous or Nonsquamous: Nivolumab with Carboplatin/Paclitaxel

Carboplatin/Paclitaxel/Nivolumab					
Agent	**Premedications; Precautions; Postmedications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Nivolumab	Monitor for hypersensitivity reactions (refer to section 7.1) Give prior to chemotherapy	360 mg (diluted in 100mL 0.9% sodium chloride)	IV over 30 minutes with 0.22 micron filter	Day 1, Q3 weeks	21 days (3 weeks)
Paclitaxel	**Give paclitaxel prior to carboplatin** Monitor for hypersensitivity reactions	200mg/m2 (diluted in 500mL 0.9% sodium chloride) Non-PVC tubing with 0.22 micron filter	IV over 3 hours	Day 1, Q3 weeks	21 days (3 weeks)
Carboplatin		AUC 6 (diluted in 250mL 0.9% sodium chloride)	IV over 30 minutes	Day 1, Q3 weeks	21 days (3 weeks)

6.1.4 Squamous: Nivolumab with Cisplatin/Gemcitabine

Day 1 Cisplatin/Gemcitabine/Nivolumab (see chart just below this chart for Day 8 gemcitabine)					
Agent	**Premedications; Precautions; Postmedications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Nivolumab	After administration of nivolumab, monitor for hypersensitivity reactions (refer to 7.1) **Give nivolumab prior to chemotherapy**	360 mg (diluted in 100 mL 0.9% sodium chloride)	IV over 30 minutes with 0.22 micron filter	Day 1, Q3 weeks	21 days (3 weeks)
Gemcitabine	**Give gemcitabine prior to cisplatin**	1000 mg/m2 (diluted in 100 mL 0.9% sodium chloride)	IV over 30 minutes	Day 1 **&** Day 8, Q3 weeks (see below for Day 8)	21 days (3 weeks)
Cisplatin	**Note: substitution of carboplatin for cisplatin is permitted for participants who cannot tolerate cisplatin**	70 mg/m2 (diluted in 500 mL 0.9% sodium chloride)	IV over 2 hours or per institutional standard	Day 1, Q3 weeks	21 days (3 weeks)

Day 8 Gemcitabine in the Cisplatin/Gemcitabine/Nivolumab regimen for squamous					
Agent	**Premedications; Precautions; Postmedications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Gemcitabine	*NOTE*: Day 8 is gemcitabine only (i.e., Nivolumab and Cisplatin are only administered on Day 1).	1000 mg/m2 (diluted in 100 mL 0.9% sodium chloride)	IV over 30 minutes	Day 1 and Day 8, Q3 weeks (for Day 1 see above)	21 days (3 weeks)

6.1.5 Squamous: Nivolumab with Carboplatin/Gemcitabine

Day 1 Carboplatin/Gemcitabine/Nivolumab (see chart just below this chart for day 8 gemcitabine)					
Agent	**Premedications; Precautions; Postmedications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Nivolumab	After administration of nivolumab, monitor for hypersensitivity reactions (refer to 7.1) **Give nivolumab prior to chemotherapy**	360 mg (diluted in 100 mL 0.9% sodium chloride)	IV over 30 minutes with 0.22 micron filter	Day 1, Q3 weeks	21 days (3 weeks)
Gemcitabine	**Give gemcitabine prior to carboplatin**	1000 mg/m2 (diluted in 100 mL 0.9% sodium chloride)	IV over 30 minutes	Day 1 and Day 8, Q3 weeks *(for Day 8 see below)*	21 days (3 weeks)
Carboplatin	**Note: substitution of carboplatin for cisplatin is permitted for participants who cannot tolerate cisplatin**	AUC 5 (diluted in 250mL 0.9% sodium chloride)	IV over 30 minutes	Day 1, Q3 weeks	21 days (3 weeks)

Day 8 Gemcitabine in the Carboplatin/Gemcitabine/Nivolumab regimen for squamous					
Agent	**Premedications; Precautions; Postmedications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Gemcitabine	*NOTE*: Day 8 is gemcitabine only (i.e., Nivolumab and Carboplatin are only administered on Day 1).	1000 mg/m2 (diluted in 100 mL 0.9% sodium chloride)	IV over 30 minutes	Day 1 and Day 8, Q3 weeks *(for Day 1 see above)*	21 days (3 weeks)

6.1.6 Growth Factor Support

The use of granulocyte colony stimulating factor (G-CSF) is allowed during induction regimens at the discretion of the treating physician if patient is considered high risk for febrile neutropenia. This will be commercially supplied and agent will be based on insurance approval/preference.

If a patient develops neutropenic fever during radiation with or without some combination of post-operative chemotherapy-nivolumab-radiation, the regimen should be stopped and G-CSF can be offered per local hospital guidelines. However, secondary prophylactic G-CSF should be avoided concurrent with radiation. The American Society of Clinical Oncology guidelines for use of CSFs should be followed for this study.

6.1.9 Carboplatin dosing

Carboplatin dose should be calculated using the Calvert formula per institutional standard.

6.2 Post-Induction

Following induction therapy, the post-induction restaging will determine whether a patient's baseline unresectable tumor has become resectable after induction chemotherapy-nivolumab.

After patients receive their induction/neoadjuvant regimen, a CT chest done with contrast is done to assess response, the primary endpoint of this study. This should be done during course 3 day 11-21. A PET/CT should also be done as feasible. If the CT shows distant metastatic disease, the patient will be taken off study. Otherwise, the next therapeutic step will be either surgery or definitive radiation concurrent with chemotherapy and nivolumab. The multi-disciplinary team will review the CT and determine whether the CT shows the cancer is potentially resectable.

However, care should be taken to not exclude patients from surgery on the basis of imaging alone when it shows new or increased post-induction lymphadenopathy. These newly or further enlarged lymph nodes after the induction regimen may reflect inflammation rather than progression. Every reasonable effort to pathologically evaluate post-induction response with a view to the patient becoming a potential surgical candidate, should be taken.

On review of post-induction CT, patients without PD at the primary site by RECIST will have tumors which are deemed potentially resectable and will have pathologic restaging. Patients with increased dimensions of the regional lymph nodes by RECIST should also get pathologic restaging as there is a risk that the increased dimensions of the lymph nodes represent inflammation. It should be noted, that if a PET/CT is done and shows increased SUV, this too may represent inflammation and should not prevent the pathologic restaging procedure.

If in the judgement of the multi-disciplinary team, the lymphadenopathy represents PD, such as if it becomes matted and/or appears to be invading local structures, a biopsy would not be necessary and the patient could proceed to definitive chemo-nivolumab-radiation on study. However, even if the patient is not deemed a surgical candidate on the basis of repeat imaging, a repeat biopsy with an effort to get core pathologic samples is strongly encouraged in order to permit post-induction molecular pathology analyses.

Post-induction repeat biopsy for restaging to determine eligibility for surgical resection:

a. A patient should still get pathologic restaging as clinically indicated. Repeat biopsy for restaging should strongly be considered even if there is PD at lymph nodes by RECIST (as above). If the primary lesion has not increased by 20% unidimensionally, as per RECIST, this would not be PD and surgery should be done if still technically resectable. If there is RECIST radiographic PD isolated to increased regional lymph nodes (including de-novo lymphadenopathy), the patient should get post induction repeat biopsy. Pseudo-PD may be especially prevalent in lymph nodes.[49] If a PET/CT is done and it shows increased SUV, this too may represent inflammation and should not prevent the pathologic restaging procedure.
b. <u>If indicated, the restaging biopsy will be done on a separate day from surgery.</u> Staging biopsies prior to planned surgery or radiation will be done as clinically indicated, and are standard of care. Choosing to do the restaging biopsy on a separate day permits analyses of formalin-fixed paraffin-embedded tumor specimens to be assessed to determine if the patient has become a post-induction chemotherapy-nivolumab surgical candidate.
c. <u>The restaging biopsy procedure (done on a separate day from surgery) should be during course 3 Days 14-28.</u> The date of this restaging biopsy should be closely coordinated with planned surgery so as to minimize the risk of delay of the surgery date. If the patient is a surgical candidate, the surgery should be scheduled.
d. <u>Path Sampling</u> (Mediastinoscopy, VATS, EBUS, other).
    i. The procedure is determined by the surgeon and multi-disciplinary team and depends on factors which include the location of the primary, the anatomy of the lymph nodes to be biopsied and other technical considerations. It should be noted that patients who had baseline mediastinoscopy typically will not be able to get a mediastinoscopy at the time of post-induction biopsy.
    ii. Every effort should be made to plan biopsy procedures which maximize the amount of tissue. This is especially relevant for those who will not go to surgery and thus, these tissue specimens will be what is available for post-induction pathologic analyses. Thus, as possible, cores, or lacking this, large aspirates to enable assessment of correlative molecular/cellular endpoints and analyses of the level of pathologic response. This can factor into the choice of post-induction biopsy procedure.
    iii. Biopsy the following
        1. All lymph node stations biopsied at baseline should be re-biopsied, if feasible. An example of infeasible would be a contralateral hilar lymph node positive at baseline but not accessible during mediastinoscopy. If a biopsy known or suspected to have had tumor at baseline is no longer identifiable, at least one lymph node from that station needs to be biopsied. Newly suspicious lymph node stations should be biopsied when feasible. In the case of a CR, and attempt at rebiospy should be made with the option to biopsy a lymph node from the same station if unclear which among the lymph nodes was initially positive.
        2. LN stations which look technically unresectable should be biopsied if feasible
        3. Biopsy of 1 representative lymph node per station is sufficient
        4. Below are comments on determining resectability in the event key lymph nodes cannot be feasibly biopsied (in "h" below).
e. Any pre-induction disease which is not technically resectable (such as contralateral hilar lymph node among others), should be shown by pathologic sampling to harbor no viable tumor in order for the patient to advance to full resection unless not feasible to biopsy (see "g"). This would include lymph nodes for which there was pre-induction biopsy proven or radiographic high suspicion of disease.
f. Note, for this purpose, if there are a number of lymph nodes or tissue nodules at a given location for which there was pre-induction therapy biopsy or radiographic evidence of tumor, sampling a representative subset can be sufficient.
g. If such technically unresectable tissue is not accessible to pathological sampling (such as a contralateral hilar lymph node etc.) the patient can still go to surgery if in the judgement of the multi-disciplinary team, this

lymph node is determined to have a low probability of harboring viable tumor based on response in other biopsied samples.

h. To be resectable, there must be 1 or fewer N2 lymph node stations which are positive. There may be more than 1 pathologic LN within this one N2 station. See d. iii, above.
i. The primary lesion needs to be confirmed by the surgeon as still resectable as assessed by radiograph or other data.
j. If resectability criteria are met, the patient will proceed to surgery; otherwise the patient will be treated with concurrent chemotherapy radiation.
   (b) If the tumor is not resectable, the patient should be treated with concurrent chemotherapy-nivolumab-radiation within Days 22-43 of the 3rd course of induction chemotherapy-nivolumab.
   (c) Surgery should be done within Days 22-43 of induction chemotherapy-nivolumab.
      a. Surgery should include all ipsilateral mediastinal stations as per institutional guidelines (as an example right sided tumor would have stations 2, 4, 7 while left sided tumors would have stations 5, 6, 7 and also also 8/9 if clinically or guideline indicated).
      b. It must include all known post-induction viable tumor.
      c. Resection must include the entire primary tumor as seen at baseline staging.
      d. All patients will have a lobectomy at a minimum. Pneumonectomy is permitted and conversion of pneumonectomy to sleeve or lobe based on treatment response is permissible.

Guidance for deciding Post-op XRT. The below is a rough guide with the decision ultimately at the discretion of the multidisciplinary team in consultation with the patient (4-8 weeks after surgery):

- pN0-N1, no PORT
- pN2 single station, to consider PORT
- pN2 >single station, to consider PORT
- pN3 PORT
- Concurrent chemo-XRT if positive margin

6.2.1 **Post-induction chemotherapy-nivolumab-radiation in patients with unresectable disease**

Patients who are not surgical candidates will receive concurrent pathology-dependent platinum doublet plus nivolumab.

- If a post-induction biopsy is not done, definitive chemo-nivolumab radiation should start by induction course 3 Days 22-57 days.
- If a biopsy is done, definitive chemo-nivolumab radiation should start ≤21-days after the tissue acquisition.

Standard 3DCRT or IMRT technique on a linear accelerator operating at >6MV beam energy will be used. The total dose of thoracic radiation therapy will be 60Gy in 30 daily fractions of 2Gy. Simulation to adjust for changes in the post-induction tumor volume may be done when appropriate. The gross tumor volume will encompass all identified tumor as detected prior to induction therapy.

Pathology-dependent platinum doublet plus nivolumab concurrent with radiation to be given as in 6.2.1.1.

6.2.1.1 Regimens concurrent with radiation: for patients whose tumors are not resectable post induction

Nonsquamous: Nivolumab with Carboplatin/Pemetrexed Concurrent with Radiation

Carboplatin/Pemetrexed Concurrent with Radiation					
Agent	**Premedications; Precautions; Postmedications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Nivolumab	Monitor for hypersensitivity reactions (refer to section 7.1) **Give prior to chemotherapy**	360 mg (diluted in 100 mL 0.9% sodium chloride)	IV over 30 minutes with 0.22 micron filter	Day 1, Q3 weeks	21 days (3 weeks)
Pemetrexed	**Give pemetrexed prior to carboplatin**	500 mg/m2 (diluted in 100mL 0.9% sodium chloride)	IV over 10 minutes	Day 1, Q3 weeks	21 days (3 weeks)
Carboplatin		AUC 5 (diluted in 250mL 0.9% sodium chloride) (See 6.1.9 for Carboplatin Dosing)	IV over 30 minutes	Day 1, Q3 weeks	21 days (3 weeks)

Squamous or Nonsquamous: Nivolumab with Carboplatin/Paclitaxel Concurrent with Radiation

Carboplatin/Paclitaxel Concurrent with Radiation					
Agent	**Premedications; Precautions; Postmedications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Nivolumab	Monitor for hypersensitivity reactions (refer to section 7.1) **Give prior to chemotherapy (on weeks when due)**	360 mg (diluted in 100 mL 0.9% sodium chloride)	IV over 30 minutes with 0.22 micron filter	Day 1, Q3 weeks	21 days (3 weeks)
Paclitaxel	**Give paclitaxel prior to carboplatin** Monitor for hypersensitivity reactions	45 mg/m2 (diluted in 250mL 0.9% sodium chloride) Non-PVC tubing with 0.22 micron filter	IV over 1 hour	Day 1, Q1 week	Duration of radiation
Carboplatin		AUC 2 (diluted in 250mL 0.9% sodium chloride) (See 6.1.9 for Carboplatin Dosing)	IV over 30 minutes	Day 1 Q1 week	Duration of radiation

6.2.2 **Surgery cohort and Definitive chemo-nivolumab-radiation cohort**

6.2.2.1 Consolidation Nivolumab:

Consolidation Nivolumab					
Nivolumab	Monitor for hypersensitivity reactions (refer to section 7.1)	480 mg (diluted in 100mL 0.9% sodium chloride)	IV over 30 minutes with 0.22 micron filter	Q4 weeks	28 days (4 weeks)

6.3 Agent Administration

Treatment will be administered outpatient. Reported adverse events and potential risks are described in Section 10. Dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Induction regimens:

Non-squamous Cell: Induction Regimen				
Agent	**Premedications; Precautions**	**Dose / Route**	**Schedule**	**Cycle Length**
Nivolumab		360mg IV	Induction: 3 cycles q3w D1	21-day
Cisplatin		70 mg/m2	Induction: 3 cycles q3w D1	
Pemetrexed		500mg/m^2 IV	Induction: 3 cycles q3w D1	
G-CSF	Optional			
NOTES: 3 cycles of the proposed nivolumab + platinum doublet will be administered followed by either: surgery (if resectable) or concurrent chemotherapy and radiation. G-CSF, if given, should be administered as per local hospital guidelines. The American Society of Clinical Oncology guidelines for use of CSFs should be followed for this study.				

Non-squamous Cell: Induction Regimen				
Agent	**Premedications; Precautions**	**Dose / Route**	**Schedule**	**Cycle Length**
Nivolumab		360mg IV	Induction: 3 cycles q3w D1	21-day
Carboplatin		AUC 5 IV	Induction: 3 cycles q3w D1	
Pemetrexed		500mg/m^2 IV	Induction: 3 cycles q3w D1	
G-CSF	Optional			
NOTES: 3 cycles of the proposed nivolumab + platinum doublet will be administered followed by either: surgery (if resectable) or concurrent chemotherapy and radiation. G-CSF, if given, should be administered as per local hospital guidelines. The American Society of Clinical Oncology guidelines for use of CSFs should be followed for this study.				

Squamous Cell: Induction Regimen				
Agent	**Premedications; Precautions**	**Dose / Route**	**Schedule**	**Cycle Length**
Nivolumab		360mg IV	Induction: 3 cycles q3w D1	21-day
Carboplatin		AUC 6 IV	Induction: 3 cycles q3w D1	
Paclitaxel *		200mg/m^2 IV	Induction: 3 cycles q3w D1	
G-CSF				
NOTES: 3 cycles of the proposed nivolumab + platinum doublet will be administered followed by either: surgery (if resectable) or concurrent chemotherapy and radiation. G-CSF, if given, should be administered as per local hospital guidelines. The American Society of Clinical Oncology guidelines for use of CSFs should be followed for this study.				

Squamous Cell: Induction Regimen				
Agent	**Premedications; Precautions**	**Dose / Route**	**Schedule**	**Cycle Length**
Nivolumab		360mg IV	Induction: 3 cycles q3w D1	21-day
Cisplatin		70 mg/m2	Induction: 3 cycles q3w D1	
Gemcitabine		1000 mg/m2	Induction: 3 cycles q3w D1, D8	
G-CSF	Optional			
NOTES: 3 cycles of the proposed nivolumab + platinum doublet will be administered followed by either: surgery (if resectable) or concurrent chemotherapy and radiation. G-CSF, if given, should be administered as per local hospital guidelines. The American Society of Clinical Oncology guidelines for use of CSFs should be followed for this study.				

Squamous Cell: Induction Regimen				
Agent	**Premedications; Precautions**	**Dose / Route**	**Schedule**	**Cycle Length**
Nivolumab		360mg IV	Induction: 3 cycles q3w D1	21-day
Carboplatin		AUC 5 IV	Induction: 3 cycles q3w D1	
Gemcitabine		1000 mg/m2	Induction: 3 cycles q3w D1, D8	
G-CSF	Optional			
NOTES: 3 cycles of the proposed nivolumab + platinum doublet will be administered followed by either: surgery (if resectable) or concurrent chemotherapy and radiation. G-CSF, if given, should be administered as per local hospital guidelines. The American Society of Clinical Oncology guidelines for use of CSFs should be followed for this study.				

Regimens given concurrent with definitive radiation

Non-squamous Cell : Concurrent Chemo-XRT				
Agent	**Premedications; Precautions**	**Dose / Route**	**Schedule**	**Cycle Length**
Carboplatin		AUC 5 IV	2 cycles q3w D1	3wks
Pemetrexed		500mg/m^2 IV	2 cycles q3w D1	
Nivolumab		360mg IV	2 cycles q3w D1	
NOTES: If a patient develops neutropenic fever during radiation with or without some combination of post-operative chemotherapy-nivolumab-radiation, the regimen should be stopped and G-CSF can be offered per local hospital guidelines. However, secondary prophylactic G-CSF should be avoided concurrent with radiation. The American Society of Clinical Oncology guidelines for use of CSFs should be followed for this study.				

