IFCT-1601 - IONESCO

A PHASE II PROSPECTIVE IMMUNE NEOADJUVANT THERAPY STUDY OF DURVALUMAB (MEDI4736) IN EARLY STAGE NON-SMALL CELL LUNG CANCER

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A phase II prospective immune adjuvant therapy study of durvalumab in early stage Non-Small Cell Lung Cancer

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et accepte de conduire cet essai en accord avec les Bonnes Pratiques Cliniques, la Loi de Recherche Biomédicale (4 aout 2004) et tel qu'il est décrit dans ce document.

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1 BACKGROUND INFORMATION AND RATIONALE

Lung cancer is still the leading cause of cancer related-deaths worldwide, with an overall all-stage 5-year survival of approximately 17%[1]. The primary treatment of early stage (I-IIIA) NSCLC is curative surgery. Although patients treated with curative surgery have a better prognosis, the 5-year survival for patients treated with surgery alone remains low, ranging from 67% (stage IA) to 23% (stage IIIA)[2].

The poor survival rates following surgical resection for patients with stage II and III disease have led several groups to investigate the utility of perioperative chemotherapy in improving lung survival outcomes. Several randomized trials comparing postoperative chemotherapy versus no chemotherapy have shown a significant overall survival benefit from postoperative chemotherapy in completely resected patients with NSCLC stage II and IIIA. A large meta-analysis based on individual patient data suggested that adjuvant cisplatin-based chemotherapy could yield an overall survival (OS) advantage of 5% at 5 years [3].

Likewise other randomized trials have demonstrated preoperative chemotherapy improves survival and recently the analyses also based on individual patients data of 15 randomized trials showed a significant benefit of preoperative chemotherapy on survival with the same survival improvement of 5% at 5 years [4]. Then, neoadjuvant chemotherapy has also become accepted in many countries [4]. Theoretically the neo-adjuvant approach has a number of advantages: it can reduce the tumour volume and facilitate the control of micro-metastatic diffusion or prevent it; The neo-adjuvant treatment allows a careful evaluation of chemotherapy response giving critical information on tumour biology in adequate tumour samples: the compliance of chemotherapy in untreated patients is certainly better than after surgery and its dose intensity higher. On the other hand, its toxicities and a delay to surgery could be disadvantages, although up to now these issues seem to be scarcely relevant.

Lastly, the observed pathological response on surgical specimen after neoadjuvant therapy has been identified as a potential surrogate of survival in patients with NSCLC [5–7]. Pathological response has been recently proposed as new primary endpoint for clinical trials, allowing the inclusion of a smaller number of patients and to obtain more rapidly main objective. This leads to a boost of interest in neoadjuvant trials.

1.1 Why targeting PD-1/PD-L1?

Despite the established benefit of adjuvant chemotherapy after curative surgery for NSCLC, 30-60% of patients, depending on stage, will eventually relapse and die of their disease [8]. Furthermore, not all patients with early stage disease are eligible or willing to undergo chemotherapy following complete surgical resection [9]. As such, the long-term prognosis of patients with NSCLC, even among those with early stage disease, remains poor. Novel therapies are, therefore, required to improve the clinical outcomes of completely resected NSCLC.

Based on their success in the advanced disease NSCLC setting [10] molecularly targeted therapies are now evaluated in the adjuvant setting following complete surgical resection for NSCLC. The lack of success of the two recent phase III adjuvant trials targeting the EGFR pathway could be related to the lack of restricting enrolment to patients with activating mutations [11,12]. Targeted therapies continue to be an attractive option in the perioperative setting given their good toxicity profiles.

In the context of early stage NSCLC, immunotherapeutic interventions that can both induce cell-mediated immunity against proliferating cancer cells following complete resection and establish immunologic memory that may guard against future recurrences through active immune surveillance is a particularly attractive therapeutic option.

Immune responses of the human immune system directly target tumours as part of the body's natural defence mechanisms. Cancers, however, can develop strategies that allow them to actively evade detection and immune-mediated apoptosis. The up-regulation of surface proteins, including programmed death-1 (PD-1), is one such strategy. Specifically, abnormal cell-surface expression of its ligand PD-L1 on tumors has been shown to induce T cell anergy, affording tumors protection against killer T-cell elimination [13]. Further, in the tumor microenvironment, PD-1 expressed on activated T cells has also been shown to limit T cell antitumor activity on cells reaching the tumor, when bound to its ligand, PD-L1[14]. Although, historically NSCLC has been considered to be non-immunogenic [15], emerging data suggests that lung cancers may actively evade attack by the immune system by expressing the PD-L1 ligand, allowing them to evade immune detection [16,17].

In preclinical studies, blocking the interaction between PD-1 and PD-L1 has been shown to induce immune responses *in vitro*[18]and enhance antitumor T cell cytotoxic activity. Clinically, blockade of the PD-1 inhibitory checkpoint pathway by inhibiting PD-L1/ PD-1 engagement, has been shown to induce tumour regression across many cancer types, including melanoma, renal cell, colon and lung cancers [16,19–22]. In lung cancer, inhibition of the PD-1 checkpoint pathway by antibodies targeting either PD-1 or its ligand PD-L1 have yielded encouraging results, suggesting a "class effect" and validating this pathway as a therapeutic target in NSCLC. Phase I NSCLC cohorts of heavily pre-treated patients have yielded dose-dependent objective responses ranging from 10%-32% [16,20–23] with responses observed in patients with both squamous and non-squamous histology. Immunotherapy with anti-PD-1 or anti-PD-L1 antibodies across many tumor types has been generally well tolerated, with common drug related adverse events mainly limited to grade 1 or 2 fatigue, diarrhea, rash, pruritus, nausea and decreased appetite.

1.2 Why targeting PD-1 in a neoadjuvant setting?

Targeting of PD-1 receptors and its ligand PD-L1, and inhibiting their engagement is an attractive therapeutic option in the early stage NSCLC, which may reactivate host immune responses and enable long-term tumor control.

We aim to launch a phase II trial, administrating PD-L1 antibody in clinical stage IB (>4cm) or stage II, NSCLC before surgery.

Neoadjuvant trial will allow the analysis in early stage NSCLC of 1) CT scan response (RECIST and immune response criteria), 2) 18-FDG metabolic response 3) pathological response 4) and to study immune response after PD-1 antibody infusions on large tissue sample such as surgical piece. We will be able to phenotype the immune reaction in responders and non-responders and we will be able to study immune reaction before (blood and tissue at diagnosis) and after PD-1 antibody infusion (blood and surgical tissue). All these observations will be correlated to disease-free survival and survival.

Some studies suggested an association between treatment response and pre-treatment PD-L1 tumor or immune cells expression[16,20], however, the role of PD-L1 expression as a biomarker for clinical response to PD-1 pathway inhibition has yet to be validated. Mandatory pre- and post-treatment tissue will allow the analysis of predictive and prognostic significance of PD-L1 expression, as well as other cytokine/biomarker expression.

Because stage IB (>4cm) or stage II NSCLC are frequently curable with surgery, we chose as first endpoint feasibility of complete surgical resection. Complete resection is defined as complete removal of the primary tumour with no residual macroscopic or microscopic tumor left behind; moreover, a systematic or lobe-specific nodal dissection must have been performed, and the highest mediastinal lymph node must be

negative [24]. We plan only three infusions of PD-L1 antibody (one infusion every two weeks) with a schedule allowing surgery to be performed not later than 6 weeks after inclusion.

The number of post-operative events and a tolerance report will be achieved on the first 15 patients to discuss the pursuit of the trial.

In the IFCT-0002 trial [25], including 528 patients with stage I and II NSCLC, 91.6% of patients achieved complete resections. In the other trials this rate was lower (see table) but with smaller number of patients, more advanced stages and also older studies. For all these reasons and because this trial is in continuity with IFCT 0002 trial, we decided to take 90% as the threshold below which the rate of complete oncologic resection observed was unacceptable.