Squamous or Non-Squamous : Concurrent Chemo-XRT				
Agent	**Premedications; Precautions**	**Dose / Route**	**Schedule**	**Cycle Length**
Carboplatin		AUC 2 IV	6 cycles q1w D1	3 wks
Paclitaxel *		45mg/m^2 IV	6 cycles q1w D1	
Nivolumab		360mg IV	2 cycles q3w D1	
NOTES: If a patient develops neutropenic fever during radiation with or without some combination of post-operative chemotherapy-nivolumab-radiation, the regimen should be stopped and G-CSF can be offered per local hospital guidelines. However, secondary prophylactic G-CSF should be avoided concurrent with radiation. The American Society of Clinical Oncology guidelines for use of CSFs should be followed for this study.				

Consolidation Single Agent Nivolumab (after either surgery or PORT or definitive chemo-nivolumab-radiation

Non-Squamous and Squamous Cell: Consolidation Regimen				
Agent	**Premedications; Precautions**	**Dose / Route**	**Schedule**	**Cycle Length**
Nivolumab		480mg IV	Induction: 12 cycles q4wks	4 wks
NOTES: This regimen is for both patients who were treated with surgery with or without PORT or for patients who were treated with definitive concurrent chemotherapy-nivolumab-radiation. After Definitive Chemo-Nivolumab-Radiation: The consolidation nivolumab is given ≥21 days and up to 12 weeks after the last dose of nivolumab given during radiation (it is ≥21 days since nivolumab was given at the lower q3wk dose concurrent with radiation). Post-surgery: It should start ≤28 days after surgery. Post-operative radiation: If a patient gets PORT, nivolumab should be held until the radiation is completed. It should start ≤28 days after the last radiation fraction. Duration of consolidation: Consolidation nivolumab is given up to 12 cycles. Thus, can be longer than a year if there are delays in its administration (See Study Calendar Section 11).				

6.3.1 Other Modalities: Surgery and Radiation

6.3.1.1 Surgery

Surgery is done in patients whose tumors were unresectable stage IIIA-C at baseline on the basis of lymphadenopathy and are determined to be resectable after responding to induction chemotherapy-nivolumab (NOTE: patients who had primary tumors which at baseline were deemed to be unresectable even if the there was a response to induction are not eligible for this study). These patients would not have been eligible for the study given the eligibility requirement of the resectability of the primary tumor before induction therapy). Post-induction resectability is demonstrated by CT thorax (with IV contrast as feasible and the addition of optional repeat PET/CT is encouraged) and subsequent restaging biopsies which together show that all known viable tumor can be completely resected.

The timing of the surgery depends on the timing of the post-induction repeat biopsy (done on a separate day from surgery). The repeat biopsy is strongly recommended if clinically indicated, since post-induction eligibility for surgery requires repeat biopsy to determine if the tumor is fully resectable. The repeat biopsy should be done between Days 14-28 of Course 3 while surgery should be done within ≤ 21-days after the repeat biopsy. Surgery needs to be tightly coordinated with the required post-induction restaging biopsy date to minimize the risk of delay. Other details as to the scheduling of the repeat CT (with IV contrast as feasible), the presurgical repeat biopsy, and the surgery itself are in the schema and in the study calendar (Section 11).

Surgical resection should include all ipsilateral mediastinal stations (right sided 2, 4, 7 vs left sided 5, 6, 7 also 8/9 if suspicious for either).

a. It must include all known post-induction viable tumor.
b. Resection must include the entire primary tumor as seen at baseline staging.
c. All patients will have a lobectomy at a minimum. Pneumonectomy is permitted and conversion of pneumonectomy to sleeve or lobe based on treatment response is permissible.
d. Post-operative radiation will be decided by the multi-disciplinary team.

6.3.1.2 Definitive or Post-operative Radiation Therapy

Post-induction work-up prior to radiation:

Patients who are not surgical candidates and do not have distant metastases detected at the time of post-induction repeat imaging will get definitive chemotherapy-nivolumab-radiation. The timing of radiation depends on the timing and outcome of any planned post-induction repeat biopsy.

If performed, the repeat biopsy should be done between Days 14-28 of course 3 to determine if the patient is a candidate for surgery. If the patient is determined to not be a candidate for surgery based on the biopsy result, then the patient will get definitive chemo-nivolumab-radiation. The patient should start radiation $\leq$21 days after the repeat (restaging) biopsy.

A few patients will be deemed to be not surgical candidates at the time of the repeat CT (with IV contrast as feasible). The details as to how to determine resectability are in the schema, the study calendar (section 11), and in surgical planning. Even if the patient is deemed to not be a surgical candidate at the time of the repeat CT and if the patient is still considered a definitive radiation candidate with regional PD but not distant PD (for example if the primary mass shows PD by RECIST or the lymph nodes become deemed to be technically unresectable by the multiple-disciplinary team), a repeat biopsy obtaining core tissue as feasible is encouraged.

6.3.1.2.1 Radiation Planning:

Prior to inclusion of any participant on this study, the radiation oncologist will evaluate the baseline thoracic CT scan, in order to ensure that the tumor at baseline is anticipated to be treatable and treatment volumes are unlikely to significantly exceed the specified normal tissue constraints, particularly that the V20 meets the threshold of 34%. Preplanning CT simulation is encouraged to permit a comparison of pre and post induction radiation fields. This will not be required but strongly encouraged. What follows is a description of post induction chemo-nivolumab-radiation planning:

Participants will receive concurrent thoracic radiation therapy using a standardized 3DCRTor IMR**T** technique on a linear accelerator operating at 2:6 MV beam energy. 6 MV photons are preferred if possible; 10 MV photons may also be used. Use of photon energies higher than l0 MV is allowed but 6 to l0 MV energies are preferred. The target total dose of thoracic radiation therapy will be 60 Gy in 30 daily fractions of 2 Gy, prescribed to the PTV. There should be no planned breaks during the radiation except for national holidays and weekends. Proton treatment is not allowed.

4-Dimensional CT scan (4DCT) simulation is preferred, if available at the institution. If a 4DCT scan simulation is not available, then a standard non-4DCT CT simulation is permitted with a motion management technique. FOG-PET/CT should be incorporated into treatment planning (i.e. image fusion).
Daily IGRT using orthogonal X-ray, CBCT, CT on rails or MRI guidance must be used for all participants, regardless of the radiation technique. CBCT is preferred.

Center credentialing will be performed according to criteria defined in the Radiation Therapy QA Manual. Participating institutions must comply with the radiation therapy QA requirements and procedures described in the manual. Sites that do not conform to the requirements of the credentialing will not be allowed to participate.

Participants will receive treatment 5 days per week, in once-daily fractions, 2 Gy per fraction, to a target dose of 60 Gy in 30 fractions. The entire PTV must be treated daily to 60 Gy.

Resimulation to adjust for changes in tumor volumes may be performed when appropriate with replanning to achieve the dose constraints provided.

When both chemotherapy and radiation are administered at the same center/location, it is recommended that radiation should follow within 30 to 60 minutes of the completion of chemotherapy, especially on Day 1 of thoracic radiation therapy.

- When the radiotherapy is delivered at a separate location, logistic considerations may result in radiotherapy being delivered prior to the administration of chemotherapy.
- On days when thoracic radiation therapy and/or chemotherapy are delayed for administrative reasons (e.g., holidays or weather), this will not be considered a protocol violation, provided the full planned dose of thoracic radiation therapy is administered.

6.3.1.2.2 Localization, Simulation, and Immobilization

Immobilization: Immobilization is necessary to assure reproducibility of the setup. An immobilization device must be used for positioning of each participant. Alpha Cradles or Vac-lok devices should be used for shoulder and upper body immobilization. Each participant will be positioned in an institutional specific immobilization device in the treatment position on a flat table.

If a conventional (non-4-dimensional CT) treatment planning CT study is performed, the GTV, clinical target volume (CTV), and PTV will be defined on all slices. If a 4DCT is available, an IGTV volume should be created to account for tumor motion. The use of 4-dimensional CT scans and 4-dimensional radiation treatment planning is strongly preferred.

Conventional CT scan simulation will be performed during quiet, uncoached respiration while the participant undertakes a normal respiration, using 3-mm or finer slice thickness through the entire target volume if no 4DCT or motion management is available. The scan volume must include the entire thorax (cricoid to L2 or base of skull to L2 based on the tumor location especially if tumor is located in the superior lung) in order to generate dose- volume histograms for the lungs, spinal cord, heart, and esophagus.
Radiotherapy must start within 2 weeks (± 2 working days) of the planning CT scan.

For planning immediately preceding initiation of radiation treatment, target volumes (GTV, IGTV, CTV), PTV, and normal organs will be outlined on all appropriate CT slices. All planning CT scans must be performed in the treatment position using the same immobilization device for set-up as is used at the linear accelerator. Optimal immobilization is critical for this protocol in order to ensure reproducibility of the daily set-up.

Intravenous contrast during the planning CT is optional, provided that a recent diagnostic chest CT was performed with contrast to delineate the major blood vessels and involved mediastinal lymph nodes. If not, intravenous contrast must be given during the planning CT. Study sites should follow the SOC when planning for RT.

Acceptable methods of accounting for tumor motion include: design of the PTV to cover the excursion of the primary lung cancer and involved nodal CTV during free breathing (motion inclusive), or the more limited excursion during a voluntary or automatic breath hold (i.e., Elekta ABC device), abdominal compression, or a gating approach (e.g., Varian RPM system).

A treatment planning FDG-PET/CT scan (or FDG-PET alone) with the participant in the treatment position is encouraged for treatment planning. Where an FDG-PET/CT is obtained in the treatment position, the CT from this study may be used as the planning CT scan.

6.3.1.2.3 Treatment Planning/Target Volumes

Target Volumes will be defined in accordance with the International Commission on Radiation Units and Measurements Report #62.[50] Elective nodal radiation is not allowed, and radiation will be confined to radiographically involved nodes only.

Gross Tumor Volume: GTV encompasses all identified tumor, identified by radiologic imaging, FDG-PET scan, bronchoscopy, or mediastinoscopy. Mediastinal lymph nodes with a short axis 2:1.0 cm or pretreatment FOG-PET scan with standardized uptake values >3 will be included in the GTV unless metastases have been shown to be absent using cytologic, histologic, or FDG-PET studies. This volume(s) may be disjointed. In the event of a collapsed lobe or lung segment, the use of FDG-PET to distinguish tumor is allowed.

Internal Gross Tumor Volume: IGTV encompasses all of the identified motion of the tumor on all ten phases of the 4DCT scan. Use of a maximum intensity projection (MIP) image is acceptable to aid in contouring the IGTV but the MIP may not be ideal in the setting of identifying cancer in the mediastinum, chest wall, heart and diaphragm. IGTV margins are enumerated in Table 13.

Clinical Target Volume: CTV is defined to be the GTV plus a 0.5cm to 1cm margin , as appropriate, to account for microscopic tumor extension. For participants with N2 disease, the CTV will include the ipsilateral hilum in the proximity of the tumor if it is felt appropriate to include this region, even if this is radiologically normal. When the hilum is normal, the lower pole of the ipsilateral hilum will be excluded if the primary tumor is located in the upper lobe and vice versa in the case of a tumor in the lower lobe. The CTV may be extended to include the entire ipsilateral hilum that is radiologically normal in cases where the location of the primary tumor makes this an appropriate consideration; for example, tumor in close proximity to the hilum. Inclusion of the hilum is encouraged for centrally located primary lung tumors. Elective treatment of the mediastinum and supraclavicular fossae will not be done.

Planning Target Volume: The appropriate CTVs (described above) will be enlarged to allow for organ motion and day-to-day setup variation to define the PTV. Typically, the CTV is expanded by 1cm in all directions (and 1.5cm superiorly or inferiorly for tumors of the lower lobe or with significant respiratory excursion) if no motion management is used (See Table 13).

Tighter PTV margins may be allowed based on assessments of CTV motion using fluoroscopy and/or 4DCT. When a 4-dimensional CT scan is performed, a participant- specific internal target volume (ITV) may be

directly derived for planning and appropriate margins added in order to derive a CTV and PTV, respectively. These determinations must be documented but are left to the discretion of the treating radiation oncologist. This PTV will be used to define the treated volume.

The final PTV accounts for participant positioning uncertainty and machine tolerance. Table 13 below provides guidance on margins of ITV and PTV.

Table 13 Planning Target Volume

Motion management	IGTV margin (cm) (superior-inferior)	IGTV margin (cm) Axial	CTV margin (cm, uniform)	Total PTV margin (superior-inferior)	Total PTV margin (axial)
Free breathing	1	0.5	0.5	1.5	1
Breath hold or gating	0.5	0.3	0.5	1	0.8
Abdominal compression	0.7	0.5	0.5	1.2	1
4D CT	Union of GTVs (IGTV)	Union of GTVs (IGTV)	0.5+IGTV	CTV+0.5 cm	CTV+0.5 cm

6.3.1.2.4 Treatment Planning

Beam-Shaping: Multi-leaf collimation will be used to spare normal tissues outside of the target volume. Heterogeneity corrections: All radiation doses will be calculated with heterogeneity corrections that take into account the density differences within the irradiated volume (e.g., air, soft tissue, or bone). Dose calculations should be performed on a non-contrast enhanced CT. Alternatively, if calculations are performed on a contrast-enhanced scan, areas of contrast enhancement adjacent to the target volumes or nearby critical structures must be over-ridden with soft tissue density.

The CT average from the 4DCT simulation should be the primary CT image that is employed for dose calculation. The CT average can also be generated from the breath hold/gated CT. The MIP image should not be used as the primary calculation dataset. It will be important to avoid using beams that pass through the uninvolved (contralateral) lung when possible to minimize the VS. Also, static IMRT may be preferred over VMAT.

Three-Dimensional Conformal Radiotherapy

The PTV is to be treated with any combination of coplanar or noncoplanar 3D conformal fields shaped to deliver the specified dose while restricting the dose to the normal tissues. The treatment plan used for each participant will be based on an analysis of the volumetric dose, including dose-volume histogram analyses of the PTV and critical normal structures. Each field is to be treated daily.

Intensity-Modulated Radiation Therapy

Intensity-Modulated Radiotherapy (IMRT) will be permitted if the appropriate credentialing has been performed. IMRT technologies such as Tomotherapy, ViewRay and Volumetric Modulated Arc Therapy (VMAT) are permitted. ViewRay with Cobalt 60 source and ViewRay Mridian are allowed. Motion management will be incorporated for an IMRT approach to control respiratory motion to a maximum excursion of 1.0cm. Acceptable approaches include abdominal compression, breath hold using the active breathing device or other computer-controlled spirometry, gating or other technologies.

The use of IMRT is permitted. As IMRT results in a greater proportion of lung receiving radiation outside the PTV, it is strongly recommended to maintain the total lung V5 level at 60% or less.

6.3.1.2.5 Image-Guided Radiation Therapy (IGRT) Treatment localization

IGRT can improve the accuracy, reduce the toxicities of therapy, and allows for assessing the need to adapt radiation therapy if there is tumor volume reduction (i.e., resimulation should be performed in this setting of tumor shrinkage). Daily IGRT allows for 3-Dimensional targeting of the tumor volume and daily alignment to the tumor volume. When able to localize to the soft tissue, the target is most appropriate for targeting (i.e., GTV/IGTV). Other landmarks such as the carina or implanted fiducial markers can aid in alignment.
Any shift of $\geq$2 mm should be adjusted before daily treatment administration.

6.3.1.2.6 Variations of Dose Prescription

The treatment plan shall be normalized such that 95% of the prescription dose covers at least 99% of the PTV (V57>99%). The minimum PTV dose must not fall below 95% of the prescription dose. All radiation doses will be calculated with inhomogeneity corrections that take into account the density differences within the irradiated volume (that is, air in the lung and bone). The maximum and minimum point doses (within the PTV) will be reported. No more than 0.03cc of the PTV may receive >120% of the prescription dose (maximum dose constraint).

No Variation: The treatment plan will be normalized so that $\geq$95% of the PTV is covered by the prescription dose. No more than 0.03cc of the PTV may receive >120% of the prescription dose (maximum dose constraint).

Variation Acceptable: Deviations of this magnitude are not desirable but are acceptable. The prescribed dose can cover <95% of the PTV provided it covers at least 90% of the PTV. The minimum dose can fall below 93% of the prescribed dose provided it is at least 90% of the prescription dose, if the areas of under-dosing are confined to regions of overlap with critical structures. The maximum dose within the PTV may exceed 120% of the prescribed dose provided it is no more than 125% of the prescription dose, and the areas exceeding 110% of the prescription dose are confined within the GTV and do not overlap with critical structures. Standard naming conventions, protocol constraints, and compliance criteria are tabulated in Table 14.

Table 14 Summary of Protocol Constraints and Compliance Criteria

Structure	Metric	Per Protocol	Variation Acceptable	Dosing Unacceptable
PTV	V60Gy	≥ 95%	≥90%	<90%
	Min dose (D99%)	≥57 Gy (95%)	≥54 Gy (90%)	<54 Gy
	Max dose (0.03cc)	≤72 Gy (l20%)	≤75 Gy (l25%)	>75 Gy
Spinal cord	**Max dose (0.03 cc)**	≤50.0 Gy	≤52 Gy	>52 Gy
Lungs (minus GTV)	V20Gy	≤34 %	None	None
	V5Gy	≤ 60%	≤ 65%	> 65%
	Mean dose	≤18Gy	≤20Gy	>20 Gy
Heart	Mean dose	≤20 Gy	≤26 Gy	>26 Gy
Esophagus	Max dose (0.03 cc)	≤60Gy	≤63Gy	>63 Gy
	Mean dose	≤34 Gy	≤35 Gy	>35 Gy
	V60Gy	Not circumferential	Circumferential for ≤2 cm contiguous length	Circumferential for >2 cm contiguous length
Brachial Plexus	Max dose (0.0 3cc)	≤63 Gy	≤66 Gy	>66 Gy

6.3.1.2.7 Documentation

Heterogeneity corrections for all study intervention planning will be done. It is recognized that differences between calculation algorithms in the different treatment planning systems may result in dose variations for individual participants.

Portal imaging of each field of 3DCRT or orthogonal images that localize the isocenter placement of IMRT must be obtained on the first day of therapy in accordance with standard department protocols. The films must be compared to localization films and discrepancies corrected. Daily CBCT image guidance is recommended. Weekly verification via orthogonal isocenter images are required to be taken. Images may be taken more often at the discretion of the treating physician. This verification information can be gathered also with a cone-beam CT or other CT devices that are present in the treatment room. Dose-volume histograms will be generated for PTV, both lungs, lungs minus PTV, spinal cord, esophagus, and heart.

6.3.1.2.8 Organs at Risk

Normal tissue constraints shall be prioritized in the following order for treatment planning:

1 = spinal cord, 2 = lungs, 3 = heart, 4 = esophagus, and 5 = brachial plexus.