Durvalumab is a novel IgG1-kappa PD-L1 inhibitor with potent and specific binding to PD-L1 at picomolar concentrations, with directed mutations in the Fc region, limiting off-target cytotoxicity in PD-L1-expressing immune cells [26]. Durvalumab has been shown to increase T-cell activation in vitro by blocking PD-L1/PD-1 engagement [26] and induces antitumor responses in tumor-bearing mice, with corresponding changes in peripheral immune markers [26]. Durvalumab has also been shown to inhibit tumor growth *in vivo* xenograft models [26].

In advanced NSCLC, inhibition of the PD-1 immune checkpoint pathway has been shown to induce durable clinical responses across all histologies and is therefore of broad clinical relevance. durvalumab has a favorable safety profile and limited off-target T cell effects and has good pharmacokinetic and pharmacodynamic properties. Results of early trials with durvalumab in advanced cancers are consistent with a class effect of early and sustained tumour control that has been observed previously with other inhibitors of the PD-1 immune checkpoint pathway. durvalumab is particularly attractive as a therapeutic strategy in the adjuvant setting as it may generate immunologic memory and persistent activity in memory B cells, allowing for active and sustained surveillance and killing of tumour cells and possibly long term cure. NSCLC patients with PD-L1 expression positive resected tumours may be particularly sensitive to immunotherapy with durvalumab given accumulating evidence in patients with advanced disease. Even if the primary endpoint is the complete resection for early stage, the expected benefit is to obtain a signal in term of response and increase of disease free survival.

Durvalumab has been shown to have a favourable pharmacokinetic (PK) profile that exhibits nonlinear PK at doses up to 1.0 mg/kg, with dose-dependent increases in target engagement and target suppression at doses ≥ 0.3 mg/kg every two weeks [22]. Immune-related reactions that are possible with this class of drug (e.g. enterocolitis, dermatitis, hepatotoxicity, endocrinopathy, pneumonitis) have been reported in less than 5% of patients on durvalumab. To date, there have been no cases of infusion reaction or IgE-induced allergic reactions with durvalumab.

Study Hypothesis: A theoretical feasibility rate equal to or lower rate of complete resection than P0=85% is considered as unacceptable after immune treatment. A test for single binomial proportion for a two-stage design with a 1:1 randomization and O'Brien-Fleming (OF) stopping rules will be used. P0 = 85% P1 = 95% Power = 90% and α risk (one-sided) = 5%. Based on these assumptions, 81 patients are required.

Table 1: Neoadjuvant trials with number of patients considered with complete resection

Trial	First author	Reference	Stage	N Neoadjuvant arm	N Surgery (%)*	N Exploratory thoracotomy	N Surgery for complete resection (%)*	N Complete resection (%)*
MIP91	Depierre	[27]	IB II IIIA	187	167 (89,3)	5	162 (86,6)	ND
S9900	Pisters	[28]	IB II IIIA non N2	169	152 (89,9)	5	147 (86,9)	142 (84)
NATCH	Felip	[29]	IA >2cm IB II T3N1	201	181 (90)	7	ND	ND
CHEST	Scagliotti	[30]	IB II T3N1	129	110 (85,2)	ND	ND	97 (75,1)
IFCT 0002	Westeel	[25]	I, II	528	509 (96,4)	4	505 (95,6)	484 (91,6)

* % has been calculated in intention to treat (ITT), i.e. using the number of patients included in the neoadjuvant arm of the corresponding trial.

2 BACKGROUND THERAPEUTIC INFORMATION

2.1 Durvalumab Background

Investigators should be familiar with the current durvalumab Investigator Brochure (IB).

Durvalumab is being developed as a potential anticancer therapy for patients with advanced solid tumors. Durvalumab is a human monoclonal antibody (MAb) of the immunoglobulin G1 kappa (IgG1κ) subclass that inhibits binding of programmed cell death ligand 1 (PD-L1) (B7 homolog 1 [B7-H1], cluster of differentiation [CD]274) to programmed cell death 1 (PD-1; CD279) and CD80 (B7-1). Durvalumab is composed of 2 identical heavy chains and 2 identical light chains, with an overall molecular weight of approximately 149 kDa. Durvalumab contains a triple mutation in the constant domain of the immunoglobulin (Ig) G1 heavy chain that reduces binding to complement protein C1q and the fragment crystallizable gamma (Fcγ) receptors involved in triggering effector function.

2.1.1 Summary of non-clinical experience

The non-clinical experience is fully described in the current version of the durvalumab Investigator's Brochure.

Durvalumab binds with high affinity and specificity to human PD-L1 and blocks its interaction with PD-1 and CD80. *In vitro* studies demonstrate that durvalumab antagonizes the inhibitory effect of PD-L1 on primary human T cells, resulting in their restored proliferation and release of interferon gamma (IFN-γ). Additionally, durvalumab demonstrated a lack of antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) in cell-based functional assays. In vivo studies show that durvalumab inhibits tumor growth in a xenograft model via a T lymphocyte (T-cell) dependent mechanism. Moreover, an anti-mouse PD-L1 antibody demonstrated improved survival in a syngeneic tumor model when given as monotherapy and resulted in complete tumor regression in > 50% of treated mice when given in combination with chemotherapy. Combination therapy (dual targeting of PD-L1 and cytotoxic T-lymphocyte-associated antigen 4 [CTLA-4]) resulted in tumor regression in a mouse model of colorectal cancer.

Cynomolgus monkeys were selected as the only relevant species for evaluation of the pharmacokinetics (PK)/pharmacodynamics and potential toxicity of durvalumab. Following intravenous (IV) administration, the PK of durvalumab in cynomolgus monkeys was nonlinear. Systemic clearance (CL) decreased and concentration half-life (t1/2) increased with increasing doses, suggesting saturable target binding-mediated clearance of durvalumab. No apparent gender differences in PK profiles were observed for durvalumab.

In general, treatment of cynomolgus monkeys with durvalumab was not associated with any durvalumab - related adverse effects that were considered to be of relevance to humans. Adverse findings in the non-Good Laboratory Practice (GLP) PK/pharmacodynamics and dose range-finding study, and a GLP 4-week repeat-dose toxicity study were consistent with antidrug antibody (ADA)-associated morbidity and mortality in individual animals. The death of a single animal in the non-GLP, PK/pharmacodynamics, and dose range-finding study was consistent with an ADA-associated acute anaphylactic reaction. The spectrum of findings, especially the clinical signs and microscopic pathology, in a single animal in the GLP, 4-week, repeat-dose study was also consistent with ADA immune complex deposition, and ADA: durvalumab immune complexes were identified in a subsequent non-GLP, investigative immunohistochemistry study. Similar observations were reported in cynomolgus monkeys administered human mAbs unrelated to durvalumab. Given that immunogenicity of human mAbs in nonclinical species is generally not predictive of responses in humans, the ADA-associated morbidity and mortality were not considered for the determination of the no-observed-adverse-effect level (NOAEL) of durvalumab.

Finally, data from the pivotal 3-month GLP toxicity study with durvalumab in cynomolgus monkeys showed that subchronic dosing of durvalumab was not associated with any adverse effects. Therefore, the NOAEL of durvalumab in all the general toxicity studies was considered to be 100 mg/kg, the highest dose tested in these studies. In addition to the *in vivo* toxicology data, no unexpected membrane binding of durvalumab to human or cynomolgus monkey tissues was observed in GLP tissue cross-reactivity studies using normal human and cynomolgus monkey tissues.

2.1.2 Summary of clinical experience

Clinical experience with durvalumab is fully described in the current version of the durvalumab Investigator's Brochure.

As of the DCO dates (15Apr2015 to 18Sep2015), a total of 1,910 subjects have been enrolled and treated in 30 ongoing durvalumab clinical studies, including 20 sponsored and 10 collaborative studies (Table 1.2-1). Of the 1,910 subjects, 1,279 received durvalumab monotherapy, 454 received durvalumab in combination with tremelimumab or other anticancer agents, 14 received other agents (1 gefitinib, 13 MEDI6383), and 163 have been treated with blinded investigational product. No studies have been completed or terminated prematurely due to toxicity.