When there is gross disease in close proximity to the esophagus or brachial plexus, it may not be possible to meet this constraint and give full dose to the gross disease. The dose coverage of the PTV will take precedence over a noncritical organ at risk (esophagus or brachial plexus only) structure in close proximity.

Spinal Cord:
The spinal canal will be contoured and taken to represent the spinal cord and should be contoured from the top of the CT simulation/cricoid to the bottom of the CT simulation/L2. **The spinal cord dose limit is the highest priority dose constraint and must be met irrespective of other constraints**. No more than 0.03cc of the spinal cord may receive greater than 50.0 Gy total dose.

Lungs:
The dose-volume constraint to the **lungs is the second highest priority and must be met, except if it conflicts with spinal cord dose constraints**. The volume of both lungs (total lung volume minus GTV) that receive more than 20 Gy (V20) must not exceed 34%. Lung V5 dosing must be limited to ≤65%.

Heart:
The heart will be contoured on all slices. The cranial border will include the infundibulum of the right ventricle and the apex of both atria and exclude the great vessels as much as possible. The caudal border is defined as the lowest part of the left ventricle that is distinguishable from the liver. The following limits are recommended: Mean heart dose, 20 Gy. A mean heart dose of up to 26 Gy will be considered a minor deviation.

Esophagus:
The esophagus contour must include the mucosa, sub mucosa, and all muscular layers out to the fatty adventitia, from the bottom of the cricoid cartilage to the gastroesophageal junction. No more than 0.03cc of the esophagus may receive >63 Gy. The mean dose to the esophagus must be ≤34 Gy. The esophagus must not be circumferentially irradiated with greater than 60 Gy at any level.

Bronchial Plexus:
The ipsilateral brachial plexus must be contoured for upper lobe tumors. No more than 0.03cc of the brachial plexus must receive >63 Gy.

Postoperative radiation therapy (PORT)
For patients who had resectable disease and had surgery, the multiple disciplinary team may recommend PORT. PORT will be administered based on a standard of care. Subjects who are recommended for PORT should have a CT scan of thorax to exclude subclinical changes suggestive of pneumonitis prior to starting PORT. In the event that subjects have radiological findings suggestive of pneumonitis on this scan then further assessment +/- treatment may be required prior to starting PORT. Considerations which are not binding which can be used in deciding on whether to offer PORT and whether to include concurrent chemotherapy are provided in the schemas. The timing of PORT can be found in the schemas and in the study Calendar. Nivolumab should not be administered during PORT but rather not begin until after PORT. The timing of the introduction of post-operative consolidation nivolumab is shown in the schemas and in the study calendar.

6.3.2 **Radiation Toxicities vs. Late Radiation Toxicities**

Toxicities documented during radiation therapy will be recorded using the CTCAE Version 5.0.

Toxicities arising more than 90-days since the completion of radiation therapy and attributed to radiation will be assessed according to CTCAE criteria and counted as late radiation toxicities.

The toxicities encountered during the post-chemotherapy-radiation treatment and during the 30-day follow-up period will not be declared "late radiation toxicities" unless the events are confined to tissue within the radiation treatment volume.

6.4 General Concomitant Medication and Supportive Care Guidelines

Concomitant use of anti-emetics should follow institutional guidelines for highly or moderately emetogenic chemotherapeutic regimens based on classification of the chemotherapy's emetic potential. Additional guidance can be found at www.nccn.org.

Prior to paclitaxel infusion, all patients should be premedicated with oral or intravenous corticosteroids, diphenhydramine, and a H2 receptor antagonist, or per institutional standard practice.

Prior to pemetrexed infusion, all patients should receive vitamin B12 supplementation, folic acid, and dexamethasone, or per institutional standard practice.

Pre- and post-cisplatin hydration should be administered per institutional standard practice.

Additional supportive measures for chemotherapy, immunotherapy and radiation are at the investigators discretion, and not restricted.

6.5 **Duration of Therapy**

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

- Completion of planned therapy
- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Clinical progression, defined as a worsening ECOG performance status or disease-related pain or

symptoms as determined by physician evaluation
- Patient non-compliance
- Pregnancy
  - All women of child-bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
  - The investigator must immediately be notified of a confirmed pregnancy in a study participant.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent

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

6.6 **Duration of Follow-Up**

Survival and treatment response: Patients will be followed for survival status and treatment response at least 24 months after the initiation of induction treatment or until death, whichever occurs first.

Patients removed from study treatment for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

See **Section 9.6** for rules regarding trial stopping criteria.

7. DOSING DELAYS/MODIFICATIONS

Chemotherapy doses should be adjusted for a >10% change from screening weight.

7.1 Nivolumab

There will not be dose modifications of Nivolumab.

Nivolumab Dose Delays during induction chemotherapy plus nivolumab:

Dose delay criteria apply for all drug-related AEs. Treatment delay is allowed for up to 2 weeks for nivolumab and up to 2 weeks for the accompanying platinum doublets; the maximum prior to the last planned dose before surgery (any dose delays greater than these will require review and approval from the UKY PI).

Nivolumab Dose Delays during concurrent radiation:

Dose delay criteria apply for all drug-related AEs. Radiation should not delayed. If concomitant chemotherapy is being given, this should not be delayed. Nivolumab may be restarted as per the criteria below. If chemotherapy is also being given, the nivolumab should be resumed on the same day as a planned chemotherapy administration.

Nivolumab Dose Delays during concurrent chemotherapy plus nivolumab with radiation:
Dose delay criteria apply for all drug-related AEs. Nivolumab may be restarted as per the criteria below. Withholding or permanently discontinuing nivolumab is at the discretion of the investigator or treating physician.

Tumor assessments for all subjects should continue as per protocol even if dosing is delayed.

Nivolumab administration should be delayed for the following:

- Any Grade ≥ 2 non-skin, drug-related adverse event, except for fatigue and laboratory abnormalities

- Any Grade ≥ 3 skin *drug-related* AE and/or
  exfoliative dermatologic conditions (suspected or confirmed Steven Johnson Syndrome, Toxic Epidermal Necrolysis or Drug Rash with Eosinophilia and Systemic Symptoms): hold for suspected; permanently discontinue for confirmed).

- Any Grade ≥ 3 drug-related laboratory abnormality with the following exceptions for lymphopenia, AST, ALT, or total bilirubin or asymptomatic amylase or lipase
  - Grade 3 lymphopenia does not require a dose delay
  - If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade 2 toxicity
  - If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity
  - Any Grade ≥ 3 drug-related amylase or lipase abnormality that is not associated with symptoms or clinical manifestations of pancreatitis does not require dose delay. The UK PI should be consulted for such Grade ≥ 3 amylase or lipase abnormalities.

- Any AE, laboratory abnormality or inter-current illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

Rescheduling:

- During induction, if the nivolumab toxicity does not resolve sufficiently to restart within a 2 week delay window, the nivolumab should be dropped and the chemotherapy be given alone that cycle. Nivolumab may be resumed at the time of the following planned platinum doublet if the nivolumab toxicities have sufficiently resolved.

Criteria to Resume Nivolumab Dosing:

- Subjects may resume treatment with nivolumab when the drug-related AE(s) resolve(s) to Grade ≤ 1 or baseline, with the following exceptions:
  - Subjects may resume treatment in the presence of Grade 2 fatigue.
  - Subjects who have not experienced a Grade 3 *drug-related* (non-radiation dermatitis) skin AE may resume treatment in the presence of Grade 2 skin toxicity.
  - Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued.
  - Drug-related pulmonary toxicity, diarrhea, or colitis must have resolved to baseline before treatment is resumed. Subjects with persistent Grade 1 pneumonitis after completion of a steroid

taper over at least 1 month may be eligible for retreatment if discussed with and approved by the UK PI.
- Subjects who received systemic corticosteroids for management of any drug-related toxicity must be off corticosteroids or have tapered down to an equivalent dose of prednisone ≤10 mg/day.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment.
- Subjects who delay study treatment due to any Grade ≥ 3 amylase or lipase abnormality that is not associated with symptoms or clinical manifestations of pancreatitis, and that is assessed by the investigator to be related to nivolumab, may resume nivolumab when the amylase or lipase abnormality has resolved to Grade < 3. The UK PI should be consulted prior to resuming nivolumab in such subjects.
- Dose delay of nivolumab which results in treatment interruption of > 2 weeks requires treatment discontinuation.

Criteria to Discontinue Nivolumab

Treatment with nivolumab should be permanently discontinued for any of the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment

- Any Grade ≥ 2 drug-related pneumonitis or interstitial lung disease that does not resolve to dose delay and systemic steroids

- Any Grade 3 drug-related bronchospasm, hypersensitivity reaction, or infusion reaction, regardless of duration

- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic toxicity, hypersensitivity reactions, infusion reactions, endocrinopathies, and laboratory abnormalities:
  - Any Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic toxicity, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation.
  - Any Grade 3 drug-related endocrinopathies adequately controlled with only physiologic hormone replacement do not require discontinuation
  - Any Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:
    - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation.
  - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
    - AST or ALT > 8x ULN
    - Total bilirubin > 5 x ULN
    - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN

- Any Grade 4 *drug-related* adverse event or laboratory abnormality, except for the following events, which do not require discontinuation:
  - Grade 4 neutropenia ≤ 7 days
  - Grade 4 lymphopenia or leukopenia

  - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset. The UK PI should be consulted for Grade 4 amylase or lipase abnormalities
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
  - Grade 4 drug-related endocrinopathy adverse events such as adrenal insufficiency, ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose controlling agents, respectively, may not require discontinuation after discussion with and approval from the UK PI.

- Dosing delays lasting > 2 weeks from the previous dose that occur for non-drug-related reasons may be allowed if approved by the UK PI. Prior to re-initiating treatment in a subject with a dosing delay lasting > 6 weeks, the UK PI must be consulted. Tumor assessments should continue as per protocol even if dosing is delayed. Periodic study visits to assess safety and laboratory studies should also continue every 6 weeks or more frequently if clinically indicated during such dosing delays.

- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing.

For subjects expected who require more than 4 weeks of corticosteroids or other immunosuppressants to manage an adverse event, consider the following recommendations

- Antimicrobial/antifungal prophylaxis per institutional guidelines to prevent opportunistic infections such as Pneumocystis and fungal infections.

- Early consultation with an infectious disease specialist should be considered. Depending on the presentation, consultation with a pulmonologist for bronchoscopy or a gastroenterologist for endoscopy may also be appropriate.

- In patients who develop recurrent adverse events in the setting of ongoing or prior immunosuppressant use, an opportunistic infection should be considered in the differential diagnosis.

Treatment of Nivolumab-Related Infusion Reactions

Since nivolumab contain only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE 5.0 guidelines.

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

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated) Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.
For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g., antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for 24 hours).

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

For Grade 3 or Grade 4 symptoms: (Severe reaction, Grade 3: prolonged [i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g., renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; pressor or ventilatory support indicated).

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

The assessment of a dose delay or discontinuation is to be made independently of the consideration of radiation treatments, please see below.

7.2 Carboplatin

Hematological toxicity
Before initiation of a new cycle of therapy, the following hematological indices must be met:

- ANC ≥ 1000/mm3
- Platelets ≥ 100,000/mm3

If the above indices have not been met, carboplatin should be delayed until recovery. The complete blood count should be checked at least once a week when the carboplatin is on hold. In patient with platelets <50,000/mm3 or ANC <500/mm3, administer 75% of the usual dose upon resumption of therapy.

7.3 Cisplatin

Hematological toxicity

Before initiation of a new cycle of therapy, the following hematological indices must be met:

- ANC ≥ 1000/mm3
- Platelets ≥ 100,000/mm3

If the above indices have not been met, cisplatin should be delayed until recovery. The complete blood count should be checked at least once a week when the cisplatin is on hold. In patient with platelets <50,000/mm3 or ANC <500/mm3, administer 75% of the usual dose upon resumption of therapy.

Neurotoxicity

Discontinue cisplatin if grade 3 or 4 peripheral neuropathy develops.

Impaired renal function

- CrCl 46-60 mL/min: Administer 75% of usual dose
- CrCl 31-45 mL/min: Administer 50% of usual dose
- CrCl 30 mL/min or less: Consider alternative agent

Electrolyte wasting (potassium, magnesium) is not a reason to discontinue treatment with cisplatin.

- Potassium and magnesium will be added to hydration fluids as indicated in section 6.
- Additional replenishment is permitted per local institutional guidelines and protocols for patients experiencing hypokalemia and hypomagnesemia.

For patients who are unable to tolerate cisplatin, the reasons for intolerability should be documented. This may include patients with baseline impaired renal function, impaired hearing, or neuropathy. Carboplatin may be substituted for cisplatin in these scenarios

7.4 Gemcitabine

Hematological toxicity

Before initiation of a new cycle of therapy, the following hematological indices must be met:

- ANC ≥ 1000/mm3
- Platelets ≥ 75,000/mm3

If ANC 500-999/mm3 or platelets 50,000-99,999/mm3, gemcitabine may be continued at 75% of the usual dose. Gemcitabine should be held until recovery for platelets ANC <500/mm3 or platelets <50,000/mm3. The complete blood count should be checked at least once a week when the gemcitabine is on hold.

Permanently discontinue gemcitabine for any of the following:

- Unexplained dyspnea or other evidence of severe pulmonary toxicity
- Severe hepatic toxicity
- Hemolytic-Uremic Syndrome or severe renal impairment
- Capillary Leak Syndrome

- Posterior reversible encephalopathy syndrome

Withhold gemcitabine or reduce dose by 50% for other severe (Grade 3 or 4) non-hematological toxicity until resolved. No dose modifications are recommended for alopecia, nausea, or vomiting.

7.5 Paclitaxel

Hematological toxicity

Before initiation of a new cycle of therapy, the following hematological indices must be met:

- ANC ≥ 1000/mm3
- Platelets ≥ 100,000/mm3

If the above indices have not been met, paclitaxel should be delayed until recovery. The complete blood count should be checked at least once a week when the paclitaxel is on hold. Paclitaxel should be reduced by 20% in patients with ANC <500/mm3.

Neurotoxicity

Reduce paclitaxel dose by 20% in patients with Grade 3 or 4 peripheral neuropathy.

Hepatotoxicity

- AST/ALT <10 times ULN and bilirubin 1.26-2 times ULN, reduce dose to 150 mg/m2
- AST/ALT <10 times ULN and bilirubin 2.01-5 times ULN, reduce dose to 100 mg/m2
- AST/ALT ≥10 times ULN or bilirubin > 5 times ULN, discontinue paclitaxel

Hypersensitivity reactions

All patients should be pretreated with a corticosteroid, antihistamine, and histamine H2 antagonist. Minor hypersensitivity reactions such as flushing, skin reactions, dyspnea, hypotension, or tachycardia do not require interruption of treatment.

Patients who experience severe hypersensitivity reactions should not be rechallenged.

7.6 Pemetrexed

In the event of toxicity, patient should be enquired about compliance with intake of folic acid. Administration of vitamin B12 every 3 cycles of therapy should be ensured.

Hematological toxicity

Before initiation of a new cycle of therapy, the following hematological indices must be met:

- ANC ≥ 1500/mm3
- Platelets ≥ 100,000/mm3

If the indices have not improved to this level, pemetrexed should be delayed until recovery. The complete blood count should be checked at least once a week when the pemetrexed is held. In patient with platelets ≥50,000/mm3 and ANC <500/mm3, or platelets <50,000/mm3 WITHOUT bleeding, administer 75% of the usual dose upon resumption of therapy. In patients who experience ≥ Grade 2 bleeding in combination with platelets <50,000/mm, the dose should be reduced by 50%.

For febrile neutropenia:

Grade 3 or 4 Febrile Neutropenia		**Pemetrexed**
	1st occurrence	**375mg/m²**
	2nd occurrence	**250mg/m²**

	3rd occurrence	**Need alternative agent**

Leucovorin can be considered for grade 4 leukopenia lasting > 3 days, grade 4 neutropenia lasting > 3 days, and immediately for grade 4 thrombocytopenia, or bleeding associated with grade 3 thrombocytopenia. The following intravenous doses and schedules of leucovorin are recommended if it is used: 100mg/m², intravenously once, followed by leucovorin, 50 mg/m², intravenously every 6 hours for 8 days.

Non-hematological toxicity

For non-hematologic toxicities (considered related to pemetrexed) ≥ grade 3, pemetrexed should be delayed until resolution to less than or equal to the patient's baseline value by the start of the cycle, before proceeding. If treatment is delayed for > 3 weeks for any pemetrexed related toxicity, the patient should be treated as follows: Discontinue all protocol treatment.

Renal Toxicity

Caution should be used when administering ibuprofen concurrently with pemetrexed to patients with mild to moderate renal insufficiency (CrCl from 45 to 79 mL/min). Patients with mild to moderate renal insufficiency should avoid taking NSAIDs with short elimination half-lives for a period of 2 days before, the day of, and 2 days following administration of pemetrexed. All patients taking NSAIDs with longer elimination half-lives should interrupt dosing for at least 5 days before, the day of, and 2 days following pemetrexed administration. If concomitant administration of an NSAID is necessary, patients should be monitored closely for renal toxicity. Renal toxicity during treatment resulting in a CrCl less than 45 mL/min requires withholding pemetrexed until CrCl has increased to at least 45 mL/min.

Stomatitis

Leucovorin may be considered for grade 3 or 4 stomatitis and can be given on the following schedule: 100mg/m², intravenously once, followed by leucovorin, 50 mg/m², intravenously every 6 hours for 8 days.

Clinically Significant Effusions

For patients who develop or have baseline clinically significant pleural or peritoneal effusions (on the basis of symptoms or clinical examination) before or during initiation of pemetrexed therapy, consideration should be given to draining the effusion prior to dosing. However, if, in the investigator's opinion, the effusion represents progression of disease, the patient should be discontinued from study therapy after confirmation of progression of disease.

Upon resolution, pemetrexed treatment will resume as follows:

- In the event of grade 3 nausea or vomiting, and/or grade 4 vomiting, pemetrexed may resume without dose reduction. Grade 3 nausea or vomiting and/or Grade 4 vomiting should be managed with appropriate changes in antiemetic regimen.
- In the event of grade 3 or 4 mucositis, pemetrexed should be resumed at 50% of the previous level.
- In the event of grade 4 transaminase elevation, grade 3 or 4 diarrhea, or any grade diarrhea requiring hospitalization, a 25% dose reduction of pemetrexed is mandatory. Thus, pemetrexed should resume at 75% of the previous dose level.
- For other grade 3 or 4 non-hematologic toxicities, treatment should resume at 75% of the previous dose level, if deemed appropriate by the treating physician.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 10.1.

Nivolumab will be supplied by Bristol-Myers Squibb directly to the specialty pharmacy of each site involved in the research, i.e., Investigational Drug Services at University of Kentucky Markey Cancer Center, and the study's sub-site (Ohio State University).

Chemotherapy (cisplatin, carboplatin, paclitaxel, pemetrexed, gemcitabine) and GCSF products at each site will be supplied commercially.