2.1.3 Pharmacokinetics and Product Metabolism

Study CD-ON-durvalumab-1108: As of 09 Feb2015, PK data were available for 378 subjects in the dose-escalation and dose-expansion phases of Study CD-ON-durvalumab-1108 following treatment with durvalumab 0.1 to 10 mg/kg every 2 weeks (Q2W) or 15 mg/kg every 3 weeks (Q3W). The maximum observed concentration (C_{max}) increased in an approximately dose-proportional manner over the dose range of 0.1 to 15 mg/kg. The area under the concentration-time curve from 0 to 14 days (AUC_{0-14}) increased in a greater than dose-proportional manner over the dose range of 0.1 to 3 mg/kg and increased dose-proportionally at ≥ 3 mg/kg. These results suggest durvalumab exhibits nonlinear PK likely due to saturable target-mediated CL at doses < 3 mg/kg and approaches linearity at doses ≥ 3 mg/kg. Near complete target saturation (soluble programmed cell death ligand 1 [sPD-L1] and membrane bound) is expected with durvalumab ≥ 3 mg/kg Q2W. Exposures after multiple doses showed accumulation consistent with PK parameters estimated from the first dose. In addition, PK simulations indicate that following durvalumab 10 mg/kg Q2W dosing, > 90% of subjects are expected to maintain PK exposure ≥ 40 µg/mL throughout the dosing interval.

As of 09 Feb2015, a total of 388 subjects provided samples for ADA analysis. Only 8 of 388 subjects (1 subject each in 0.1, 1, 3, and 15 mg/kg cohorts, and 4 subjects in 10 mg/kg cohort) were ADA positive with an impact on PK/pharmacodynamics in 1 subject in the 3 mg/kg cohort.

Safety

The safety profile of durvalumab as monotherapy and combined with other anticancer agents was consistent with the pharmacology of the target and other agents in the immune checkpoint inhibitor class. No tumor types appeared to be associated with unique AEs. Immune-related AEs (irAEs), which are important risks of immune checkpoint inhibitors, have been observed with durvalumab and include colitis, pneumonitis, hepatitis/hepatotoxicity, neuropathy/neuromuscular toxicity, endocrinopathy, dermatitis, and nephritis. In addition, pancreatitis is an important potential risk particularly with durvalumab and tremelimumab

combination therapy. These events are manageable by available/established treatment guidelines as described in the study protocols (see paragraph 7.5).

AEs reported with durvalumab monotherapy in key clinical studies are described below.

Adverse Event Profile of durvalumab Monotherapy

Study CD-ON-durvalumab-1108: The safety profile of durvalumab monotherapy in the 694 subjects with advanced solid tumors treated at 10 mg/kg Q2W in Study CD-ON-durvalumab-1108 has been broadly consistent with that of the overall 1,279 subjects who have received durvalumab monotherapy (not including subjects treated with blinded investigational product) across the clinical development program. The majority of treatment-related AEs were manageable with dose delays, symptomatic treatment, and in the case of events suspected to have an immune basis, the use of established treatment guidelines for immune-mediated toxicity. As of 07 May2015, among the 694 subjects treated with durvalumab 10 mg/kg Q2W in Study CD-ON-durvalumab-1108, a total of 378 subjects (54.5%) experienced a treatment-related AE, with the most frequent (occurring in ≥ 5% of subjects) being fatigue (17.7%), nausea (8.6%), diarrhea (7.3%), decreased appetite (6.8%), pruritus (6.3%), rash (6.1%), and vomiting (5.0%). A majority of the treatment-related AEs were Grade 1 or Grade 2 in severity with ≥ Grade 3 events occurring in 65 subjects (9.4%). Treatment-related ≥ Grade 3 events reported in 3 or more subjects (≥ 0.4%) were fatigue (12 subjects, 1.7%); increased aspartate aminotransferase (AST; 7 subjects, 1.0%); increased gamma-glutamyltransferase (GGT; 6 subjects, 0.9%); increased alanine aminotransferase (ALT; 5 subjects, 0.7%); and colitis, vomiting, decreased appetite, and hyponatremia (3 subjects, 0.4% each). Six subjects had treatment-related Grade 4 AEs (upper gastrointestinal hemorrhage, increased AST, dyspnea, neutropenia, colitis, diarrhea, and pneumonitis) and 1 subject had a treatment-related Grade 5 event (pneumonia). Treatment-related serious adverse events (SAEs) that occurred in ≥ 2 subjects were colitis and pneumonitis (3 subjects each). A majority of the treatment-related SAEs were ≥ Grade 3 in severity and resolved with or without sequelae. AEs that resulted in permanent discontinuation of durvalumab were considered as treatment related in 18 subjects (2.6%), with colitis being the most frequent treatment-related AE resulting in discontinuation (3 subjects). A majority of the treatment-related AEs resulting in discontinuation of durvalumab were ≥ Grade 3 in severity and resolved with or without sequelae.

Study D4191C00003/ATLANTIC: The safety profile of durvalumab monotherapy in Study CD-ON-durvalumab-1108 is generally consistent with that of Study D4191C00003/ATLANTIC in subjects with locally advanced or metastatic non-small-cell lung cancer (NSCLC) treated with durvalumab 10 mg/kg Q2W. As of 05May2015, 264 of 303 subjects (87.1%) reported any AE in Study D4191C00003/ATLANTIC. Overall, events reported in ≥ 10% of subjects were dyspnea (18.8%), fatigue (17.8%), decreased appetite (17.5%), cough (14.2%), pyrexia (12.2%), asthenia (11.9%), and nausea (11.2%). Nearly two-thirds of the subjects experienced AEs that were Grade 1 or 2 in severity and manageable by general treatment guidelines as described in the current durvalumab study protocols. Grade 3 or higher AEs were reported in 107 of 303 subjects (35.3%). A total of 128 subjects (42.2%) reported AEs that were considered by the investigator as related to investigational product. Treatment-related AEs (all grades) reported in ≥ 2% of subjects were decreased appetite (6.6%); fatigue (5.9%); asthenia (5.0%); nausea (4.6%); pruritus (4.3%); diarrhea, hyperthyroidism, hypothyroidism, and pyrexia (3.3% each); rash (2.6%); weight decreased (2.3%); and vomiting (2.0%). Treatment-related Grade 3 AEs reported in ≥ 2 subjects were pneumonitis (3 subjects) and

increased GGT (2 subjects). There was no treatment-related Grade 4 or 5 AEs. Ninety-four of 303 subjects (31.0%) reported any SAE. SAEs that occurred in ≥ 1.0% of subjects were dyspnea (6.6%); pleural effusion, general physical health deterioration (2.3% each); pneumonia (2.0%); hemoptysis, pulmonary embolism (1.3% each); and pneumonitis, respiratory failure, disease progression (1.0% each). Nine subjects had an SAE considered by the investigator as related to durvalumab. Each treatment-related SAE occurred in 1 subject each with the exception of pneumonitis, which occurred in 3 subjects. Fifteen of 303 subjects (5.0%) have died due to an AE (pneumonia [3 subjects]; general physical health deterioration, disease progression, hemoptysis, dyspnea [2 subjects each]; pulmonary sepsis, respiratory distress, cardiopulmonary arrest [verbatim term (VT)], hepatic failure, and sepsis [1 subject each]). None of these events was considered related to durvalumab. Twenty-three of 303 subjects (7.6%) permanently discontinued durvalumab treatment due to AEs. Events that led to discontinuation of durvalumab in ≥ 2 subjects were dyspnea, general physical health deterioration, and pneumonia. Treatment-related AEs that led to discontinuation were increased ALT and increased hepatic enzyme, which occurred in 1 subject each.

Efficacy

Study CD-ON-durvalumab-1108: Overall, 456 of 694 subjects treated with durvalumab 10 mg/kg Q2W were evaluable for response (defined as having ≥ 24 weeks follow-up, measurable disease at baseline, and ≥ 1 follow-up scan, or discontinued due to disease progression or death without any follow-up scan). In PD-L1 unselected patients, the objective response rate (ORR), based on investigator assessment per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, ranged from 0% in uveal melanoma (n = 23) to 20.0% in bladder cancer (n = 15), and disease control rate at 24 weeks (DCR-24w) ranged from 4.2% in triple-negative breast cancer (TNBC; n = 24) to 39.1% in advanced cutaneous melanoma (n = 23). PD-L1 status was known for 383 of the 456 response evaluable subjects. Across the PD-L1-positive tumors, ORR was highest for bladder cancer, advanced cutaneous melanoma, hepatocellular carcinoma (HCC; n = 3 each, 33.3% each), NSCLC (n = 86, 26.7%), and squamous cell carcinoma of the head and neck (SCCHN; n = 22, 18.2%). In the PD-L1-positive subset, DCR-24w was highest in advanced cutaneous melanoma (n = 3, 66.7%), NSCLC (n = 86, 36.0%), HCC and bladder cancer (n = 3 each, 33.3% each), and SCCHN (n = 22, 18.2%).

Study D4190C00007: Of the 32 subjects with myelodysplastic syndrome (MDS) treated in Study D4190C00007, 21 subjects had at least 1 post-baseline disease assessment. Among these subjects, the best overall responses were marrow complete remission (mCR) in 4 subjects (19.0%); stable disease (SD) in 4 subjects (19.0%); and progressive disease (PD) in 5 subjects (23.8%). The remaining 8 subjects (38.1%) did not meet the criteria for complete remission (CR), mCR, partial remission (PR), SD, or PD at the date of assessment.

Study CD-ON-durvalumab-1161: Of the 65 subjects with metastatic or unresectable melanoma treated with the combination of durvalumab and BRAF inhibitor (BRAFi; dabrafenib)/MEK inhibitor (MEKi; trametinib), 63 subjects were evaluable for response. A total of 35 subjects (55.6%) had a best overall response of confirmed or unconfirmed PR. The disease control rate (DCR; CR + PR [regardless of confirmation] + SD ≥ 12 weeks) was 79.4%.

Fixed Dosing

A population PK model was developed for durvalumab using monotherapy data from a Phase 1 study (*study 1108; N=292; doses= 0.1 to 10 mg/kg Q2W or 15 mg/kg Q3W; solid tumors*). Population PK analysis indicated

only minor impact of body weight (WT) on PK of durvalumab (coefficient of ≤ 0.5). The impact of body WT-based (10 mg/kg Q2W) and fixed dosing (750 mg Q2W) of durvalumab was evaluated by comparing predicted steady state PK concentrations (5^{th}, median and 95^{th} percentiles) using the population PK model. A fixed dose of 750 mg was selected to approximate 10 mg/kg (based on median body WT of ~75 kg). A total of 1000 patients were simulated using body WT distribution of 40–120 kg. Simulation results demonstrate that body WT-based and fixed dosing regimens yield similar median steady state PK concentrations with slightly less overall between-subject variability with fixed dosing regimen.

Similar findings have been reported by others [Ng et al 2006, Wang et al. 2009, Zhang et al, 2012,Narwal et al 2013]. Wang and colleagues investigated 12 monoclonal antibodies and found that fixed and body size-based dosing perform similarly, with fixed dosing being better for 7 of 12 antibodies [3]. In addition, they investigated 18 therapeutic proteins and peptides and showed that fixed dosing performed better for 12 of 18 in terms of reducing the between-subject variability in pharmacokinetic/pharmacodynamics parameters [Zhang et al 2012].

A fixed dosing approach is preferred by the prescribing community due to ease of use and reduced dosing errors. Given expectation of similar pharmacokinetic exposure and variability, we considered it feasible to switch to fixed dosing regimens. Based on average body WT of 75 kg, a fixed dose of 750 mg Q2W durvalumab (equivalent to 10 mg/kg Q2W), 1500 mg Q4W durvalumab (equivalent to 20 mg/kg Q4W) is included in the current study. Fixed dosing of durvalumab is recommend only for subjects with > 30kg body weight due to endotoxin exposure. Patients with a body weight less than or equal to 30 kg should be dosed using a weight-based dosing schedule .

2.2 Pharmaceutical Data durvalumab

Supplied as a vialed liquid solution containing 500 mg (nominal) durvalumab. The solution contains 50 mg/mL durvalumab, 26 mM histidine/histidine-HCl, 275 mM trehalose dihydrate, 0.02% (w/v) polysorbate 80, at pH 6.0.

Stability:

The shelf life of durvalumab will be indicated on the vial label. Durvalumab must be used within the individually assigned expiry date on the label.

Storage:

durvalumab must be stored at 2°C to 8°C.

It is recommended that durvalumab is stored in opaque containers during storage to prevent excessive exposure to light.

Solution Preparation:

The dose of investigational product for administration must be prepared using aseptic technique. Total in-use storage time from needle puncture of durvalumab vial to the start of administration should not exceed 4 hours at room temperature or 24 hours at 2 to 8°C (36 to 46°F). If in-use storage time exceeds these limits, a new dose must be prepared from new vials. durvalumab does not contain preservatives and any unused portion must be discarded.

No incompatibilities have been observed between durvalumab and polyethylene, polypropylene, polyvinylchloride (PVC), or polyolefin copolymers. Saline bags must be latex free.

Doses will be administered using a 250 mL IV bag of normal saline (0.9% (weight/volume) sodium chloride injection) and delivered through an IV administration set with a 0.2-µm in-line filter via an infusion pump into a peripheral vein.

Patient weight at baseline should be used for dosing calculations in patients ≤30 kg unless there is a ≥ 10% change in weight. Dosing day weight can be used for dosing calculations instead of baseline weight per institutional standard.

For patients <30kg, calculate the dose volume of durvalumab and number of vials needed for the subject to achieve the accurate dose according to Appendix VI.

Patient weight at baseline should be used for dosing calculations unless there is a ≥ 10% change in weight. An additional volume of 0.9% (weight/volume) saline equal to the calculated volume of durvalumab to be added to the IV bag must be removed from the bag prior to addition of durvalumab. The calculated volume of durvalumab is then added to the IV bag, and the bag is mixed by gentle inversion to ensure homogeneity of the dose in the bag. Infusion solutions must be allowed to equilibrate to room temperature prior to commencement of administration.

Route of Administration:

Intravenous.

Following preparation of durvalumab the entire contents of the IV bag should be administered as an IV infusion over approximately 60 minutes (±5 minutes), using a 0.2-µm in-line filter. The IV line will be flushed with a volume of normal saline equal to the priming volume of the infusion set used after the contents of the IV bag are fully administered, or complete the infusion according to institutional policy to ensure the full dose is administered and document if the line was not flushed.

2.3 Justification of durvalumab Dose/s, Schedule and Duration of Administration

The optimal duration of an immunotherapy used as neoadjuvant therapy to improve complete resection of the tumour is unknown.

For this trial, to minimize the delay for surgery, durvalumab will be administered at a dose of 750 mg for 3 planned doses, every 2 weeks for one month. Only early NSCLC will be included Stage IB (only T = 4 cm in greatest dimension, N0), Stage IIA (T2b,N0) and some of Stage IIB : (T1-2,N1) and (T3 : > 5 cm and ≤ 7 cm in greatest dimension surrounded by lung or associated with separate tumor nodule(s) in the same lobe but **without** mediastinum or chest wall involvements, or superior sulcus tumors, N0) on the basis of clinical evaluation (8th classification TNM, UICC 2015).

3 OBJECTIVES

3.1 Primary Objective

To assess the impact of neo-adjuvant therapy with durvalumab given by intravenous infusion for one month on the complete resection (see section 10).