Procurement of medications:

Prescriptions for medications will be written by the site PI, treating physician, preferably the medical oncologist, using study-approved standardized chemotherapy order sets at each site per intuitional procedures. Specialty pharmacy at each site will review and approve these orders per published polices. Specialty pharmacy at each site will order and dispense Nivolumab during the treatment phase of this trial. Drug accountability will be maintained by each site's Specialty Pharmacy on a Drug Accountability Report Form (DARF), per institutional policy.

Storage & Drug Accountability:

The specialty pharmacist at each site will ensure that Nivolumab is stored in a secured, limited access storage area, under recommended storage conditions in accordance with applicable labeling and regulatory requirements and as detailed below. Under no circumstances should the investigator or other site personnel supply study drug to other investigators, patients, or clinics. Adequate records documenting receipts, use, return, loss, or other disposition of study provided drugs must be kept. The University of Kentucky Investigational Drug Service will supply drug accountability forms (DARF) that will be used, or may approve use of standard institution forms for an external site. The drug accountability forms will be maintained at each site to contain current and accurate inventory records and must be readily available for inspection. Unless otherwise authorized by the sponsor, at the end of the clinical trial all drug supplies unallocated or unused by sites or patients must be returned to the specialty pharmacy at each site for final actions in accordance with sponsor instructions.

8.1 Nivolumab: Investigational Agent

Nivolumab (also referred to as BMS-936558 or MDX1106) is a human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 is a negative regulatory molecule expressed by activated T and B lymphocytes.1 Binding of PD-1 to its ligands, programmed death–ligands 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens. Nivolumab is expressed in Chinese hamster ovary (CHO) cells and is produced using standard mammalian cell cultivation and chromatographic purification technologies. The clinical study product is a sterile solution for parenteral administration. OPDIVO™ (nivolumab) is approved for use in multiple countries including the United States (US, Dec-2014), the European Union (EU, Jun-2015), and Japan (Jul-2014).

Nivolumab Acquisition:

Nivolumab will be supplied by Bristol-Myers Squibb directly to each site involved in the research (to IDS at the University of Kentucky Markey Cancer Center and to the research specialty pharmacy at each external sub-site).

Nivolumab: Formulation, Packaging, and Labeling

Nivolumab, also referred to as BMS-936558-01 or BMS-936558, is a soluble protein consisting of 4 polypeptide chains, which include 2 identical heavy chains and 2 identical light chains. The physical and chemical properties of nivolumab are:

BMS Number	*BMS-936558-01*
Other Names	Nivolumab, BMS-936558, MDX1106, ONO-4538, anti-PD-1
Molecular Weight	146,221 daltons (143,619.17 daltons, protein portion)
Appearance	Clear to opalescent, colorless to pale yellow liquid, light (few) particulates may be present
Solution pH	5.5 to 6.5

Nivolumab Injection, 100 mg/10 mL (10 mg/mL) is a clear to opalescent, colorless to pale yellow liquid, which may contain light (few) particulates. The drug product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/mL in sodium citrate, sodium chloride, mannitol, diethylenetriaminepentaacetic acid (pentetic acid), and polysorbate 80 (Tween™ 80), pH 6.0 and includes an overfill to account for vial, needle, and syringe holdup. It is supplied in 10-cc Type I flint glass vials, stoppered with butyl rubber stoppers and sealed with aluminum seals. The only difference between the two drug product presentations is the vial fill volume.

Product Storage and Stability

Recommended Storage and Use Conditions
Nivolumab Injection
Vials of nivolumab injection must be stored at 2°C to 8°C (36°F to 46°F) and protected from light and freezing. The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours.

Undiluted Nivolumab Injection and Diluted Nivolumab Injection in the IV Container
The administration of nivolumab infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored under refrigeration conditions (2°C to 8°C, 36°F to 46°F) for up to 24 hours, and a maximum of 8 hours of the total 24 hours can be at room temperature (up to 25°C, 77°F) and room light. The maximum of 8 hours under room temperature and room light conditions includes the product administration period.

Frequency of Treatment:

For the duration of the study, nivolumab is to be administered during induction and during consolidation. During induction, it will be given on a Q3 weeks schedule with platinum-based chemotherapy which are also given Q 3 weeks, all on the same day. Nivolumab administration will precede chemotherapy administration. During concurrent chemoradiation, nivolumab will be given on a Q3 weeks schedule with platinum-based chemotherapy. During consolidation, it will be given as a single agent Q4 weeks.

Nivolumab: Product Description					
Product Description and Dosage Form	**Potency**	**Primary Packaging (Volume)/ Label Type**	**Secondary Packaging (Qty) /Label Type**	**Appearance**	**Storage Conditions (per label)**
Nivolumab BMS-936558-01 Solution for Injection	100 mg (10 mg/mL)	10 mL vial	5-vials per carton/ Open-label	Clear to opalescent colorless to pale yellow liquid. May contain particles	2 to 8°C. Protect form light and freezing

Drug Destruction:

Sponsor/Investigator drug destruction is allowed provided the following minimal standards are met:

- On-site disposal practices must not expose humans to risks from the drug.
- On-site disposal practices and procedures are in agreement with applicable laws and regulations, including any special requirements for controlled or hazardous substances.
- Written procedures for on-site disposal are available and followed. **The procedures must be filed** with the Sponsor SOPs and a copy provided to BMS upon request.
- Records are maintained that allow for traceability of each container, including the date disposed of, quantity disposed, and identification of the person disposing the containers. The method of disposal, i.e., incinerator, licensed sanitary landfill, or licensed waste disposal vendor, must be documented.
- Accountability and disposal records are complete, up-to-date, and available for BMS to review throughout the clinical trial period as per the study agreement.
- If conditions for destruction cannot be met, please contact BMS.
- It is the Sponsor Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

8.2 **Commercially Available Agents**

Carboplatin, Cisplatin, Pemetrexed, Paclitaxel and Gemcitabine are commercially available.

Refer to the package inserts for administration and for the current comprehensive list of adverse events for each of these commercially available agents.

8.2.1 Carboplatin
Expected Toxicities/Adverse Events:
- Myelosuppression (anemia, thrombocytopenia, neutropenia)
- Nausea/vomiting
- Diarrhea
- Neurotoxicity
- Hepatotoxicity
- Hypersensitivity reactions

8.2.2 Cisplatin
Expected Toxicities/Adverse Events:
- Myelosuppression (anemia, thrombocytopenia, neutropenia)
- Nausea/vomiting
- Diarrhea
- Nephrotoxicity
- Ototoxicity
- Neurotoxicity
- Hepatotoxicity

8.2.3 Pemetrexed
Expected Toxicities/Adverse Events:
- Fatigue
- Myelosuppression (anemia, thrombocytopenia, neutropenia)
- Nausea/vomiting
- Diarrhea
- Rash
- Hepatotoxicity

8.2.4 Paclitaxel
Expected Toxicities/Adverse Events:
- Hypersensitivity reactions
- Myelosuppression (anemia, thrombocytopenia, neutropenia)
- Neurotoxicity
- Nausea/vomiting
- Alopecia

8.2.5 Gemcitabine
Expected Toxicities/Adverse Events:
- Myelosuppression (anemia, thrombocytopenia, neutropenia)
- Nausea/vomiting
- Flu-like syndrome
- Hepatotoxicity

8.3 Useful Links and Contacts

- OnCore account: Mr. Hany Elkholy, hany.elkholy@uky.edu; Mr. Mark Stevens, mark.stevens@uky.edu
- UK IDS website: https://idsc.sharepointsite.net.
- UK IDS email: IDS@uky.edu
- UK IDS phone and hours of service: (859) 218-5562
- UK IDS Fax: (859) 323-4765

- UK IDS hours Monday through Friday between 8:30 am and 4:30 pm (ET)

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

This is a non-randomized phase II study of 3 cycles of chemotherapy with nivolumab in the neoadjuvant setting in unresectable stage IIIA through IIIC NSCLC. The primary endpoint is overall response rate (ORR) based on RECIST v1.1 after induction platinum doublet + nivolumab on course 3 day 11-21.

9.2 Sample Size/Accrual Rate

A total of 37 patients will be accrued over 18 months at a rate of 2 patients per month across 2 cancer centers (UK Markey, Ohio State University).

9.3 Analysis of Primary Endpoint

Assuming a 35% historic overall response rate, a single arm study with 37 patients will have over 80% power to detect a 20% increase (55%) in overall radiographic response rate with 5% one-sided type 1 error rate (see discussion on page 17). [21,36,37,40,41] Detailed power analysis on various scenarios are summarized in table below.

Numeric Results for Testing One Proportion using the Exact Test Alternative Hypothesis: One-Sided (H0: $P \leq P0$ vs. H1: $P > P0$)						
Power	n	P_0	P_1	P_1-P_0	Alpha	Reject H0 if Resp. ≥
0.80051	145	0.35	0.45	0.1	0.05	61
0.80222	65	0.35	0.5	0.15	0.05	30
*0.8074	37	0.35	0.55	0.2	0.05	19
0.8012	23	0.35	0.6	0.25	0.05	13
* Denotes the selected study design parameters						

9.4 Analysis of Secondary Endpoints

Safety data will be summarized descriptively with AE/SAE frequency and percent.

Parameters of disease responses, including rate of converting non-surgical stage II(A-C) to surgically resectable disease, pCR, major pathologic response (MPR) rate will be estimated along with appropriate 95% confidence intervals. These disease response endpoints will be evaluated formally only in those patients who have complete surgical resection with the surgery itself serving as the trigger to evaluate this endpoint.

We will tabulate the total number of patients who convert to surgery and the total number of patients who do not convert to surgery.

The rate and extent of post-induction radiation field decrease will be summarized by descriptive statistics.

The distribution of progression-free survival and overall survival will be estimated using the Kaplan-Meier method. Estimates of median PFS and OS will be obtained from the Kaplan-Meier analysis.

Confidence intervals and survival curves from secondary endpoints will only be used for descriptive/hypothesis generating.

Patient-reported Quality of Life as measured by FACT-TOI will be summarized by subscale scores. Internal consistency will be measured by Cronbach alpha.

PDL1 status will be reported by descriptive statistics, as will TMB if it is captured (where tissue available).

9.5 Analyses of Exploratory/Correlative Endpoints

Exploratory/correlative analysis will be only used for descriptive/hypothesis generating. Baseline (pre-treatment) and post-baseline changes in markers, including measures on ctDNA, cytokines via peripheral blood and PKs, circulating T-cells, macrophages polarity, extracellular vesicles and tumor infiltrating neutrophil, lymphocyte and monocyte counts will be summarized descriptively. Baseline (pre-treatment) marker values and changes in markers will be summarized by response status. Association of continuous marker levels with response will be evaluated using logistic regression. Differences between response groups with respect to change in markers will be evaluated using two-sample tests (e.g., t-tests or Wilcoxon rank sum tests). When longitudinal marker measurements are available, responders will be compared to non-responders using mixed effects linear regression. Data collected from the FACT-TOI (all participants) will be summarized descriptively and tested for intraindividual variation (minimal clinically important differences). Data collected from 6 Markey participants for the remote symptom monitoring sub-study will be summarized with descriptive statistics, comprising 5 symptoms adapted PRO-CTCAE items (patient self-report via app) and heart rate (via iOS-connected SmartWatch).

9.6 Trial Stopping Criteria

9.6.1 Considerations for suspending accrual

Accrual will be temporarily halted if any patient does not get definitive therapy (chemo-radiation or surgery) for any reason.
If accrual is halted, the investigative team will reassess circumstances and determine if it is safe to resume accrual.

The FDA approved standard of care for patients with stage III unresectable NSCLC is concurrent chemo-radiation followed by 1 year of consolidation durvalumab. In the current study, we diverge from this in a number of ways which both offer promise and pose risk. To address these risks, we will continuously monitor patients and be ready to hold accrual as below.

1) All patients will have a delay in their definitive curative intent therapy whether it is concurrent chemo-nivo-xrt or whether it is surgery. The patients who get surgery will have the option for post-op radiation or chemo-radiation. This approximate 9-week delay poses a risk of not getting definitive therapy. This can occur due to progression of disease, excess toxicity, or death. To address this risk from delay, accrual will be held when any patient during or immediately after induction chemo-nivolumab has:
    a. progression of disease preventing definitive therapy, whether concurrent chemo-nivo-radiation or surgery
    b. any grade 4 non-hematologic toxicity whether or not it delays definitive therapy. Likewise, accrual will be held if there is a hematologic toxicity which prevents definitive therapy or delays it by 2 weeks or more (11 weeks after first dose of the induction regimen)
    c. died
2) Patients assigned to surgery rather than concurrent chemo-nivo-radiation risk inferior therapy since they are getting a therapy which is not yet established as effective as the current FDA approved standard of care. To address this risk accrual will be held if
    a. any patient has a post-op death defined as dying within 30 days after surgery
    b. any patient for whom a post-op radiation containing regimen is recommended but which cannot be offered because their medical condition does not permit it. Note, if a patient refuses therapy but is otherwise medically able to have such post-op radiation, accrual does not need to halt

   c. any patient found to have progression at the time of surgery who would not be eligible for post-op definitive radiation
3) Patients who are assigned chemo-nivo-radiation are receiving the added agent, nivolumab, to the standard chemo-radiation regimen which poses risk. Accrual will be held if during or within a month of completion of radiation a patient:
   a. dies
   b. has a grade 4 non-hematologic toxicity
   c. has a grade 4 hematologic toxicity which delays radiation for a week or results in a ≥10% reduction in planned radiation dose or ≥ 1/3 reduction in total planned chemotherapy dose
   d. note, if a patient elects to stop definitive therapy not due to toxicity, the accrual will not be halted
4) For more long-term endpoints such as PFS, OS, and chronic morbidity from the treatment regimens, risk will be assessed by comparing to the up-front chemo-radiation followed by durvalumab historical controls. However, given the long lag time for these events and thus their analyses, there will not be a hold in the accrual for these outcomes. These include:
   a. Patients assigned to surgery who will be compared to both the historical controls and to the cohort of patients assigned to chemo-nivo-radiation on this study.
   b. Patients assigned to chemo-nivo-xrt to the historic controls
5) All patients will be receiving consolidation nivolumab rather than durvalumab. It is not yet established whether there is higher risk or lower efficacy of nivolumab compared to durvalumab. This study is not designed to differentiate the impact nivolumab.

9.6.2 Stopping Criteria

There is a risk of patients not receiving definitive therapy (whether it be chemo-radiation or surgery) after administration of the neoadjuvant immunotherapy regimen. To address this risk, we include a formal stopping rule based on Phase 3 trial of neoadjuvant Nivolumab in resectable NSCLC patients. [21]

If any of the following stopping criteria are met during the DSMC review of the trial, the study should be suspended or terminated:

1. The lower bound of the 95% confidence intervals (CI) for the rate of treatment-related AEs leading to discontinuation of treatment is greater than or equal to 10.2%*. CIs will be calculated using the Clopper-Pearson method.
2. The upper bound of the 95% confidence interval for the percent of patients who did not receive a definitive procedure (surgery or definitive radiation concurrent with chemotherapy and nivolumab) is lower than 83%**.
3. The 1-year event-free survival (progression, death, recurrence) of participants is significantly worse than 77%***, determined by the log-rank test.

The stopping criteria are determined based on the observed outcomes from a Phase 3 trial [21] (see notations below):

* Table 2, Treatment related AEs leading to discontinuation of treatment in the Nivolumab plus Chemotherapy group.

** Table S7, for stage IIIA patients in the Nivolumab plus Chemotherapy group, 83.2% of patients received definitive surgery.

*** Figure S4, for stage IIIA patients in the Nivolumab plus Chemotherapy group, the EFS is 77% at 1 year.

10. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 10.1) and the characteristics of an observed AE (Sections 10.2 and 10.3) will determine whether the event requires expedited reporting to Overall PI and Markey DSMC via the OnCore **in addition** to routine reporting.

NOTE: ***The below Section 10.1 is from the BMS Investigator-Sponsored Research Protocol and Adverse Event reporting guidelines:***

10.1 BMS Guide to SERIOUS AE COLLECTION AND REPORTING

All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through *100* days of discontinuation of dosing must be reported to BMS Worldwide Safety, whether related or not related to study drug**. If applicable,** SAEs must be collected that relate to any follow-up protocol-specified procedure (e.g., a follow-up skin biopsy).

- Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The Investigator should report any SAE occurring after these aforementioned time periods, which is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its seriousness;
- If the Investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours \ 1 Business Day of becoming aware of the event. SAEs must be recorded on either CIOMS, MedWatch, or approved study specific/institutional SAE form.

Extra Instructions for EXTERNAL SITE:

Information about all SAEs is collected by the external site; the external site enters the SAE information into the trial's database housed in Markey's OnCore system. The information about an SAE is **also recorded** on a MedWatch 3500A Form, which is reviewed, completed and signed by the external site PI.
The study site PI or designee e-mails the completed MedWatch form along with relevant de-identified source documentation to Markey's Multi-Center Research Unit Project Manager at **MarkeyMRU@uky.edu** within 24 hours of knowledge of the event. The MRU Project Manager will communicate all SAE information to the Markey PI within 24 hours of receipt. The MRU Project Manager will facilitate submission of external site SAE to BMS.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: +1-609-818-3804

If only limited information is initially available, follow-up reports are required. (**Note**: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours \ 1 Business Day to BMS using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

aepbusinessprocess@bms.com

BMS: Expedited and periodic safety update reporting by BMS:

It is the Sponsor-Investigator's responsibility to report events to their Local HA. In addition, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).

In accordance with local regulations, BMS will notify Sponsor-Investigator of all reported SAEs that are suspected (related to the investigational product) and unexpected (i.e., not previously described in the IB). An event meeting these criteria is termed a **Suspected, Unexpected Serious Adverse Reaction (SUSAR)**. Sponsor-Investigator notification of these events will be in the form of either a SUSAR Report or a Semi-Annual SUSAR Report. Sponsor-Investigator (or delegate) will receive these reports through the FastTrack portal.

Other important findings which may be reported by BMS as an Expedited Safety Report (ESR) include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (e.g., animal) study, important safety recommendations from a study data monitoring committee, or Sponsor-Investigator or BMS decision to end or temporarily halt a clinical study for safety reasons.

Upon receiving an ESR from BMS, the Sponsor-Investigator **must review and retain the ESR with the IB**. Where required by local regulations or when there is a central IRB/IEC for the study, the Sponsor-Investigator will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.

BMS: NON-SERIOUS ADVERSE EVENT COLLECTION AND REPORTING

The collection of non-serious AE information should begin following the subject's written consent to participate in the study. All non-serious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 30 days following the last dose of study treatment.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

Non-serious Adverse Events (AE) are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [e.g., IND US trial] as part of an annual reporting requirement.

BMS: PREGNANCY

If, following initiation of the investigational product, it is subsequently discovered that a study participant is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 5 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for participant).

The Sponsor-Investigator must immediately notify **Worldwide.Safety@bms.com** of this event and complete one of the following forms **within 24 hours of awareness** of the event via either the CIOMS, MedWatch or appropriate Pregnancy Surveillance Form in accordance with **SAE reporting procedures.**

External Site(s): The study site PI or designee e-mails the completed MedWatch form along with relevant de-identified source documentation to Markey's Multi-Center Research Unit Project Manager at **MarkeyMRU@uky.edu** within 24 hours of knowledge of the event. The MRU Project Manager will communicate all SAE information to the Markey PI within 24 hours of receipt. The MRU Project Manager will facilitate submissions of external site SAEs to BMS when applicable.

Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the **CIOMS, MedWatch, BMS Pregnancy Surveillance Form, or approved site SAE form**. A BMS Pregnancy Surveillance Form may be provided upon request.

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. In order for Sponsor-Investigator or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

BMS: LABORATORY TEST ABNORMALITIES

All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported to BMS as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (e.g., anemia versus low hemoglobin value).

BMS: OTHER SAFETY CONSIDERATIONS

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

BMS Guide to ADVERSE EVENT REPORTING FOR SPECIFIC SITUATIONS:

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, **must be reported as SAEs.**

Potential drug-induced liver injury is defined as:

1) AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)

AND

2) Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)

AND

3) No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

- **Immune-Mediated AEs**

Immune-Mediated AEs are required in **ISR Protocols with Registrational Intent**.
Every AE must be assessed by the investigator with regard to whether it is considered immune-mediated. For events which are potentially immune-mediated, additional information must be collected.

Immune-mediated adverse events (IMAEs) are AEs consistent with an immune-mediated mechanism or immune-mediated component for which non-inflammatory etiologies (e.g., infection or tumor progression) have been ruled out. IMAEs can include events with an alternate etiology which were exacerbated by the induction of autoimmunity. Information supporting the assessment will be collected.

- **AEs of Special Interest**

BMS: ADVERSE EVENT RECONCILIATION PROCESS
The Sponsor-Investigator (or designee) will reconcile the clinical database AE cases (case level only) transmitted to BMS Global Pharmacovigilance (Worldwide.Safety@bms.com).

- The Sponsor-Investigator will request the SAE reconciliation report (and include the BMS protocol number) from BMS GPV&E (aepbusinessprocess@bms.com) every 3 months and prior to data base lock or final data summary
- GPV&E will send the Sponsor-Investigator the report to verify and confirm all AE and SAEs have been transmitted to BMS GPV&E.
- The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the Sponsor-Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to BMS (Worldwide.Safety@bms.com).

10.2 Nivolumab

10.2.1 Adverse Event list for Nivolumab

Adverse Event List for <u>nivolumab</u>: Fatigue, rash, musculoskeletal pain, pruritus, diarrhea, nausea, asthenia, cough, dyspnea, constipation, decreased appetite, back pain, arthralgia, upper respiratory tract infection, pyrexia, headache, abdominal pain, and vomiting. Immune-mediated pneumonitis, Immune-mediated colitis, Immune-mediated hepatitis, Immune-mediated endocrinopathies, Immune-mediated nephritis and renal dysfunction, Immune-mediated skin adverse reactions, Immune-mediated encephalitis, Infusion reactions.

10.2.2 Prevalence of Adverse Events: Nivolumab

Very common side effects of Nivolumab: (Occurring in 10% or more of patients)

- Diarrhea
- Fatigue
- Itching
- Rash

Common Side Effects of Nivolumab: (Occurring in 1% to fewer than 10% of patients)

- Abdominal pain
- Alkaline phosphatase increased: lab test result associated with liver or bone abnormalities
- Allergic reaction/hypersensitivity
- ALT Increased: lab teste result associated with abnormal liver function
- Amylase increase: lab test associated with pancreas inflammation_
- AST Increased: lab teste result associated with abnormal liver function
- Bilirubin (liver function blood test) increased
- Chills
- Constipation
- Cough
- Creatinine increased: lab test associated with abnormal kidney function
- Decreased appetite
- Dizziness or vertigo (feeling off balance, which can lead to dizziness)
- Dry skin
- Fever
- Headache
- High Blood Pressure
- Increased Blood Sugar
- Inflammation of the colon
- Inflammation of the mouth
- Infusion related reactions
- Joint pain or stiffness

- Lipase increased: lab test result associated with pancreatic inflammation
- Loss of color (pigment) from areas of skin
- Lung inflammation (pneumonitis—see details below)
- Musculoskeletal pain
- Nausea
- Redness (of the skin)
- Shortness of breath
- Sodium levels in the blood low
- Swelling including face, arms, legs
- Thyroid gland function decreased/thyroid stimulating hormone increased (lab test associated with abnormal thyroid function)
- Thyroid gland function increased
- Tingling, numbness, burning or weakness
- Upper respiratory tract infections
- Vomiting

Uncommon Side Effects of Nivolumab: (Occurring in 0.1% to less than 1% of patients)

- Adrenal gland function decreased
- Bronchitis
- Cranial nerve disorder
- Diabetes
- Dry eyes and/or blurred vision
- Hair loss
- Heart rate increased
- Hear rhythm abnormal
- High blood pressure
- Hives
- Inflammation of the eyes
- Inflammation of the kidney
- Inflammation of the pancreas
- Inflammation of the pituitary gland
- Inflammation of the stomach, intestines, colon
- Inflammation of the thyroid gland
- Liver inflammation
- Low Blood pressure
- Lung infiltrate associated with infection or inflammation
- Pituitary gland function decreased
- Psoriasis, a skin condition characterized by scaly patches of skin.
- Kidney failure or kidney injury
- Respiratory failure

Rare side effects of Nivolumab: [Occurring in 0.01% to less than 0.1% of patients]

- Anaphylactic reaction (severe allergic reaction)
- Damage to the protective covering of the brain, nerves and spinal cord.
- Diabetes complication resulting in increased blood acid and diabetic coma
- Guillain-Barre syndrome, an autoimmune disorder associated with progressive muscle weakness or paralysis
- Inflammation of blood vessel
- Inflammation of the brain, potential life threatening or fatal
- Inflammation of the heart
- Muscle inflammation
- Myasthenic syndrome (neurologic syndrome characterized by muscle weakness) including myasthenia gravis, a nerve disease that may cause weakness of eye, face, breathing, and swallowing muscles.
- Pemphigoid: blistering of the skin or mouth caused by the immune system attacking healthy tissue.
- Rhabdomyolysis: muscle fiber released into blood stream, which could damage kidneys
- Rosacea: acne-like skin condition resulting in redness of face
- Sarcoidosis, a disease involving abnormal collections of inflammatory cells (granulomas) in organs such as lungs, skin, and lymph nodes
- Stevens Johnson syndrome: inflammatory disorder of skin and mucous membranes, resulting in blistering and shedding of skin
- Toxic epidermal necrolysis: a potentially fatal disease characterized by blistering and peeling of the top layer of skin resembling a severe burn
- Histiocytic necrotizing lymphadenitis or Kikuchi lymphadenitis: disorder of the lymph nodes, which causes the lymph nodes to become enlarged, inflamed and painful, commonly affecting lymph nodes of the neck and possibly associated with fever or muscle and joint pains.
- Vogt Koyanagi Harada syndrome; a disease that affects the pigmented tissue; this may affect the eye, leading to swelling, pain and/or blurred vision; the ear, leading to hearing loss, ringing in the ears; and /or the skin, leading to loss of skin color

Lung Inflammation (pneumonitis):

It is possible that nivolumab may cause inflammation of the tissues of the lung. This adverse effect has been reported in those treated with nivolumab. While many patients with x-ray or CT abnormalities have not developed any symptoms, some patients have developed mild to severe symptoms and in rare cases, death has occurred as a result of their lung inflammation. Signs and symptoms of lung inflammation may include difficulty breathing, pain or discomfort while breathing, chest pain, cough, shortness of breath, increased rate of breathing, fever, low blood oxygen levels, or fatigue. Your study doctor and nurse will watch you closely for changes in your ability to breathe and for other signs or symptoms that might show you are developing this type of lung inflammation.

10.2.3 Supportive Care Guidelines for Nivolumab toxicity

Opportunistic Infections: It is rare for a patient receiving immunosuppression for nivolumab-related AEs to develop an opportunistic infection. Subjects with inflammatory events of any organ category expected to require more than 4 weeks of corticosteroid or other immunosuppressive agents to manage the AE should be considered for antimicrobial/antifungal prophylaxis, per institutional guidelines, to prevent opportunistic infections such as Pneumocystis and fungal infections.

Immune-Mediated Pneumonitis: Nivolumab can cause immune-mediated pneumonitis, defined as requiring use of corticosteroids and no clear alternate etiology. Fatal cases have been reported.
Monitor patients for signs with radiographic imaging and for symptoms of pneumonitis. Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents for moderate (Grade 2) or more severe (Grade 3-4) pneumonitis, followed by corticosteroid taper. Please refer to section 6 for dose modification guidance.

Immune-Mediated Colitis: Nivolumab can cause immune-mediated colitis, defined as requiring use of corticosteroids with no clear alternate etiology. Monitor patients for signs and symptoms of colitis. Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents followed by corticosteroid taper for severe (Grade 3) or life- threatening (Grade 4) colitis. Administer corticosteroids at a dose of 0.5 to 1 mg/kg/day prednisone equivalents followed by corticosteroid taper for moderate (Grade 2) colitis of more than 5 days duration; if worsening or no improvement occurs despite initiation of corticosteroids, increase dose to 1 to 2 mg/kg/day prednisone equivalents. Refer to section 6 for dose modification guidance.

Immune-Mediated Hepatitis: Nivolumab can cause immune-mediated hepatitis, defined as requiring use of corticosteroids and no clear alternate etiology. Monitor patients for abnormal liver tests prior to and periodically during treatment. Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents followed by corticosteroid taper for severe (Grade 3) or life-threatening (Grade 4) transaminase elevations, with or without concomitant elevation in total bilirubin. Administer corticosteroids at a dose of 0.5 to 1 mg/kg/day prednisone equivalents for moderate (Grade 2) transaminase elevations. Refer to section 6 for dose modification guidance.

Immune-Mediated Endocrinopathies: Nivolumab can cause immune-mediated hypophysitis. Monitor patients for signs and symptoms of hypophysitis. Administer hormone replacement as clinically indicated and corticosteroids at a dose of 1 mg/kg/day prednisone equivalents followed by corticosteroid taper for moderate (Grade 2) or greater hypophysitis. Refer to section 6 for dose modification guidance.

Adrenal Insufficiency: Nivolumab can cause immune-mediated adrenal insufficiency. Monitor patients for signs and symptoms of adrenal insufficiency. Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents followed by a corticosteroid taper for severe (Grade 3) or life-threatening (Grade 4) adrenal insufficiency. Please refer to section 6 for dose modification guidance.

Immune-Mediated Nephritis and Renal Dysfunction: Nivolumab can cause immune-mediated nephritis, defined as renal dysfunction or Grade 2 increased creatinine, requirement for corticosteroids, and no clear alternate etiology. Monitor patients for elevated serum creatinine prior to and periodically during treatment. Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents followed by corticosteroid taper for life-threatening (Grade 4) increased serum creatinine. Administer corticosteroids at a dose of 0.5 to 1 mg/kg/day prednisone equivalents for moderate (Grade 2) or severe (Grade 3) increased serum creatinine, if worsening or no improvement occurs, increase dose of corticosteroids to 1 to 2 mg/kg/day prednisone equivalents. Please refer to section 6.

Immune-Mediated Encephalitis: Nivolumab can cause immune-mediated encephalitis with no clear alternate etiology. Evaluation of patients with neurologic symptoms may include, but not be limited to, consultation with a neurologist, brain MRI, and lumbar puncture.

Withhold OPDIVO in patients with new-onset moderate to severe neurologic signs or symptoms and evaluate to rule out infectious or other causes of moderate to severe neurologic deterioration. If other etiologies are ruled out, administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents for patients with immune-mediated encephalitis, followed by corticosteroid taper. Permanently discontinue OPDIVO for immune-mediated encephalitis

Infusion Reactions: Nivolumab can cause severe infusion reactions, which have been reported in less than 1.0% of patients in clinical trials. Discontinue OPDIVO in patients with severe or life-threatening infusion reactions. Interrupt or slow the rate of infusion in patients with mild or moderate infusion reactions

10.3 Adverse Event Lists for Other Commercially Available Agents

Please refer to the latest package inserts for toxicities and dose reduction for the following commercially available agents:

Carboplatin
Cisplatin
Pemetrexed
Paclitaxel
Gemcitabine

10.4 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

10.5 MCC Expedited Adverse Event Reporting Guidelines

Investigators and staff within MCC will report SAEs directly to the MCC DSMC per the MCC DSMC SOP and the University of Kentucky IRB reporting policy, as specified in the table in below. Use the MCC protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

External sites are not permitted to report directly to the UK IRB, FDA, or BMS. All external site SAEs are to be reported to the MCC Multi-Center Unit Project Manager. The MCC Multi-Center Unit Project Manager will facilitate submission of external site SAEs to the UK IRB, FDA, and BMS.

Information about all SAEs is collected by the site and the site enters the SAE information into OnCore. The information about an SAE is **also recorded** on a MedWatch 3500A Form, which is reviewed, completed and signed by the site PI.

FOR EXTERNAL SITES: The study site PI or designee e-mails the completed MedWatch form along with relevant de-identified source documentation to Markey's Multi-Center Research Unit Project Manager at **MarkeyMRU@uky.edu**. The MRU Project Manager will communicate all SAE information to the Markey PI within 24 hours of receipt. The MRU Project Manager will facilitae submissions of external site SAEs to the UK IRB, FDA, and BMS when applicable.

External sites must report all SAEs to their local IRB per their local IRB and institutional policy.

10.5.1 Required forms and reporting structure

Study type	Expedited reporting to MCC	Expedited reporting to External Agency	Non-expedited AE	Form	UK IRB
Multi-site IIT by MCC investigator sponsored by industry	• Grade 3 Unexpected AE regardless of attribution • Grade 3 Expected AE that is Possibly, Probably or Definitely Related • ALL Grade 4 • ALL Grade 5 (fatal) Events	*FDA*: Suspected AE that is both Serious and Unanticipated (i.e., not listed in package insert or informed consent form)	OnCore and MCC DSMC reporting only	Voluntary Medwatch 3500 for Serious and Unanticipated OnCore for all AEs, including SAEs	Per SOPs

Note: A death on study requires <u>both</u> routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

10.5.2 MCC Expedited Reporting guidelines for MCC IITs

Investigators within MCC will report SAEs directly to the MCC DSMC per the MCC DSMC SOP and the University of Kentucky IRB reporting policy.

Table 10.4.2 – MCC Reportable Adverse Events. (AEs)

Attribution	Gr. 1 AEs & Gr. 2 AEs	Grade 3 AE		Grade 4 AE	Grade 5 AE
		Expected Gr. 3	Unexpected Gr. 3		
Unrelated Unlikely	Routine reporting	Routine Reporting only	5 calendar days	5 calendar days	24-hours *
Possible Probable Definite	Routine reporting	5 calendar days	5 calendar days	5 calendar days	24-hours *

NOTES:
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 24 business hours of learning of the event.

10.5.3 Expedited Reporting to External Agencies

Overall PI will comply with the policies of all external funding agencies and the UK IRB regarding expedited reporting, as per the UK IRB's Mandated Reporting to External Agencies SOP C4.0150.

10.5.4 Expedited Reporting to the Food and Drug Administration (FDA)

The FDA deemed this trial as non-exempt. The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

10.5.5 Expedited Reporting to Hospital Risk Management

Participating investigators and study staff will report to Overall PI any participant safety reports or sentinel events that require reporting. The Overall PI will then report these participant safety reports and/or sentinel events to the UK Office of Risk Management per institutional policy.

10.6 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions.

NOTE: **AEs reported expeditiously to the PI and Markey's DSMC via OnCore must also be reported in routine study data submissions.**

10.6.1 Pregnancy

Pregnancy is considered an unanticipated events and pregnancy as well as its outcome must be documented and reported to overall PI and Markey DSMC and UK Office of Research Integrity, as well the FDA and BMS sponsor in according to

reporting requirements. Any pregnancy occurring in a patient or patient's partner from the time of consent to 90 days after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old.

10.6.2 Second and Secondary Malignancies

10.6.3 Second Malignancy

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

10.6.4 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm. All secondary malignancies that occur following treatment with an agent under an NCI IND/IDE must be reported to overall PI and Markey DSMC and UK Office of Research Integrity, as well the FDA and sponsor in according to reporting requirements. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

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

11. STUDY CALENDAR

	Screening	Induction / Neoadjuvant with Nivo and Chemo			Post-Induction Reassessment	Post-Induction Biopsy	Surgery or XRT (~ Week 10)	Nivo Consolidation (Q4wks x 12 cycles)	Observation per Std of care (a)
		Wk 1 C1D1	Wk 4 C2D1	Wk 7 C3D1					
Induction Platinum Doublet + Nivolumab **(b)**		X	X(**c**)	X(**c**)					
Concurrent Chemo/ Nivolumab/XRT and/or Surgery							X(**e,f**)		
Consolidation Nivo (12 cycles; Q4wks)								X(**g,h**)	
Informed Consent w/i 4-wks	X								
Medical History w/i 4-wks	X								
Demographics w/i 4-wks	X								
PE (vitals, wt, BMI)	X, w/i 2 weeks		X(**i**)	X(**i**)	X(**i**)			X(**j**)	X
ECOG PS	X, w/i 2 weeks		X(**i**)	X(**i**)	X(**i**)			X(**j**)	X
Concurrent meds (**i**)	X, w/i 2 weeks		X(**i**)	X(**i**)	X(**i**)				
PET-CT **(k)**	X, w/i 4 weeks								
Thoracic CT **(l, m)**	X, w/i 4 weeks				**X (l)**			**X(m)**	X
MRI brain	X, w/i 4 weeks								
Viral load studies (Hep B/C, HIV)	X								
CBC w/ diff, platelets	X, w/i 2 weeks		X(i)	X(i)	X(i)			X(j)	X
CMP **(n)**	X, w/i 2 weeks		X(i)	X(i)	X(i)			X(j)	X
Magnesium **(n)**	X, w/i 2 weeks		X (n)	X (n)	X (n)				
Phosphate **(n)**	X, w/i 2 weeks								
Amylase **(n)**	X, w/i 2 weeks								

Lipase **(n)**	X, w/i 2 weeks								
LDH **(n)**	X, w/i 2 weeks								
PT/PTT	X, w/i 2 weeks				X(i)				
TSH **(o, p)**	X, w/i 2 weeks		X(i)	X(i)	X(i)			X(o,p)	X
T4 **(q)**	X, w/i 2 weeks								
T3 **(q)**	X, w/i 2 weeks								
Cortisol	X, w/i 2 weeks								
EKG (electrocardiogram)	X, w/i 2 weeks				X(i)				
Pregnancy Test (childbearing women)	X, w/i 72 hrs								
PFT to assess pulmonary reserve	X, w/i 4 weeks								
Adverse Event Evaluation	X		X(i)	X(i)	X(i)			X(r)	
QOL via FACT-TOI **(s)**	X		X	X	X			X(s)	
Daily Symptom Report via mobile app and sensor (6 Ss only) **(t)**		X(t)	X(t)	X(t)	X(i)				
Biopsy Baseline – archival tissue	X, if available								
Post Chemo/Nivo Induction restaging biopsy (pre surg/XRT)						**X(d)**			
Post-surgical lung tumor tissue (Path review to assess response)							**X**		
Radiation							X(e)		
RC 1.3.1 : Circulating tumor DNA **(u)**	**X**		**X**	**X**	**X**				
RC 1.3.2a: PKs Nivolumab **(v, w)**	X **(v)**		**X(v)**	**X(v)**	**X(w)**				
RC 1.3.3: PBMC, cytokines, T-cells & macrophages polarity **(x)**	**X**		**X**	**X**	**X**				
RC 1.3.4: Extracellular Vesicles **(y)**	**X**		**X**	**X**	**X**				
RC 1.3.2b: FcRn (neonatal Fc Receptor) **(z)**	**X**								

RC 1.3.5: Tumor infilt neut, lymph, monocytes **(aa, bb)**	**X (aa)**				**X (bb)**		**X (bb)**		

Calendar Footnotes – Continued

RC = research correlative

(a) After consolidation nivolumab is completed, patients will be on observation with surveillance every 3 months for at least 2 years from the start of the initiation of induction therapy or until death, whichever comes first.
(b) Gemcitabine, pemetrexed or paclitaxel. Gemcitabine will also be given on day 8 of each cycle with standard CBC with differential and CMP within 36 hours before day 8 gemcitabine infusion. G-CSF is recommended but not obligatory
(c) If any of the agents in the neoadjuvant/induction courses 2 and 3 is delayed more than 7 days, the dose should be skipped and the patient would be then dosed for the next scheduled course if criteria for restarting are met.
(d) Patients without PD by pre-surgical repeat thoracic CT can have presurgical repeat biopsy as clinically indicated on a separate day. Schedule on course 3 between Days 14-28. Repeat biopsy for restaging should strongly be considered even if there is PD at lymph nodes by RECIST (as above).
(e) If the tumor is not resectable, definitive chemo-nivolumab-radiation should start:
- ≤ 21 days after repeat pathology sampling, if performed.
- If repeat pathology sampling is not done, it should start course 3 Day 22-57.
- Chemotherapy plus nivolumab should start on the first day of radiation.
- The second course (if the q3wk carboplatin pemetrexed nivolumab regimen is used) may be delayed as long as radiation is still being given. In the case of weekly carboplatin/paclitaxel with nivolumab, the delays can extend as long as the radiation is still being given. For both the q3wk and weekly chemotherapy regimens, chemotherapy should not be delayed in the event of nivolumab induced toxicity.