3.2 Secondary Objectives

- Tolerance, adverse effects and tolerability of durvalumab.

- Delay between start of treatment and surgery.

- Response Rate (RECIST 1.1).

- Metabolic response rate on TEP-FDG on pre-treatment PET-CT and pre-operative PET-CT after 3 durvalumab infusions, on both primary tumor and eventual nodes.

- Post-operative adverse events (AE).

- Pathological response (as assessed by microscopic histological examination, with at least one slide assessed by cm of tumor in the greatest diameter, *i.e*. 5 to 30 slides assessed). Major Pathological Response on surgical tissue (tumor + nodes) as centrally reviewed by a panel of IFCT Pathologists, on both tumor and resected nodes, and defined as semi-quantitative evaluation of less than 10% or remaining tumor cells, in surgical primary tumor and nodes samples.

- Disease Free Survival (DFS)

- Overall survival (OS)

3.3 Exploratory objective

- Evaluation of predictive/prognostic significance of PD-1/PD-L1 expression (using at least SP263 antibody) on both pre-operative and surgical samples, and both tumor and infiltrating immune cells

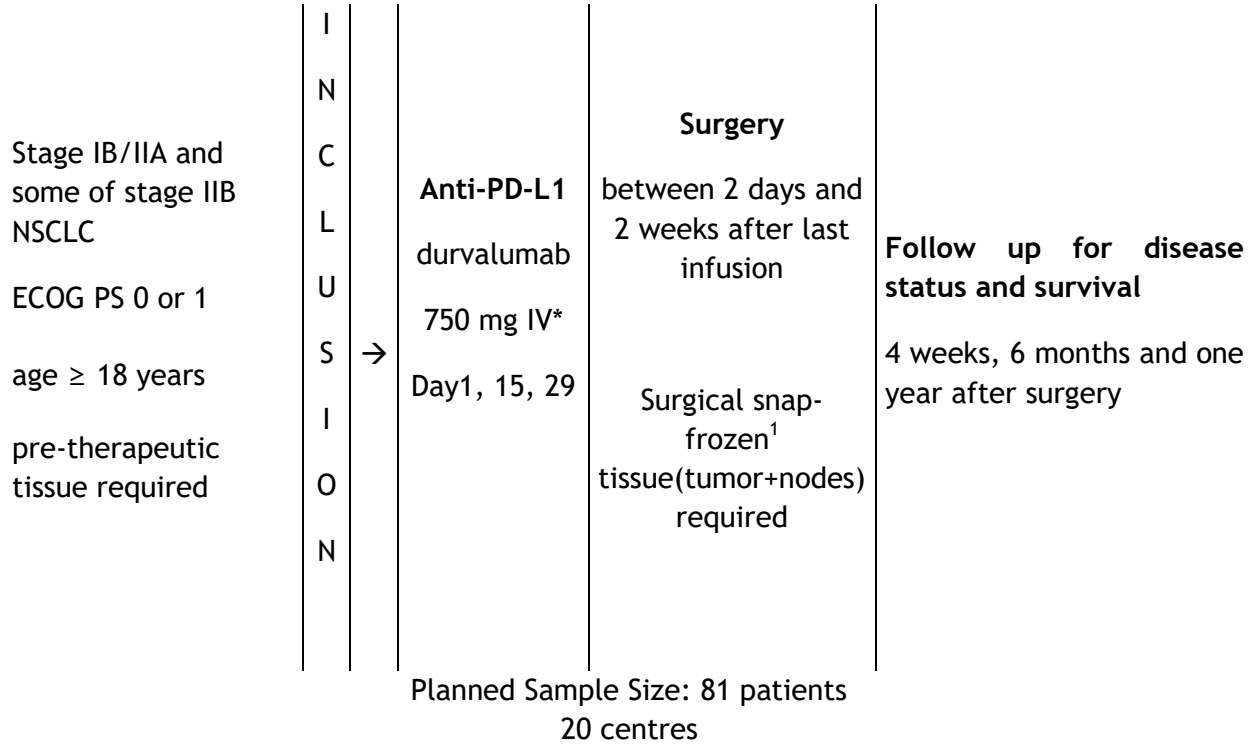
- Evaluation of changes in plasma/serum cytokines and other biomarkers before and after treatment

- Characterization of the immune reactivity of tumors

- Analysis of the intestinal microbiome

4 TRIAL DESIGN

This is a multi-centre, prospective, single-arm phase II trial of the human monoclonal antibody directed against programmed cell death ligand-1 (PD-L1) durvalumab in neoadjuvant treatment in primary resectable stage IB (only T = 4 cm in greatest dimension, N0), stage IIA (T2b,N0) and some of stage IIB : (T1-2,N1) and (T3 : > 5 cm and ≤ 7 cm in greatest dimension surrounded by lung or associated with separate tumor nodule(s) in the same lobe but **without** mediastinum or chest wall involvements, or superior sulcus tumors, N0) non-small cell lung cancer patients.



** 10 mg/kg for patient weighting less than 30 kg (see appendix VI).*

1. Optional

5 STUDY POPULATION

This trial will study patients with clinically Stage IB (only T = 4 cm in greatest dimension, N0), Stage IIA (T2b,N0) and some of Stage IIB : (T1-2,N1) and (T3 : > 5 cm and ≤ 7 cm in greatest dimension surrounded by lung or associated with separate tumor nodule(s) in the same lobe but **without** mediastinum or chest wall involvements, or superior sulcus tumors, N0) non-small cell lung cancer (NSCLC). Staging will be according to the TNM staging system for lung cancer (8th edition).

5.1 Eligibility Criteria

There will be NO EXCEPTIONS to eligibility requirements at the time of inclusion. Questions about eligibility criteria should be addressed prior to inclusion.

The eligibility criteria for this study have been carefully considered. Eligibility criteria are standards used to ensure that patients who enter this study are medically appropriate candidates for this therapy.

Patients must fulfil all of the following criteria to be eligible for admission to the study:

1 Histologically confirmed diagnosis of primary non-small cell carcinoma of the lung.
2 Tissue block of diagnosis must be available for submission after inclusion (one HES slide and one paraffin embedded block).
3 Patients must be classified clinically as Stage IB (only T = 4 cm in greatest dimension, N0), Stage IIA (T2b,N0) and some of Stage IIB : (T1-2,N1) and (T3 : > 5 cm and ≤ 7 cm in greatest dimension surrounded by lung or associated with separate tumor nodule(s) in the same lobe but **without** mediastinum or chest wall involvements, or superior sulcus tumors, N0) on the basis of clinical evaluation (8th classification TNM, UICC 2015). In case of invasion of the main bronchus (distance < 2 cm from carina), a biopsy of the carina is required. A pre-surgical PET scan of the thorax and a MRI or CT scan of the brain as well as thorax abdomen pelvis CT scan must be done prior to surgery and before inclusion. **If preoperative CT and/or PET are suspicious for mediastinal nodal involvement, invasive mediastinal staging with mediastinoscopy or EBUS-TBNA must be performed.** Station 5 or 6 lymph nodes may be accessed by anterior mediastinotomy or VATS.
4 Pre-operative (neo-adjuvant) platinum based or other chemotherapy except the treatment of the protocol is not permissible. Pre-operative radiation therapy is not permissible
5 The patient must have an ECOG performance status of 0, 1.
6 Hematology (done within 14 days prior to inclusion and with values within the ranges specified below): If anemic, patients should be asymptomatic and should not be decompensated. Transfusions are permissible.
Haemoglobin ≥ 9,0 g/dL
Absolute neutrophil count ≥ 1.5 x 10^9/L or ≥ 1,500/μl
Platelets ≥ 100 x 10^9/L or ≥ 100,000/μl
7 Biochemistry (done within 14 days prior to inclusion and with values within the ranges specified below):

Total bilirubin*	within normal institutional limits
Alkaline phosphatase	≤ 2.5 x institutional upper limit of normal
AST(SGOT) and ALT(SGPT)	≤ 2.5 x institutional upper limit of normal
Creatinine Clearance	≥ 40 ml/min

TSH	within normal institutional limits

** excluding Gilbert's syndrome*

Creatinine clearance to be measured directly by 24 hour urine sampling or as calculated by Cockcroft Formula:

$$\text{Females: GFR} = \frac{1.04 \times (140 - \text{age}) \times \text{weight in kg}}{\text{serum creatinine in } \mu\text{mol/L}}$$

$$\text{Males: GFR} = \frac{1.23 \times (140 - \text{age}) \times \text{weight in kg}}{\text{serum creatinine in } \mu\text{mol/L}}$$

8 Other investigations detailed in Section 6 must have been performed within the timelines indicated.

9 Patient consent must be appropriately obtained in accordance with applicable local and regulatory requirements. Each patient must sign a consent form prior to inclusion in the trial to document their willingness to participate.