(f) Surgery should be done within ≤21 (inclusive of day 21) days after repeat pathology sampling.
- Post-operative radiation will be decided by the multi-disciplinary team. Whether to add concurrent chemotherapy is to be determined by the team. Nivolumab will not be given during PORT.

(g) Start consolidation nivolumab ≤28 days after surgery in patients who did not have PORT. For patients who do get PORT, start consolidation nivolumab ≤28 days after last fraction. May delay subsequent doses up to 8 weeks, i.e., 12 weeks between nivolumab doses
(h) Patients who get definitive chemotherapy-nivolumab-radiation, will start consolidation nivolumab ≥21 days and up to 12 weeks after the last dose of nivolumab given during radiation. Consolidation nivolumab is given up to 12 cycles (thus can be longer than a year if there are delays in its administration between cycles). Nivolumab can be given no earlier than day 26 of the prior consolidation cycle.
(i) ≤3 days before each induction chemo-nivo and ≤ 17 days before repeat biopsy. In the event the patient does not have a repeat biopsy, the repeat draw should be done before the start of chemo-nivo-radiation. The PK and FcRn samples to be sent to Ohio State and will be partially supported by separate funding through Ohio State.
(j) ≤7 days before each consolidation nivolumab dose.

Calendar Footnotes, Continued

(k) If cannot do baseline PET/CT, then do CT chest/abdomen/pelvis and bone scan. Post-induction PET/CT is encouraged but not obligatory and should be done course 3 Day 11-21. Repeat PET/CT post-surgery or definitive chemo-nivo-XRT are optional but are encouraged at a time as per institutional standard.

(l) Repeat CT should be done on course 3 Day 11-21

(m) Repeat Thoracic CT after surgery or definitive chemo-nivolumab radiation, should be done before consolidation nivolumab cycles 1,4,7, 11, and within 30 days after the last consolidative nivolumab dose.

(n) Standard CMP at Markey includes: Albumin, AST, ALK Phos, Total Bilirubin, BUN, Creatinine, Calcium, Na, Cl, Glucose.
At Pre-Treatment Baseline only, also collect and record the following labs (not included in Markey's standard CMP):
Magnesium, Phosphate, Amylase, Lipase, and LDH.
NOTE: For cisplatin-based induction, magnesium is standard of care for each induction cycle.

(o) After the baseline, only TSH needs to be repeated. If the first course of consolidation nivolumab is delayed, repeat blood endocrine draws should be done $\leq$ 12 wks up to the start of consolidation nivolumab.

(p) TSH Endocrine blood draws for Nivo administration per routine care should be done every odd-numbered consolidation nivolumab cycle or <12 wks after the prior draw, whichever is a shorter interval.

(q) T3 & T4 notes: T4 may be ordered as medically necessary (e.g., if TSH level is outside acceptable ranges) as clinically indicated. T3 may be ordered as medically necessary (e.g., if TSH level is outside acceptable ranges) as clinically indicated.

(r) Every 4 week consolidation nivolumab cycle and within 8 weeks after the last cycle.

(s) Quality of Life (patient-reported outcomes) via FACT-TOI:
ALL Participants: The FACT-TOI (Functional Assessment of Cancer Treatment – Trial Outcome Index) is a 21-item patient-reported quality of life questionnaire derived from the original FACT-L by Dave Cella. The FACT-TOI comprises the Physical WellBeing subscale (7 items), the Functional WellBeing subscale (7 items), and the Lung Cancer Subscale (7 of 9 items). Please see **Appendix F.1** for the FACT-TOI questionnaire and scoring guide. FACT-TOI will be completed by all study participants at 6 timepoints: baseline (pre-treatment); course 2 of induction; course 3 of induction; at time of post-induction restaging biopsy; Cycle 1 of consolidation nivolumab; Cycle 3 of consolidation nivolumab.

(t) Daily Symptom Report (sub-study of 6 Markey participants) via app and mobile sensor:
Remote symptom monitoring will be conducted in a subset of six participants enrolled at Markey Cancer Center. The participant will be prompted to report five symptoms daily via an app on their mobile device (note: participants will be required to own an iOS device). Symptoms will be reported beginning on day 1(of course 1 induction) to the clinic visit during week 7 (also during induction). The clinical team will review data with participants at the routine clinic visits (week 4 and week 7 during induction). The details are in **Appendix F.2** re: remote symptom monitoring.

Calendar Footnotes – Continued

(u) Research Correlative 1.3.1 CtDNA (Zhang): Collect a total of 15mL of whole blood in two Streck cell-free DNA Blood Collection tubes (prior to dosing) at the following 4 timepoints and process/ship per instructions in Section 5:

- Pre-Treatment (within two weeks before C1D1)
- After Cycle 1 of neoadjuvant IO: at the C2D1 visit
- After Cycle 2 of neoadjuvant IO: at the C3D1 visit
- After completion of Cycle 3 of neoadjuvant IO: prior to surgery/chemoradiation therapy

(v) Research Correlative **1.3.2a** Nivo PKs (Phelps): Peripheral blood in 4mL red top Vacutainer tubes (with clot activator).
First PKs Sample Collection: Pre-treatment sample (4mL) can be collected up to 2-weeks before C1D1 OR on C1D1 prior to Nivo dosing.
On C1D1 collect post-Nivo dosing sample: Draw 4mL of peripheral blood at 30-mins post-Nivo administration.

Second PKs Sample: On C2D1, Draw 4mL of peripheral blood into one red top Vacutainer tube (w clot activator) prior to Nivo dosing.
At 30-mins post-Nivo administration, draw 4mL into a second tube. **NOTE:** A total of 8mL will be collected on C2D1.

Third PKs Sample: On C3D1, Draw 4mL of peripheral blood into one red top Vacutainer tube (w clot activator) prior to Nivo dosing.
At 30-mins post-Nivo administration, draw 4mL into a second tube. **NOTE:** A total of 8mL will be collected on C3D1.

NOTE: A total of 8mL (2 tubes x 4mL are collected, 4mL at pre-dosing and 4mL at 30-minutes post infusion of neoadjuvant Nivo) at 3 timepoints.
Process and ship these research blood samples per instructions in Section 5 and Appendix G.

(w) Research Correlative **1.3.2a** Nivo PKs (Phelps): At the clinic visit following the completion of 3 cycles of neoadjuvant Nivo, draw 4mL peripheral blood in red top Vacutainer tube (with clot activator). This visit typically occurs just before the patient will go to surgery or initiate chemoradiation therapy. Process and ship these research blood samples per instructions in Section 5 and Appendix G.

(x) Research Correlative 1.3.3 Cytokines (Woodward): Four 8mL tubes (BD Vacutainer CPT tube w/ sodium citrate glass tube with blue rubber stopper with black stripes). (prior to dosing). *NOTE*: Draw a total of 32mL at each of the 4 timepoints, as follows, and process and ship as per instructions in Section 5:

- Pre-Treatment (within two weeks before C1D1)
- After Cycle 1 of neoadjuvant IO: at the C2D1 visit
- After Cycle 2 of neoadjuvant IO: at the C3D1 visit
- After completion of Cycle 3 of neoadjuvant IO (ie prior to surgery/chemoradiation therapy)

Calendar Footnotes – Continued

(y) Research Correlative 1.3.4 Extracellular Vesicles (D. St. Clair):
Collect 15 mL peripheral blood in heparin tube to obtain plasma at each of the following 4 timepoints. Process/ship per instructions in Section 5:

- Pre-Treatment (within two weeks before C1D1)
- After Cycle 1 of neoadjuvant IO: at the C2D1 visit
- After Cycle 2 of neoadjuvant IO: at the C3D1 visit
- After completion of Cycle 3 of neoadjuvant IO (ie prior to surgery/chemoradiation therapy)

(z) Research Correlative **1.3.2b** Neonatal Fc Receptor (FcRn; Phelps):
Collect a total of 12mL of peripheral blood (into two 6mL green-top tubes with heparin) to assess PBMC for FcRn. (prior to dosing).
Process and ship per instructions in Section 5 and Appendix G the blood samples collected at the following timepoint:

- Pre-Treatment (within two weeks before C1D1)

(aa) Research Correlative **1.3.5** Tumor infiltrating neutrophils, lymphocytes and monocytes (Brainson):
PRE-TREATMENT → The pre-treatment analysis will be conducted on archival tumor tissue (if available).

(bb) Research Correlative **1.3.5** Tumor infiltrating neutrophils, lymphocytes, and monocytes (Brainson):
DURING study treatment → The analysis of tumor infiltrating factors *during treatment* will be conducted **if** tumor tissue is available in the course of 2 routine biopsies, ***a)*** restaging post-induction, i.e., just prior to surgery/chemoradiation; and/or ***b)*** tissue that is biopsied during surgical resection. Have the option to do both but would prefer the surgical specimen. If the surgical specimen shows a pCR, may elect to choose a LN at repeat post-induction biopsy if it has viable tumor. Can also have the option to both. Even a specimen that is pCR may have very interesting biology.

12. MEASUREMENT OF EFFECT

12.1 Antitumor Effect

For the purposes of this study, patients should be re-evaluated for response after completion of the 3 courses of induction chemotherapy-nivolumab which will be at about 9 weeks after its initiation at course 1. A CT of thorax with contrast should be used. This post-induction chemotherapy-nivolumab repeat image serves to evaluate the primary endpoint of this study which is clinical response. After completion of induction chemotherapy-nivolumab, patients get either surgery or definitive chemotherapy-radiation. Thus, further imaging after these definitive procedures, will serve principally as surveillance.

Other than repeat CT, a repeat PET/CT should be considered as post-induction imaging for this study to assist in the identification of sites of potential residual viable post induction tumor. It also can permit an analysis of PET/CT findings against pathologic response in patients who get surgery.

MRI may be relevant to further evaluate response in selected patients with T4 disease.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[51] Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria

Most of the modalities and definitions included below will be unlikely to apply to patients on this. However, they serve as a reference for the rare patient for whom theses definitions and modalities will be relevant and are included as a reference below.

12.1.1 Definitions

Evaluable for Toxicity. All patients will be evaluable for toxicity from the time of their first treatment with Nivolumab + platinum doublet. This will be done at each clinic visit. Thus, toxicity will be evaluated during induction (every three weeks with each new course), just prior to surgery or radiation, and with each clinic visit during radiation. Among the patients who will receive consolidation Nivo, toxicity will be evaluated via monthly visits during 12-months consolidation therapy.

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

12.1.2 Disease Parameters

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

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

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

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

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

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

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

12.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

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

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

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

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

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

12.1.4 Response Criteria

12.1.4.1 Evaluation of Best Overall Response

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

12.1.4.2 Evaluation of Target Lesions

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

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

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

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

12.1.4.3 Evaluation of Non-Target Lesions

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

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

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

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

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

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

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

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

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

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

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

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

12.1.5 Duration of Response

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

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

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

12.1.6 Progression-Free Survival

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

12.1.7 Over-All Survival

OS is defined as the duration of time from start of treatment to time of r death.

12.2 Pathologic response assessment.

At time of post-induction re-biopsy, assessment of evidence of viable tumor in lymph nodes will be done by a pathologist as per standard of care.

In patients who have surgery, a formal assessment of the extent of pathologic response in the resected primary tumor and dissected lymph nodes will be done (see section 5.3).

13. REGULATORY APPROVAL, OVERSIGHT & DATA REPORTING

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 10.0 (Adverse Events: List and Reporting Requirements).

13.1 Protocol Review and Monitoring Committee & Institutional Review Board

Before implementing this study, the protocol must be reviewed by the Markey Cancer Center's Protocol Review and Monitoring Committee and the protocol, the proposed informed consent form and other information to subjects, must be reviewed by the University of Kentucky Institutional Review Board (IRB). A signed and dated UK IRB initial review approval memo must be maintained in the Markey Cancer Center Clinical Research Office (MCC CRO) regulatory binder. Any amendments to the protocol, other than administrative ones, must be reviewed and approved by the PRMC, study sponsor and the UK IRB.

13.2 **Quality Assurance**

The Principal Investigator at the MCC will hold the role of Study Chair. The Study Chair is responsible for the overall conduct of the trial and for auditing its safety and progress at all participating sites.

The MCC places the highest priority on ensuring the safety of subjects participating in clinical trials and on the quality of data obtained from clinical and translation research. The MCC Quality Assurance (QA) Office oversees the maintenance of quality standards in clinical cancer research through clinical data monitoring of Investigator-Initiated Trials (IITs) and routine quality assurance audits.

13.2.1 Data Monitoring

The MCC QA Office will collaborate with the PI, Biostatisticians and Lead OnCore® Data Management Specialist in determining what data points to be source verified during routine audits using a risk-based approach.

The QA auditor/monitor assigned to the trial will perform the routine monitoring tasks in accordance with the protocol-specified routine audit plan.

13.2.2 Audit

To ensure compliance with Good Clinical Practices and all applicable regulatory requirements, the MCC Audit Committee will conduct a quality assurance audit. A minimum of 25% of patients enrolled in the study may be selected for review. The purpose of a routine audit is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice guidelines of the International Conference on Harmonization, and any applicable regulatory requirements.

In addition, routine audits provide research staff and the PI with the opportunity to evaluate the progress of the study, verify the accuracy and completeness of the case report forms, assure that all protocol requirements, including applicable regulations and investigator's obligations are being fulfilled, and prompt resolution of any inconsistencies in the study records.

Multi-Center Trials: Audits may be accomplished in one of two ways:
1. On site/In person audit at the participating site.
The participating sites are responsible for having all source documents, research records, all IRB approval documents, drug accountability record forms, patient registration lists, response assessments scans, x-rays, etc., available for the auditor.

2. Off-site/Remote audit.
For continued oversight of patient safety, there may be circumstances when remote auditing (i.e., off-site) is necessary. To the extent possible, this approach should include remote access to the site's Electronic Medical Records (EMRs) system. Due to logistical issues and unfamiliarity with the site's EMR system related to conducting remote audits, it may require extending the audit duration (i.e., # of days). Use of other secure portals to send source documents (i.e. OnCore, share drive) may also be used in combination with other approaches.

The use of the above approaches is primarily intended for review of patient cases. It is at the discretion of the MCC Quality Assurance Office on how the review of the regulatory and pharmacy documentation are provided and reviewed.

13.3 Data and Safety Monitoring Committee

The MCC Data and Safety Monitoring Committee (DSMC) will oversee the conduct of this trial. The MCC DSMC performs routine real-time data monitoring and safety review of all trials, with a special focus upon investigator-initiated trials (IITs). The MCC DSMC will conduct review of the trial on a schedule determined by the MCC Protocol Review & Monitoring Committee (PRMC). The MCC DSMC will monitor the following elements of the trial: adverse event analysis, serious adverse events, protocol deviations/violations, and accrual. In addition, when applicable will review QA audits and monitoring reports, previous reviews by the DSMC, suggested actions by other committees, such as the IRB, UK Risk Management Committee, and other parameters and outcomes as determined by the DSMC. If appropriate, the DSMC will designate and monitor corrective action(s) based on review outcome. The MCC DSMC has the authority to amend, temporarily suspend, or terminate the trial based upon patient safety or compliance matters.

13.4 Data Reporting

13.4.1 Method

This study will require data submission and reporting via the OnCore Enterprise Research Clinical Trials Management System, which is the official database of the Markey Cancer Center. Instructions for submitting data is listed in study-specific guidance documents authored by a member of the MCC Data Management Team. These guidance documents may include any of the following, as appropriate for the scope of the study: eCRF Completion Guidelines, Data Management Specifications, Subject Console Guide, and Query Resolution Guide. These guidance documents will be approved and housed within OnCore to ensure access to approved versions to facilitate data submission.

13.4.2 Responsibility for Data Submission

This trial will be monitored by the MCC Data and Safety Monitoring Committee (DSMC) on a schedule determined by the Protocol Review and Monitoring Committee at the initial PRMC review. Study staff are responsible for submitting study data and/or data forms to OnCore as per the Markey Cancer Center SOPs. Study staff are responsible for compiling and submitting data for all participants and for providing the data to the Principal Investigator for review.

13.5 Data Management

Data management will be performed by cross-team members at MCC. These team members will include representatives from the Data Management Team, Biostatistics and Bioinformatics SRF, and the Quality Assurance Office. They will work closely with study staff to ensure timely and accurate data submission. A protocol specific Data Management Plan (DMP) will be authored by a senior data manager in collaboration with the biostatistician and Principal Investigator with each expected to review and approve the finalization of the DMP. In order to maintain best clinical practices in data management, the DMP may include, but not be limited to CRF/eCRF design, database build and design, database training, edit check/validation specifications, study database testing/release, data and paper workflow, report, metrics, query/discrepancy management, management of external (including lab) data, medical coding, SAE handling/reconciliation, data transfers and database lock. The protocol specific DMP will additionally define the schedule at which data will be accessed by data management and study statistician to perform statistical programming for conduct of data quality, data control, data management, generation of interim reports and statistical analysis. Cross-team members will collaborate to

establish procedures and timelines for quality control, audits, query resolution, annual reports, interim analysis and final data analysis.

13.6 Compliance with Laws and Regulations

The study will be conducted in accordance with U.S. Food and Drug Administration (FDA) and International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP), the Declaration of Helsinki, any applicable local health authority, and Institutional Review Board (IRB) requirements. The PI or designee will be responsible for obtaining continuing and not less than annual IRB re-approval throughout the duration of the study. Copies of the Investigator's annual report to the IRB and copies of the IRB continuance of approval must maintained by the MCC CRO. The PI or designee is also responsible for notifying the Data and Safety Monitoring Committee of the MCCC and the UK IRB of any significant adverse events that are serious and/or unexpected, as per SOP's of those entities. MCC DSMC will review all adverse events of this IIT as per its SOP.

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APPENDIX A. ECOG PERFORMANCE STATUS

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

APPENDIX B. RENAL FUNCTION FORMULA

Formula to estimate renal function using serum creatinine provided.

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

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

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

APPENDIX C. CIRCULATING TUMOR DNA

Circulating tumor DNA (ctDNA) has proven to be an adequate source for biomarker testing and tumor bulk monitoring (Dawson et al. 2013, PMID:23484797). Previous studies had shown a prognostic value of monitoring the plasma T790M ctDNA level in non-small cell lung cancer patients (Provencio et al. 2018, PMID:29416630; Zheng et al. 2016, PMID:26867973). In this study, in addition to the CT monitoring of tumor progression after chemotherapy-nivolumab, we also propose to monitor tumor progression using ctDNA as an exploratory approach. Due to the diversity of mutation profile of individual tumors and the cost of ctDNA sequencing, it is challenging to use an "all-in-one" ctDNA assay for all tumors. Previously, we carried out an analysis for the "common" molecular markers among 1,708 tumors from Markey Cancer Center, we found that one or different combinations of four genes (TP53, KRAS CDKN2A, PIK3CA) are present in almost 100% of tumors (Dr. J.C. Jeon personal communication). This finding enables us to use one ctDNA assay containing these four genes to monitor the molecular progression for all tumors. The Fluxion Spotlight 59TM ctDNA kit contains four genes that we previously identified for all MCC patients. Using the Fluxion Spotlight 59TM ctDNA assay we can monitor the ctDNA level for all subjects in this study regardless the diverse mutation profile in individual's tumor. We will correlate the ctDNA level with the findings on CT and pathology examination. This result will provide alternative approach to monitor the disease progression after chemotherapy-nivolumab treatment. It will also provide a preliminary data for the feasibility of non-invasive monitoring of tumor progression.

Blood collection and ctDNA isolation: Consented patients will have blood collected prior to dosing during induction cycles 1, 2, and 3 and prior to surgical resection or definitive radiation treatment begins. At each time point,7 mL of whole blood will be collected into two Streck Cell-Free DNA Blood Collection tubes and transported to the Markey BPTP for processing to plasma and freezing a -70C or lower. Plasma samples will be batch dispensed to Markey Oncogenomics core in batches for analysis. The Streck cell-free DNA collection tube will stabilize cell-free DNA for up to 14 days at 6°C to 37°C. Cell free DNA will be isolated at Genomics Core using QIAamp MinElute ctDNA kit according to manufacturer's instruction. Isolated Cell free DNA will be stored at -20°C for downstream sequencing.

ctDNA Next Generation Sequencing (NGS): ctDNA will be sequenced from the baseline and follow-up specimens. We will use the FluxionBio Spotlight 59 ™ liquid biopsy panel for targeted DNA enrichment. This panel includes 59 most commonly mutated genes in solid tumors (https://liquidbiopsy.fluxionbio.com/spotlight-59). The sequencing libraries will be prepared at the Genomics Core using Bravo Automated Liquid Handling platform from Agilent. Libraries will be sequenced using NextSeq 2000 sequencer from Illumina. The average sequencing depth for this panel is estimated to be ~15,000x. With the bioinformatics platform from Fluxion (ERASE-Seq), this assay is expected to detect alleles at ~0.1% frequency.

APPENDIX D. CYTOKINES AND T-CELL POLARITY & MACROPHAGES (1.3.2-1.3.3)

We plan a series of in-depth correlative studies to help identify cell biomarkers correlating with clinical responses. We achieve this by in-depth immunophenotyping and functional interrogations of PBMCs. The, exploratory studies will be performed on peripheral blood tissues and biopsy tissue in a subset to subjects to examine 1) the correlation between immune biomarkers in the periphery and treatment outcome; 2) the correlation between immunologic factors in the tumor microenvironment and the treatment outcome.

Peripheral blood (four 8mL tubes per collection timepoint) will be obtained at 4 timepoints: at baseline; and on clinic visits of C2D1 (i.e., after Cycle 1 completion); and C3D1 (i.e., after Cycle 2 completion); and after completion of Cycle 3 prior to surgery/chemoradiation therapy for cytokine (List attached) and PBMC analyses.

Currently, no biomarkers have been identified that correlate very well to clinical benefit from either of these immune checkpoint inhibitors. We anticipate hypothesis-generating data that can correlate with clinical response.

The study is based on peripheral blood to assess immune activation. The following steps for processing and cryopreservation of blood samples listed below are to be performed in Flow Cytometry and Immune Monitoring (FCIM) SRF. FCIM has technical expertise in human immune monitoring and routinely perform these assays on a fee for services basis. FCIM will be process the blood samples to isolate PBMCs and plasma. These biospecimens will be cryopreserved and stored at the Biospecimen and Tissue Procurement (BSTP) SRF until all samples for a subject have been collected. Once all samples are collected, all stored samples for a subject will have thawed and analyzed simultaneously by FCIM to obtain correlative endpoints as described above.

Immune studies: Peripheral blood will be drawn in four 8mL blood collection tubes (BD Vacutainer ™ CPT ™ Tube with Sodium Citrate) at the 4 above-mentioned timepoints. This would be a total of four collection times and should yield approximately 32 million PBMCs for each blood draw that will be analyzed by flow cytometry. We will evaluate four different panels of immune cell markers to identify clinical benefit.

Isolation of Mononuclear Cells from Peripheral Blood

Blood will be collected from patients at indicated times (Whole Blood tube w/ Anticoagulant, BD Vacutainer™ CPT™ Tube with Sodium Citrate). This collection is performed in clinic and handed to Markey FCIM.

- The FCIM group will perform the following steps to process for cryopreservation.
- CPT blood tubes will be centrifuged at 400 x g for 30 minutes at room temperature
- Plasma will be collected (1-2 mL aliquots) and frozen for future use.
- Aspirate buffy coat at interface, resuspend in sterile saline and wash by centrifugation. Repeat wash procedure twice.
- Resuspend pellet containing PBMCs in ice cold 100% fetal calf serum (FCS)
- Determine cell concentration and viability
- Store on ice for cryopreservation by FCIM.

NOTE: PBMCs from all blood collections from each patient will be cryopreserved by FCIM and banked by BPTP SRF until all blood collections are completed. Flow cytometric analysis by FCIM will be performed in bulk on all collected samples per patient.

- Following determination of cell number and viability, PBMCs in FCS will be adjusted to a concentration of 2×10^6 viable cells/ml.
- 2×10^6 viable cells will be added to cryopreservation vials containing DMSO to obtain a final concentration

of 10% DMSO.

- Vials will be transferred to cryopreservation containers for slow freezing in a -80°C freezer.
- Following overnight freezing, vials will be transferred to liquid nitrogen freezers maintained by BPTP for banking until all patient blood samples are collected.

Flow cytometric analysis (performed by FCIM SRF).

For immune biomarker assessment, Frozen samples will be thawed and processed for flow cytometry. For analysis, samples will be labeled with a cocktail of fluorochrome conjugated antibodies specific to immune biomarkers (to identify, T cell and their subsets, B cells, NK cells and subsets, and monocytes). Following this step, samples will be analyzed by the MCC Flow Cytometry & Cell Sorting Shared Resource Facility for percentage of cells expressing each marker and for mean fluorescence intensity for each marker. PBMC samples will be analyzed on a BD FACS Symphony flow cytometer that can detect up to 27 parameters. Flow cytometric data sets will be interpreted by the MCC Biostatistics and Bioinformatics Shared Resource Facility via computational flow cytometry using SPADE algorithm to automatically identify populations.

Antibody panels

T cell markers

Cell surface markers: CD3, CD4, CD8, CD25, CD33, CD45RO, CD45RA, CD69, CD56, CD62L, CD127

Suppression markers: PD-1, PD-L1, CTLA-4 Intracellular markers: IFNγ, IL-17a, Granzyme Cell subsets identified with these panels:

- CD4+ helper T cells (CD3+, CD4+)
- CD8+ cytotoxic T cells (CD3+, CD8+)
- Regulatory T cells (CD3+, CD4+, CD25hi, CD127lo)
- NK (CD3-, CD56+) and NKT cell subsets (CD3+, CD56+)
- Naïve, Effector and memory cell subsets: CD45RO, CD62L, CD45RA, CCR7

Myeloid cell markers

Cell markers: CD1c, CD11a, CD11b, CD11c, CD14, CD15, CD16, CD33, CD86, CD303, HLA-DR

Suppression markers: PD-1, PD-L1, CTLA-4 Cell subsets identified with these panel s:

- Monocytes: CD14+, CD11b+, CD16−,CD33+,HLA-DR
- Myeloid-derived suppressor cells (PMN): CD14−,CD11b+, CD15+
- Myeloid-derived suppressor cells (monocytic): CD14−,CD11b+, CD15-
- Classical dendritic cells (CD1c+, CD11c+, HLA-DR+)
- Plasmacytoid dendritic cells (CD303+, HLA-DR+, CD11c−)

Outside facilities- ship to BPTP

APPENDIX E. TUMOR INFILTRATING NEUTROPHIL, LYMPHOCYTE & MONOCYTE COUNTS

In order to understand the tumor immune microenvironments before and after treatment, analysis of FFPE biopsies and resections will be performed. In the initial analysis, a board-certified pathologist will assess the biopsy or tissue H&E stained cross-sections for percentages of tumor, fibrosis, inflammation and necrosis and give descriptive analysis of the types of immune cell infiltrates. Categories can include chronic inflammation, acute inflammation, neutrophilic and lympho-plasmocytic infiltrates. We will also use the artificial intelligence (AI) module of HALO AI™ software from Indica Labs to classify cell types in H&E stained sections using advanced deep learning neural network algorithms. We are in the process of training the classification of numerous immune cell types with the assistance of a board-certified pathologist. Lastly, key immune cell markers will be stained on cross-sections of biopsies and resected tumors that have sufficient cellularity. Immuno-staining is routinely performed in the Brainson laboratory and markers could include CD3, PD1, PD-L1, LAG3, CD86 or MPO. Additional tissue based studies, including single or multiplex immunohistochemistry or immunofluorescent staining, or spatial analyses (such as Nanostring Digital Spatial Profiling) may be performed to characterize the immune microenvironment of the tumor samples in collaboration with Dr. Brainson and the Markey BPTP-SRF.

All available tissue samples will be analyzed- pre-study archival tumor tissue, on-study tumor biopsy tissue, and/or resection tumor tissue. If sufficient tissue is available, 10 slides, cut at 4 um and mounted on plus charged slides, will be prepared by Markey BPTP for staining or dispense to Dr. Brainson' lab for staining. Outside facilities should submit an FFPE tumor block or unstained slides when possible. In the event that a specimen does not have sufficient material to prepare slides, diagnostic H&E slides may be submitted for review. Diagnostic materials and FFPE blocks will be returned to submitting facility.

- Submission of FFPE block is preferred
- If a block cannot be released, unstained slides should be cut at 4 um and mounted onto plus-charged slides and *air dried* overnight before shipping.
- Label slides with surgical ID and patient study ID
- Please minimize the time between sectioning and shipping.
- Please submit all available blocks from tumor, tumor bed, or lymph nodes samples for each time point (pre-study/baseline, on-study restaging, resection)
- Please include a pathology report for each case
- Ship all samples from 1 patient together as soon as they are all available.
- Package blocks and slides securely to protect from breakage and include a cold pack in warm weather.
- Ship overnight to:

  Shipping Address:
  MCC Biospecimen Procurement and Translational Pathology SRF
  744 Rose Street, Combs 107
  Lexington, KY 40506
  859-323-7374 or 859-257-4717
  Markey.bstp@uky.edu

APPENDIX F.1. QOL: TRIAL OUTCOME INDEX (FACT-TOI) W/ SCORING

The FACT-TOI is a 21-item patient report questionnaire assessing quality of life and well-being in patients with lung cancer. The FACT-TOI (Trial Outcome Index) comprises items from the standardized, validated FACT-L, Lung Cancer form of the Functional Assessment of Cancer Treatment (D Cella). The FACT-TOI comprises 7 Physical Wellbeing items plus 7 Functional Wellbeing items plus 7 of the 9 items on the Lung Cancer Subscale (LCS) of the FACT-L. The FACT-TOI omits two items from the 9-item LCS: B5 (bothered by hair loss) and L5 (ever smoked? If Yes to "ever smoked?", then item stem is "I regret my smoking."

The next page: the 21-item FACT-TOI questionnaire (with original item codes & questions on a 5-point Likert scale).
The following page: Scoring guidelines for the FACT-TOI.

Below is a list of statements that other people with your illness have said are important**. Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Somewhat	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea …….....	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family.....	0	1	2	3	4
GP4	I have pain …….....	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill …….....	0	1	2	3	4
GP7	I am forced to spend time in bed …….....	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Somewhat	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling.....	0	1	2	3	4
GF3	I am able to enjoy life.....	0	1	2	3	4
GF4	I have accepted my illness.....	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun.....	0	1	2	3	4
GF7	I am content with the quality of my life right now.....	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Very much**
B1	I have been short of breath	0	1	2	3	4
C2	I am losing weight …….....	0	1	2	3	4
L1	My thinking is clear	0	1	2	3	4
L2	I have been coughing	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
L3	I feel tightness in my chest	0	1	2	3	4
L4	Breathing is easy for me	0	1	2	3	4

FACT-TOI Scoring Guidelines [*Derived from FACT-L Scoring Guidelines (Version 4*)]

Instructions: *
1. Record answers in "item response" column. If missing, mark with an X
2. Perform reversals as indicated, and sum individual items to obtain a score.
3. Multiply the sum of the item scores by the number of items in the subscale, then divide by the number of items answered. This produces the subscale score.
4. Add subscale scores to derive total scores (TOI).
5. **The higher the score, the better the QOL.**

Subscale	Item Code	Reverse item?	Item response	Item Score	
PHYSICAL WELL-BEING (PWB)	GP1	4	-	_____	=_____
	GP2	4	-	_____	=_____
	GP3	4	-	_____	=_____
	GP4	4	-	_____	=_____
Score range: 0-28	GP5	4	-	_____	=_____
	GP6	4	-	_____	=_____
	GP7	4	-	_____	=_____

Sum individual item scores: _____
Multiply by 7: _____
Divide by number of items answered: _____=**PWB subscale score**

Subscale	Item Code	Reverse item?	Item response	Item Score	
FUNCTIONAL WELL-BEING (FWB)	GF1	0	+	_____	=_____
	GF2	0	+	_____	=_____
	GF3	0	+	_____	=_____
	GF4	0	+	_____	=_____
Score range: 0-28	GF5	0	+	_____	=_____
	GF6	0	+	_____	=_____
	GF7	0	+	_____	=_____

Sum individual item scores: _____
Multiply by 7: _____
Divide by number of items answered: _____=**FWB subscale score**

Subscale	Item Code	Reverse item?	Item response	Item Score	
LUNG CANCER SUBSCALE (LCS)	B1	4	-	_____	=_____
	C2	4	-	_____	=_____
	L1	0	+	_____	=_____
	L2	4	-	_____	=_____
Score range: 0-28 (7-item LCS)	C6	0	+	_____	=_____
	L3	4	-	_____	=_____
	L4	0	+	_____	=_____

Sum individual item scores: _____
Multiply by 7: _____
Divide by number of items answered: _____=**LC Subscale score**

To derive a FACT-L Trial Outcome Index (TOI):
Score range: 0-84

_____ + _____ + _____ =_____=**FACT-L TOI**
(**PWB** score) (**FWB** score) (**LCS** score)

FACT-L scoring template 05.21.03

*For guidelines on handling missing data and scoring options, please refer to the Administration and Scoring Guidelines in the manual or on-line at www.facit.org

APPENDIX F.2. REMOTE SX MONITORING SUB-STUDY – IOS APP

Cancer treatments (chemotherapy, radiation, and surgery) may inevitably cause risks of toxicity or symptoms. These symptoms, if left unmonitored and uncontrolled, may lead to unnecessary and preventable harms, suffering, and healthcare utilization. To ensure the safety and evaluate the tolerability of our study participants receiving the proposed cancer treatments, a technology-assisted remote symptom management protocol will be applied. We will pilot test the method of allowing participants to report 5 symptoms remotely (on a daily basis); symptom data during two routine clinic visits (Week 4 and Week 7 during nivolumab induction). A total of 6 patients from Markey Cancer Center who are willing to participate in exploratory symptom monitoring who also currently own a compatible smartphone with Internet access will be recruited to join this sub-study.

Procedures

Participant Recruitment. Patients who will enroll in the main trial will be approached about volunteering for this exploratory sub-study by a research assistant (from Dr. Chih's team). To take part in this remote symptom monitoring sub-study, a trial participant must currently own a smartphone and have reliable Internet access. IF the participant is interested in the sub-study and meets these conditions (Internet, smartphone), then a research assistant will provide a loaner smartwatch with the symptom app pre-installed and connect the smartwatch to the participant's smartphone. The partcipant will receive training and safety instruction from the research assistant about how to use the smartwatch and the app in conjunction with their smartphone. The trial participant will use this symptom reporting app during the induction period with nivolumab (i.e., weeks 1 – 7). They will return the smartwatch at the routine clinic visit during week 7.

Remote Data Collection. Participants will be prompted daily to filled out a brief symptom report on the smartwatch app and use the smartwatch (see Table 1, below) for data collection. The smartwatch app prompts patients to report five symptoms, including nausea, vomiting, diarrhea, dizziness, and fatigue. These 5 symptom questions were adopted from Patient-Reported Outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE™, https://healthcaredelivery.cancer.gov/pro-ctcae/). Previous studies showed PRO-CTCAE offers valid and reliable symptom measurement, can be collected electronically, and can be used to further ensure safety and inform tolerability during cancer trials.

Patient Data Reviewing. The monitoring period stretches from day 1 cycle 1 to their visit to the clinic on week 7. The care team will be asked to review these patient-reported data with the patient during the clinic visit in the induction period (i.e., week 4 and week 7). A research assistant working with Dr. Chih will bring the data report to the clinic team during patient's clinic visit to facilitate this patient data review process. Clinicians will address patient's concerns if needed. Markey Cancer Center providers on study will receive training on how to use these data report.

Table 1. Components of the Remote Symptom Management System

Component	*Toxicity/Symptom measured by component*
Smartwatch app for symptom reporting	Patient-Reported Outcomes of five symptoms: Nausea / Vomiting / Diarrhea / Dizziness / Fatigue *NOTE*: 5 symptom items adapted from the PRO-CTCAE
	heart rate; physical activities.