10 Patients must be accessible for treatment and follow-up. Investigators must assure themselves the patients included on this trial will be available for complete documentation of the treatment, adverse events, and follow-up.

11 Protocol treatment is to begin within 7 days of patient inclusion.

12 Age of at least 18 years.

13 Female subjects must either be of non-reproductive potential (ie, post-menopausal by history: ≥60 years old and no menses for ≥1 year without an alternative medical cause; OR history of hysterectomy, OR history of bilateral tubal ligation, OR history of bilateral oophorectomy) or must have a negative serum pregnancy test upon study entry.

14 Females of childbearing potential who are sexually active with a non-sterilized male partner or men who are sexually active with women of childbearing potential must use a highly effective method of contraception prior the first dose of investigational product, and must agree to continue using such precautions for 4 months after the final dose of investigational product. Periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of contraception.

5.2 Ineligibility Criteria

Patients who fulfil any of the following criteria are not eligible for admission to the study:

1 Patients with a history of other malignancies, except: adequately treated non-melanoma skin cancer, curatively treated in-situ cancer, or other solid tumours curatively treated with no evidence of disease for $\geq$ 5 years following the end of treatment and which, in the opinion of the treating physician, do not have a substantial risk of recurrence of the prior malignancy.

2 A combination of small cell and non-small cell lung cancer or pulmonary carcinoid tumour.

3 History of autoimmune disease, including but not limited to myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, vascular thrombosis associated with antiphospholipid syndrome, Wegener's granulomatosis, Sjögren's syndrome, Guillain-Barré syndrome, multiple sclerosis, vasculitis, or glomerulonephritis. NOTE: patients with Grave's disease and/or psoriasis not requiring systemic therapy within the last two years from inclusion are not excluded.

4 History of primary immunodeficiency, history of allogenic organ transplant, use of immunosuppressive agents within 28 days of inclusion* or a prior history of severe (grade 3 or 4) immune mediated toxicity from other immune therapy.

** NOTE: Intranasal/inhaled corticosteroids or systemic steroids that do not to exceed 10 mg/day of prednisone or equivalent dose of an alternative corticosteroid are permissible.*

5 Live attenuated vaccination administered within 30 days prior to inclusion.
6 History of hypersensitivity to durvalumab or any excipient.
7 Patients who have experienced untreated and/or uncontrolled cardiovascular conditions and/or have symptomatic cardiac dysfunction (unstable angina, congestive heart failure, myocardial infarction within the previous year or cardiac ventricular arrhythmias requiring medication, history of 2nd or 3rd degree atrioventricular conduction defects). Patients with a significant cardiac history, even if controlled, should have a LVEF > 50% within 12 weeks prior to inclusion.
8 Concurrent treatment with other investigational drugs or anti-cancer therapy.
9 Patients with active or uncontrolled infections or with serious illnesses or medical conditions which would not permit the patient to be managed according to the protocol. This includes but is not limited to:

- known prior history of tuberculosis;
- known acute hepatitis B or C by serological evaluation;
- known Human immunodeficiency virus infection.

10 Current or prior use of immunosuppressive medication within 28 days before the first dose of durvalumab, with the exceptions of intranasal and inhaled corticosteroids or systemic corticosteroids at physiological doses, which are not to exceed 10 mg/day of prednisone, or an equivalent corticosteroid
11 Any previous treatment with a PD1 or PD-L1 inhibitor, including durvalumab
12 Active or prior documented inflammatory bowel disease (e.g., Crohn’s disease, ulcerative colitis)
13 Known history of previous clinical diagnosis of tuberculosis
14 Female subjects who are pregnant, breast-feeding or male or female patients of reproductive potential who are not employing an effective method of birth control
15 Any condition that, in the opinion of the investigator, would interfere with evaluation of study treatment or interpretation of patient safety or study results

6 INCLUSION

Prior to the inclusion of any new patients in the IFCT-1601 IONESCO trial, the site should first submit by e-mail to ionesco@ifct.fr the reports of initial CT scan and PET scan as well as the report of the multidisciplinary meeting if possible. A review of the imaging will take place by the steering committee if there is any doubt about the eligibility of the patient. Validation of the inclusion of the new patient will be sent to the site as soon as possible.

7 PRE-TREATMENT EVALUATION

Investigations		Timing
History and Physical Exam including:	• history including cigarette smoking • physical examination including height, weight, pulse, blood pressure • performance status • Concomitant medication	Within 14 days prior to inclusion
Haematology	• CBC including haemoglobin, differential leukocyte, platelet count	Within 14 days prior to inclusion
Biochemistry	• AST (SGOT) and ALT (SGPT) • Alkaline phosphatase • Total bilirubin (if total bilirubin is ≥ 2xULN (and no evidence of Gilbert's syndrome) then fractionate into direct and indirect bilirubin • Gamma glutamyltransferase • albumin • creatinine • Urea • CRP • TSH [1] • INR/APTT • Lactate dehydrogenase • Uric acid • Lipase • Amylase (if lipase > 2,5N) • Magnesium • Potassium • Bicarbonate sodium • Calcium • Chloride • Total protein	Within 14 days prior to inclusion
	• HIV antibody • Hepatitis A antibody, • Hepatitis B surface antigen • Hepatitis C antibody	

Radiology	• baseline CT chest and upper abdomen (must include adrenal glands) [2]	Within 28 days prior to inclusion
	• MRI or CT of brain • PET scan	Within 28 days prior to inclusion
	• other baseline imaging (e.g. bone scan)	Required, within 28 days of inclusion, only if suspicious signs/symptoms are present
Other Investigations	• ECG	Within 2 months prior to inclusion if clinically indicated
	• urinalysis • lung function tests: FEV1, FVC, TLCO and SaO2 on room air • pregnancy test if applicable	Within 2 months prior to inclusion
	• LVEF [3]	Within 12 weeks prior to inclusion
	• Serum, plasma for correlatives • Feces • Tumor block available	Prior to Cycle 1, day 1 dosing [4]

1 If TSH is abnormal, both T3 and T4 should be measured (both are recommended but at least one of T3 or T4 is mandatory if TSH is abnormal).

2 Must be contrast enhanced CT scan; low dose surveillance CT is not acceptable. To ensure comparability, the baseline scans and subsequent scans to assess response must be performed using identical techniques (i.e. scans performed immediately following bolus contrast administration using a standard volume of contrast, the identical contrast agent, and preferably the same scanner). CT appearance consistent with post-operative change is expected and does not preclude inclusion.

3 MUGA or ECHO, only for patients with significant cardiac history.

4 Serum, plasma and feces for correlatives must be taken after consent is obtained but prior to day 1 dosing of cycle 1 – may be done after inclusion providing it is done prior to day 1 dosing or within 14 days prior to inclusion.

8 TREATMENT PLAN

The responsibility for treatment of patients rests with the individual investigator. Protocol treatment is to begin within 7 working days of inclusion.

8.1 Drug Administration

Agent(s)	Route	Duration	Dose and Schedule**
durvalumab	IV	60 minutes*	**750 mg*****on weeks 0, 2, 4

* See Section 7.5.1 for duration in the case of an infusion reaction.