Safety and Security

The research assistant from Dr. Chih's team provide the following safety instruction to the 6 participants in this sub-study:

1. The participant will be prompted to complete daily symptom survey from the smartwatch app, however it is not mandatory (i.e., the patient doesn't need to complete it if they don't want to).

2. The data collected in the smartwatch app will only be reviewed during two routine clinic visits (at week 4 and week 7 of nivolumab induction). NOTE: If there is an emergency or the patient has any concern about his/her health, the patient should contact 911 or the provider's office directly.

3. The participant can contact Dr. Chih's lab (859-218-0482) to leave a message if the participant has any issue with using the smartwatch or app. Dr. Chih's lab will contact the participant to provide solutions.

The smartwatch app was developed in-house at Markey Cancer Center. This smartwatch app has being tested in a feasibility study at UK Markey Cancer Center. The smartwatch can be linked to a patient's personal smartphone. The smartwatch app will send and store all the data to a secure server at Markey Cancer Center via a secure Internet connection. Patient-reported symptom data from the smartwatch will be deidentified in the app and on the server; specifically, each trial participant is assigned a unique study number, which will also be used for the 6 symptom sub-study participants' data (in the app and on the server). Dr. Chih will keep a table that links the unique study number to the actual participant in a locked cabinet. Before a scheduled clinic visit (either week 4 or week 7), a research assistant from Dr. Chih's lab will retrieve the correct data stored on the server, generate a report, and bring this report to the patient's clinical team during the visit as mentioned earlier.

NOTE: The research staff in Dr. Chih's lab maintain requisite certification on human subject protection.

References for Appendix F.2

1. Basch E, Reeve BB, Mitchell SA, et al. Development of the National Cancer Institute's Patient-Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE). *JNCI: J Natl Cancer Inst*2014;**106**:dju244–dju244.
2. Dueck AC, Mendoza TR, Mitchell SA, et al. Validity and Reliability of the US National Cancer Institute's Patient-Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE). *Jama Oncol*. 2015;1(8):1051.
3. Basch E, Schrag D, Henson S, et al. Effect of Electronic Symptom Monitoring on Patient-Reported Outcomes Among Patients With Metastatic Cancer. *Jama* 2022;**327**.

NCI-PRO-CTCAE® CUSTOM SURVEY

Item subset derived from PRO-CTCAE® Item Library Version 1.0
English
Form Created on 03-August-2023
https://healthcaredelivery.cancer.gov/pro-ctcae/builder.html

As individuals go through treatment for their cancer they sometimes experience different symptoms and side effects. For each question, please select the one response that best describes your experiences over the past 7 days…

1a. In the last 7 days, how OFTEN did you have NAUSEA?				
O Never	O Rarely	O Occasionally	O Frequently	O Almost constantly
1b. In the last 7 days, what was the SEVERITY of your NAUSEA at its WORST?				
O None	O Mild	O Moderate	O Severe	O Very severe

2a. In the last 7 days, how OFTEN did you have VOMITING?				
O Never	O Rarely	O Occasionally	O Frequently	O Almost constantly
2b. In the last 7 days, what was the SEVERITY of your VOMITING at its WORST?				
O None	O Mild	O Moderate	O Severe	O Very severe

3a. In the last 7 days, how OFTEN did you have LOOSE OR WATERY STOOLS (DIARRHEA/DIARRHOEA)?				
O Never	O Rarely	O Occasionally	O Frequently	O Almost constantly

4a. In the last 7 days, what was the SEVERITY of your DIZZINESS at its WORST?				
O None	O Mild	O Moderate	O Severe	O Very severe
4b. In the last 7 days, how much did DIZZINESS INTERFERE with your usual or daily activities?				
O Not at all	O A little bit	O Somewhat	O Quite a bit	O Very much

5a. In the last 7 days, what was the SEVERITY of your FATIGUE, TIREDNESS, OR LACK OF ENERGY at its WORST?				
O None	O Mild	O Moderate	O Severe	O Very severe
5b. In the last 7 days, how much did FATIGUE, TIREDNESS, OR LACK OF ENERGY INTERFERE with your usual or daily activities?				
O Not at all	O A little bit	O Somewhat	O Quite a bit	O Very much

OTHER SYMPTOMS					
Do you have any other symptoms that you wish to report?					
O Yes		O No			
Please list any other symptoms:					
1.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	O None	O Mild	O Moderate	O Severe	O Very Severe
2.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	O None	O Mild	O Moderate	O Severe	O Very Severe
3.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	O None	O Mild	O Moderate	O Severe	O Very Severe
4.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	O None	O Mild	O Moderate	O Severe	O Very Severe
5.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	O None	O Mild	O Moderate	O Severe	O Very Severe

The PRO-CTCAE® items and information herein were developed by the Division of Cancer Control and Population Sciences in the NATIONAL CANCER INSTITUTE at the NATIONAL INSTITUTES OF HEALTH, in Bethesda, Maryland, U.S.A. Use of the PRO- CTCAE® is subject to NCI's Terms of Use

APPENDIX G. PHARMACOKINETICS & FCRN

From this study, we would like to address the following questions:

1. Does time-varying clearance of nivolumab exist in patients enrolled in this study? If yes, is it associated with efficacy endpoints? Rationale: Evidence has shown that ICI response is associated with elevated systemic plasma clearance of ICI. Specifically, increased CL of pembrolizumab[1], ipilimumab[2], nivolumab[3], and durvalumab[4] are all associated with reduced response. Pharmacokinetic models for various ICIs have also demonstrated that ICI clearance changes over time. Meanwhile, incorporating longitudinal lab values, such as serum albumin, LDH, and body weight, as covariates for time-varying clearance of ICIs significantly improved model fit[5]. However, this phenomenon of ICIs has not been tested at neoadjuvant setting among surgery-eligible patients.
2. Is there a difference in nivolumab clearance and treatment efficacy between cachectic vs non-cachectic patients in either of the treatment arms? Rationale: Cancer-induced cachexia is a multifactorial muscle wasting syndrome, characterized by irreversible losses in skeletal muscle and adipose tissue[6,7]. In an early report by Turner et al., patients with high baseline pembrolizumab CL developed rapid weight loss and hypoalbuminemia consistent with cachexia[8]. Turner et al. also found that elevated baseline CL of pembrolizumab was the strongest predictor of overall survival in advanced NSCLC and melanoma patients, even when corrected for tumor burden and all other known factors associated with response. Furthermore, several clinical studies using multiple ICIs suggest patients with cachexia have a poorer response to ICI [9–11]. In preclinical setting, our recently published data in pre-clinical models of cachexia has also confirmed increased CL of pembrolizumab[12]. Therefore, these studies unveil a mechanistic link driving cachexia, increased CL and poor ICI response. It is worthwhile to investigate this complex interaction in surgery-eligible NSCLC patients undergoing nivolumab treatment.
3. Can elevated ICI drug clearance be explained by changes in FcRn? Rationale: It is well established that the Fc region of monoclonal antibodies plays a critical role in the clearance of ICIs by interacting with neonatal Fc Receptor (FcRn)[13]. Binding of IgGs to FcRn is the primary means of IgG salvage from the endosomal-lysosomal system such that IgG:FcRn interactions largely govern the circulating half-life of therapeutic IgGs at clinical doses. Importantly, FcRn is also involved in antigen processing and immune response, which may impact ICI response[15]. Therefore, we propose to measure FcRn expression in peripheral blood cells to determine if FcRn expression correlates with ICI clearance, if it differs in cachectic vs. non-cachectic patients, and also whether or not it changes over time.
4. Does nivolumab clearance differ pre vs. post-surgery? Rationale: Since ICIs have been extensively explored in advanced cancer, not enough evidence is available to demonstrate the association of nivolumab clearance with ICI efficacy in neoadjuvant and adjuvant setting. Though tumor volume is not likely to significantly impact ICI clearance, the relationship of nivolumab clearance with health condition improvement and efficacy of maintenance therapy post-surgery may provide us with more insights.
5. What other baseline and on-treatment covariates would significantly impact nivolumab clearance as well as pCR at the time of surgery? Rationale: Two previously established pharmacokinetics models have reported various covariates of nivolumab clearance, which includes body weight, eGFR, gender, albumin, and LDH[3,16]. Since both models were based upon patients with advanced or metastatic cancers, it would be interesting to investigate if those covariates still associate with clearance in neoadjuvant setting and if there are new covariates of interest.

To answer questions above, we would like to request the following data:

Sample collection timepoints: baseline, and then again just before surgery or radiation.

Samples to collect:

1. Nivolumab PKs concentration in plasma: pre-Nivo dose and again at 30-minutes post Nivo administration collect 4mL at three timepoints (pre-treatment sample, on C2D1 and on C3D1) for a total of 8mL (2 tubes x 4mL per collection). After Cycle 3 is completed, collect 4mL only.
2. Baseline and subsequent body weight, albumin, and LDH values
3. CT images for L3 to determine skeletal muscle index (SMI) as a measure of cachexia
4. Peripheral blood samples to quantify FcRn levels

Nivolumab PKs concentration in plasma - pre-treatment, and post-administration

Collect peripheral blood - pre-treatment and 30-min post administration (4 mL each) of each dose in red top vacutainer with clot activator. Samples will be collected at 4 timepoints - at baseline (2 draws); and on clinic visit for C2D1 (2 draws; i.e., after Cycle 1 completion); on clinic visit for C3D1 (2 draws; i.e., after Cycle 2 completion); and after completion of Cycle 3 just prior to surgery/chemoradiation therapy (1 draw only).

Directions on the collection - Venous whole blood will be collected into 4 mL red-topped tubes with clot activator. Before each PK sample is collected, the line should be flushed by saline and then 1 mL of whole blood will be collected and discarded to avoid artificial dilution. Collect each PK sample as close as possible to the planned (nominal) time relative to dosing. The post-infusion sample should be drawn between 15-30 minutes after end-of-infusion. Clock-times for infusion start time, infusion stop time, as well as sample collection time should be recorded.

Processing at the collection site:

a. Allow blood to clot upright at room temperature for at least 30 minutes (maximum 60 minutes) prior to processing.
b. If the blood is not immediately processed after the clotting period, then tubes should be stored (after the 30-60 minutes of clotting time) at 4°C for no longer than 4 hours.
c. Centrifuge for 10 minutes at 1,200 × g at room temperature.
d. Using a clean transfer pipette, aliquot serum into the labeled cryovials (~2-3) at an aliquot volume of 1 mL per tube. Labeling should be printed and include at a minimum the Study ID, Patient ID, sample type, sample collection date, exact sample collection time.
e. Avoid picking up red blood cells when aliquoting by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube.
f. Tightly secure the cap of the vials before storage.
g. Aliquoting and freezing of serum specimens should be completed within 1 hour of centrifugation.
h. Store serum cryovials upright in a specimen box or rack in a -70°C to -90°C or colder freezer. Do not allow specimens to thaw after freezing.

PK samples will be shipped to OSUCCC PhASR lab.

Shipping Instructions:

- Specimens should be stored at -70C or colder through the end of the last timepoint and shipped as a batch by participant (more than one participant/shipment is acceptable).
- A participant's samples should be shipped to the OSUCCC PhASR lab within 2 weeks of the last sample's collection date. (i.e., if C6D15 sample is collected on 9/1/2021, all of that participant's samples should be at the OSUCCC PhASR lab by 9/15/2021).
- The OSUCCC PhASR lab may contact the study team to request shipment off-schedule.
- Please ship only 1 aliquot to the OSUCCC PhASR laboratory within each shipment. Once receipt is confirmed, the back-up aliquot(s) may also be shipped. The back-up aliquots can be shipped at a later date with subsequent batches of samples for other participants.
- Samples should be stored in cardboard boxes (5 1/8" x 5 1/8" x 2", LxWxH) with dividers. (e.g., VWR Box item number is 82021-114; divider item number is 82007-154.)
- Please organize the samples by Patient and Time point in the box.
- Do not store in plastic bags (they break on dry-ice and labels will detach).
- A copy of each of the pharmacokinetic sample collection forms for the respective patients or a sample list should be included with each shipment. To prevent problems with illegible writing on tubes, consider numbering them (in addition to sample label) and numbering samples on the sample sheet.
- Note the study number, PI, and the drugs used/to be measured
- A name, phone number and email address should be included with samples so that receipt can be acknowledged.
- All samples should be shipped via overnight express courier in insulated containers with enough dry ice to maintain the samples in a frozen state.
- Overnight shipments should occur on Monday through Wednesday except when the following day is a holiday.
- Please notify the OSUCCC PhASR lab by email (PhASR@osumc.edu) within 24 hours prior to shipment.

Address:

The OSUCCC Pharmacoanalytical Shared Resource
Attn: Kasey Hill, Ph.D.
441 Biomedical Research Tower
460 West 12th Avenue
Columbus, OH 43210
Phone: (614) 688-0578
Fax: (614) 292-7766

Contact Information:

OSUCCC PhASR lab
PhASR@osumc.edu
Kasey Hill, PhD or Nicole Abbott, PhD can be reached at: (614) 688-0578
Mitch Phelps, PhD can be reached at: (614) 832-2547

Peripheral blood samples to quantify FcRn levels

- Collect 12mL of peripheral blood in 2 x 6mL Green Top tubes with sodium heparin (to collect PBMC) at baseline (pre-treatment).
- Samples should be shipped the same day as collection and can be stored at ambient temperature until shipped.
- To ensure quality of the cells and plasma, it is imperative that samples be shipped on Monday – Thursday only to avoid problems with weekend/holiday delivery.
- For shipment of blood samples, specimens will remain in the green top tubes, which are to be placed into a cardboard box containing stryofoam holders that stabilize the glass tubes. An ice pack should be added to each shipment to reduce temperature for shipment.
- The box containing blood and cold pack should be contained within a Fed-Ex UN3373 Clinical Pak.
- All blood specimens should be shipped via Fed-Ex priority overnight the same day of collection for next day processing and analysis to the address below.

Address:

The OSUCCC Pharmacoanalytical Shared Resource
Attn: Kasey Hill, Ph.D.
441 Biomedical Research Tower
460 West 12th Avenue
Columbus, OH 43210
Phone: (614) 688-0578
Fax: (614) 292-7766

Contact Information:

OSUCCC PhASR lab
PhASR@osumc.edu
Kasey Hill, PhD or Nicole Abbott, PhD can be reached at: (614) 688-0578
Mitch Phelps, PhD can be reached at: (614) 832-2547

Data analysis plan

A nonlinear mixed effect approach with NONMEM software will be used to estimate nivolumab baseline and time-varying clearance for each patient. Nivolumab base PK model will be obtained from previous publications of Bajaj G et al. and Zhang J et al. Percentage change of clearance from baseline will be calculated using the estimated clearance at each time point. The association of estimated nivolumab baseline clearance and clearance with covariates such as albumin, LDH, cachexia, surgical intervention, FcRn level will be assessed. Identification of patients with cancer cachexia will be based on BMI, weight loss, and/or sarcopenia as determined based on L3 CT scan. Significant covariates will be subsequently added into nivolumab base PK model to investigate if those covariates would improve model fitting and describe significant portions of inter-individual variability of nivolumab PK. The association of nivolumab clearance and cachexia with efficacy endpoints such as pCR, MPR, clinical response, PFS and OS will also be assessed.

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APPENDIX H. EXTRACELLULAR VESICLES

Sample will be collected pre-dose (where applicable) at 4 timepoints: pre-treatment baseline, C2D1 (after Cycle 1), C3D1 (after Cycle 2) and the final timepoint is at the visit after Cycle 3 is completed (prior to surgery or radiation).
At each of the 4 timepoints, draw 15 mL total blood in green top tubes containing heparin (any combination of tube size totaling 15 mL). Hold samples on ice until processing. Within 2 hours of draw, centrifuge at 1500-1700 RCF for 12 minutes. Prepare 1 mL aliquots of plasma in screw-top cryovial. Label each aliquot using indelible ink or cryo-label with study/protocol number, patient study sequence number, plasma, timepoint (e.g. pre-Tx baseline, C3D1), date and time of collection. Freeze immediately after aliquoting at -70C or colder. Hold until requested by investigator.

SHIPMENT

- Frozen plasma for EV specimens should be batch shipped together on dry ice upon investigator request.
- Multiple patients should be shipped together.
- Please ship only 1 aliquot to the BPTP-SRF laboratory in the initial EV shipment. Once receipt is confirmed, the back-up aliquot(s) may also be shipped
- Samples should be shipped in cardboard boxes (5 1/8" x 5 1/8" x 2", LxWxH) with dividers. (e.g., VWR Box item number is 82021-114; divider item number is 82007-154.)
- Please organize the samples by Patient and Timepoint in the box
- Be sure to include a detailed manifest which includes the following information for each sample shipped- patient study ID, timepoint, number of specimens for each timepoint, date and time of collection for each timepoint.
- If samples are labelled with additional identifiers (internal specimen number, or similar), please include in manifest list.
- A name, phone number and email address should be included with samples so that receipt can be acknowledged.
- All samples should be shipped via overnight express courier in insulated containers with enough dry ice to maintain the samples in a frozen state
- Ship specimens Monday-Wednesday only for priority overnight delivery.
- Do not ship specimens for delivery on a holiday. The University of Kentucky holiday schedule is available at https://www.uky.edu/hr/hr-home/official-staff-holiday-schedule.
- Prior to shipment, send an email to markey.bstp@uky.edu. Please provide the study number, tracking number and a copy of the shipment manifest.

Shipping Address:
MCC Biospecimen Procurement and Translational Pathology SRF
744 Rose Street, Combs 107
Lexington, KY 40506
859-323-7374 or 859-257-4717
Markey.bstp@uky.edu

APPENDIX I. BMS DRUG REQUEST FORM FOR NIVOLUMAB

DRUG REQUEST FORM FOR:
Nivolumab (BMS-936558) (US STUDIES ONLY)
BMS Protocol # CA209-6K4

Please mark ONE of the following boxes:

☐ Request for Initial Drug Shipment to Site
☐ Request for Resupply Drug Shipment to Site

All dates should be in dd-mmm-yyyy format

BMS Protocol Number: CA209-6K4	Site #: 0001	Date of Request: ___/___/_____
Investigator Name:	Shipment Must Reach Destination By: *(Please allow 5 – 7 business days. Deliveries are not made on Monday)* ___/____/_____	
Investigator Address (Please include contact name):	Ship Supplies To (If Different From Investigator Address):	

Product Strength/Dose Form:	**Nivolumab** Solution for Injection 10mg/mL
Order Unit:	100mg Vials (10mL)
# of Vials Requested (Please order in multiples of 5):	

SPECIAL INSTRUCTIONS:

Please Note: These are refrigerated supplies. Please transfer to **+2°C/+8°C** storage immediately upon receipt.

Transmission of this Form serves as assurance that all required regulatory and contractual documentation for this Site/Study is complete.
By submission for Re-Supply requests, the sponsor/site assures the site remains in good regulatory standing.

- Please e-mail this document with the subject heading "CA209-6K4 SUPPLY REQUEST" to the following:
  distribution.allentown@thermofisher.com
  tasha.alex@BMS.com