** From inclusion.

*** Two week interval between infusions must be maintained; if infusion cannot be administered, it should be omitted until the next planned infusion.

Do not administer durvalumab unless treatment day tests meet the following criteria:

Haemoglobin ≥ 9 g/dL

Absolute neutrophil count ≥ 1.0 x 10^9/L

Platelets ≥ 100 x 10^9/L

Liver function tests ≤ grade 1 or as per Section 7.5.4

All other related adverse events should be ≤ grade 2. See Section 7.5.4 for additional details.

8.2 Premedication

Premedication is not expected to be required. See Section 7.5.1 with respect to premedication of patients that have had a prior ≤ Grade 2 infusion-related reaction.

8.3 Patient Monitoring

8.3.1 Monitoring of dose administration

Subjects will be monitored before, during and after the infusion with assessment of vital signs at the times specified in the Schedule of Assessment. Subjects are monitored (pulse rate, blood pressure) every 30 minutes during the infusion period (including times where infusion rate is slowed or temporarily stopped).

In the event of a ≤Grade 2 infusion-related reaction, the infusion rate of study drug may be decreased by 50% or interrupted until resolution of the event (up to 4 hours) and re-initiated at 50% of the initial rate until completion of the infusion. For subjects with a ≤ Grade 2 infusion-related reaction, subsequent infusions may be administered at 50% of the initial rate. Acetaminophen and/or an antihistamine (e.g., diphenhydramine) or equivalent medications per institutional standard may be administered at the discretion of the investigator. If the infusion-related reaction is Grade 3 or higher in severity, study drug

will be discontinued. The standard infusion time is one hour, however if there are interruptions during infusion, the total allowed time from infusion start to completion of infusion should not exceed 4 hours at room temperature, with maximum total time at room temperature not exceeding 4 hours (otherwise requires new infusion preparation).

As with any antibody, allergic reactions to dose administration are possible. Appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis. The study site must have immediate access to emergency resuscitation teams and equipment in addition to the ability to admit subjects to an intensive care unit if necessary.

Patients will be monitored during the infusion and for a 30 minute observation period after the infusion with assessment of vital signs as per local procedures.

All patients should be closely monitored according to guidelines in Section 8 and be advised to contact the treating centre in the case of significant toxicities.

8.4 Dose Adjustments

The major toxic effects of durvalumab which are anticipated to limit dosing are hypersensitivity/ infusion related reactions and possible class related immune related AEs, based on the mechanism of action of durvalumab leading to T-cell activation and proliferation. Potential immune related AEs include enterocolitis, dermatitis, hepatitis, pneumonitis and endocrinopathies *[Brahmer 2010; Hodi 2010]*. Patients should be monitored for signs and symptoms of immune related AEs. In the absence of an alternate aetiology (e.g. infection or relapse) signs or symptoms of enterocolitis, dermatitis, hepatitis, and endocrinopathy should be considered to be immune-related.

The guidelines which follow outline dose adjustments for several of these toxic effects. If a patient experiences several adverse events and there are conflicting recommendations, please use the recommended dose adjustment that requires the greatest dose hold or discontinuation (see Appendix II). Adverse events will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) (see Appendix III).

Dose reductions are not permitted, but dose adjustments (slowing/interruption of infusion rate, omission of a dose, or permanent discontinuation) will be made for hematologic and other adverse events**.**

If the infusion cannot be administered, it should be omitted until the next planned infusion.

8.5 Management of Toxicity

The following general guidance should be followed for management of toxicities (see Appendix II):

1. Treat each of the toxicities with maximum supportive care (including slowing / interrupting / omitting the agent suspected of causing the toxicity where required).

2. If the symptoms promptly resolve with supportive care, consideration should be given to continuing the same dose of durvalumab along with appropriate continuing supportive care.

In addition to the dose adjustments shown in Section 7.4, the following are recommended:

- Patient evaluation to identify any alternative aetiology.

- In the absence of a clear alternative aetiology, all events of an inflammatory nature should be considered to be immune-related.
- Symptomatic and topical therapy should be considered for low-grade events.
- Systemic corticosteroids should be considered for a persistent low-grade event or for a severe event.
- More potent immunosuppressives should be considered for events not responding to systemic steroids.

Investigators should exercise clinical judgment in managing actual patients alongside the guidelines presented in the protocol(s). An event that exhibits rapid progression and/or the likelihood for high morbidity/mortality requires that clinical judgment be exercised above and beyond toxicity management guidelines to ensure that treatment is optimally tailored to any given patient's specific case. For example, the general principles outlined in the toxicity management guidelines describe prompt initiation of corticosteroids for both Grade 2 events (that have persisted for 4-5 days) and Grade 3-4 events; clinical judgment applied to this baseline guidance for an event that exhibits rapid progression and/or the likelihood for high morbidity/mortality – such as myocarditis – would warrant prompt initiation of high-dose corticosteroids without delay even for grade 2 cases. Similarly, clinical judgment for patients with suspected myocarditis should lead investigators to obtain a cardiology consult and institute a thorough diagnostic work-up (that includes exclusion of other alternate causes such as infection), and the appropriate management that includes discontinuing drug (permanently if biopsy-proven immune-mediated myocarditis) and, as already noted, the prompt use of steroids or other immunosuppressives.

8.6 Duration of Therapy

Treatment with durvalumab will continue for 1 month taken from date of first infusion irrespective of treatment omissions.

8.7 Patient Compliance

Trained medical personnel will administer durvalumab. Treatment compliance will be monitored by drug accountability, as well as recording durvalumab administration in the patient's medical record and case report form (CRF).

8.8 Concomitant Therapy

8.8.1 Permitted

- Concomitant medications or treatments (e.g. acetaminophen, diphenhydramine) deemed necessary to provide adequate prophylactic or supportive care except for those medications identified as "excluded" as listed in Section 7.8.2.
- Use of immunosuppressive medications for the management of acute immune related adverse events as outlined in Section 7.5 is acceptable. In addition, intranasal/inhaled corticosteroids or systemic steroids that do not to exceed 10 mg/day of prednisone or equivalent dose of an alternative corticosteroid are permissible.

8.8.2 Not Permitted

- The chronic use of immunosuppressive medications including, but not limited to systemic corticosteroids at doses beyond 10 mg/day of prednisone or equivalent, methotrexate, azathioprine, and tumour necrosis factor alpha blockers.
- Live attenuated vaccines within 30 days of dosing with study agent. Inactivated viruses such as those in the influenza vaccine are permitted.

Prohibited and Rescue Medications	
Rescue/supportive medication/class of drug:	**Usage:**
Concomitant medications or treatments (eg, acetaminophen or diphenhydramine) deemed necessary by the Investigator to provide adequate prophylactic or supportive care, except for those medications identified as "prohibited" as listed above	To be administered as prescribed by the Investigator
Best supportive care (including antibiotics, nutritional support, growth factor support, correction of metabolic disorders, optimal symptom control, and pain management [including palliative radiotherapy, etc])	Should be used when necessary for all patients

- Concurrent administration with other anti-cancer therapy, radiation therapy or investigational treatment.

Blood donation

Subjects should not donate blood while participating in this study and for at least 90 days following the last infusion of durvalumab.

9 EVALUATION DURING AND AFTER PROTOCOL TREATMENT

All patients entered on study must be evaluated according to the schedule outlined in Appendix I.

9.1 Evaluation during Protocol Treatment

Investigations		Timing
Physical Exam including:	• physical examination including blood pressure • performance status • Concomitant medication	Prior to each infusion until off protocol therapy.
Hematology[1]	• CBC including haemoglobin, differential leukocyte, platelet count	Prior to each infusion until off protocol therapy.
Biochemistry[1]	• AST (SGOT) and ALT (SGPT) • alkaline phosphatase • Total bilirubin (if total bilirubin is ≥ 2x ULN (and no evidence of Gilbert's syndrome) then fractionate into direct and indirect bilirubin • Gamma glutamyltransferase • albumin • creatinine • Urea • CRP • Magnesium • Potassium • Bicarbonate sodium • Calcium • Chloride • Total protein	Prior to each infusion until off protocol therapy.
	• TSH [2]	one month after the first infusion
	• INR/APTT	As clinically indicated.
Radiology[4]	• CT scan chest and upper abdomen[3]	prior to surgery
	• PET scan	prior to surgery
Other Investigations	• ECG • urinalysis • pregnancy test if applicable	As clinically indicated.
	• lung function tests: FEV1, FVC, TLCO[4] and SaO2 on room air	Only if pulmonary symptoms/suspicion of pneumonitis (see Section 7.5).
Correlatives	• serum, plasma	At the time of 3rd infusion
Adverse Events	Patients must be evaluated after each cycle for adverse events	

1 **Bloodwork** Timing: Pre-treatment blood draws may be done the day prior to treatment if necessary, and when treatment is to begin on a Monday, may be done on the previous Friday (maximum 72 hours prior to treatment).
2 If TSH is abnormal, both T3 and T4 should be measured (both are recommended but at least one of T3 or T4 is mandatory if TSH is abnormal).
3 Must be contrast enhanced CT scan; low dose surveillance CT is not acceptable. To ensure comparability, the baseline scans and subsequent scans to assess response must be performed using identical techniques (i.e. scans performed immediately following bolus contrast administration using a standard volume of contrast, the identical contrast agent, and preferably the same scanner). When relapse is first documented at any site, complete restaging is required to identify all sites of relapse.
4 These exams should have been analysed before surgery by primary physician and surgeon to confirm the operability.

9.2 Evaluation after protocol treatment but prior to disease relapse/new invasive primary malignancy

The following investigations are to be performed for the period after discontinuation of durvalumab and surgery.

All patients must be seen at 4 weeks after surgery. Follow up visits then occur at 6 months and one year and then according to current guidelines.

Investigations		Timing
Physical Exam including:	• physical examination including blood pressure • performance status • Concomitant medication • Adverse events	Every follow up visit
Hematology	• CBC including hemoglobin, differential, platelet count	Only at 4 weeks after surgery. Thereafter as clinically indicated only or to follow durvalumab related AEs until resolution to grade 0-1.
Biochemistry	• AST (SGOT) and ALT (SGPT) • alkaline phosphatase • Total bilirubin (if total bilirubin is ≥ 2x ULN (and no evidence of Gilbert's syndrome) then fractionate into direct and indirect bilirubin • Gamma glutamyltransferase • albumin • creatinine • Urea • CRP • Magnesium • Potassium • Bicarbonate sodium • Calcium • Chloride • Total protein	Only at 4 weeks after surgery. Thereafter as clinically indicated only or to follow durvalumab related AEs until resolution to grade 0-1.
	• TSH[1]	At 4 week after surgery and then as clinically indicated, or to follow durvalumab related AEs until resolution to grade 0-1.[2]
	• INR/APTT	As clinically indicated.
Radiology[3]	• CT scan chest and upper abdomen [3] • other imaging as clinically indicated if suspicion of relapse or new primary invasive malignancy [4]	6 monthly for one year and then according to current guidelines.
Other Investigations	• ECG	4 weeks after surgery then as clinically indicated
	• urinalysis • pregnancy test if applicable	As clinically indicated.
	• lung function tests: FEV1, FVC, TLCO and SaO2 on room air	Only if pulmonary symptoms or suspicion of pneumonitis (see Section 7.5 for full recommendations).

1 If TSH is abnormal, both T3 and T4 should be measured (both are recommended but at least one of T3 or T4 is mandatory if TSH is abnormal).
2 TSH may be completed within 7 days prior to visit.
3 Must be contrast enhanced CT scan, low dose surveillance CT is not acceptable. To ensure comparability, the baseline scans and subsequent scans to assess response must be performed using identical techniques (i.e. scans performed immediately following bolus contrast administration using a standard volume of contrast, the identical contrast agent, and preferably the same scanner).
4 When relapse is first documented at any site, complete restaging is required to identify all sites of relapse.

9.3 Evaluation after disease relapse or new invasive primary malignancy (during or after protocol treatment)

Should a patient relapse or develop a new invasive primary malignancy (as defined in Section 10) during the trial, details should be entered in the EDC disease event form and patient follow-up will be altered. All study patients must have a visit 4 weeks, 6 months and one year after surgery.

Treatments received by the patient after relapse and for new primary malignancies will be left to the discretion of the patient and his/her physician, but all treatments will be recorded in the EDC case report forms. All sites of NSCLC relapse or new primary malignancies will be recorded on the case report forms.

Follow up visit according to the centre practice.

Investigations		Timing
Physical Exam including:	• subsequent anti-cancer therapy • physical examination including pulse, blood pressure • performance status	Every follow up visit.
Hematology	• CBC including hemoglobin, differential, platelet count	Only at 4 week post surgery. Thereafter as clinically indicated only or to follow durvalumab related AEs until resolution to grade 0-1.
Biochemistry	• AST (SGOT) and ALT (SGPT) • alkaline phosphatase • bilirubin • creatinine	Only at 4 week post surgery. Thereafter as clinically indicated only or to follow durvalumab related AEs until resolution to grade 0-1.
	• TSH[1]	At 4 week and then as clinically indicated, or to follow durvalumab related AEs until resolution to grade 0-1.[2]
	• INR/APTT	As clinically indicated.
Radiology	• CT scan chest and upper abdomen other imaging as clinically indicated if suspicion of relapse or new primary invasive malignancy	According to current guidelines.
Other Investigations	• ECG • urinalysis • pregnancy test if applicable	As clinically indicated.
	• Lung function tests: FEV1, FVC, TLCO and SaO2 at room atmosphere	Repeat if pulmonary symptoms/suspicion of pneumonitis.
Adverse Events	Patients must be evaluated for durvalumab related adverse events at each visit, including late events.	

1 If TSH is abnormal, both T3 and T4 should be measured (both are recommended but at least one of T3 or T4 is mandatory if TSH is abnormal).
2 TSH may be completed within 7 days prior to scheduled visit.

10 SURGERY AND PATHOLOGICAL ASSESSMENT

10.1 Surgery

After durvalumab administration and prior to surgery, a second PET scan as well as a second thorax CT scan must be done to evaluate Response to durvalumab treatment.

We plan only three infusions of PD-L1 antibody (one infusion every two weeks) with a schedule allowing surgery to be performed not later than 6 weeks after first infusion. Surgery will be performed between 2 days and 2 weeks after the last infusion.

Before surgery, all adverse events must recover to a grade ≤ 1.

Surgery may consist of lobectomy, sleeve resection, bilobectomy or pneumonectomy as determined by the attending surgeon based on the intraoperative findings. Patients who have had only segmentectomies or wedge resections are not eligible for this study. Resection may be accomplished by open or VATS techniques.

Lymph node mapping is defined by The International Association for the Study of Lung Cancer (IASLC) lymph node map. The nodal tissue must be labelled according to the recommendations of the American Thoracic Society. Surgeons are encouraged to dissect or sample all accessible nodal levels in accordance with the European Society of Thoracic Surgeons guidelines. Accordingly, a minimum of three lobe specific mediastinal nodal stations (N2), one of which must include station 7, and at least one N1 station - inclusive of the ones removed with the pulmonary specimen - must have been sampled at the end of the procedure.

Description of surgical resection (complete or not) is mandatory (see section 10).

10.2 Pathological assessment

A standard approach to post neoadjuvant therapy pathologic assessment is required to use pathological complete response as a demonstration of treatment efficacy. This requires a multidisciplinary communication and information on the pre-treatment tumor size and location, pre-treatment cTNM and evaluation of the clinical response on tumor and mediastinal nodes, critical for the pathologist to perform adequate sampling of the presumed tumor bed and immediately adjacent tissue. Residual tumor is often softer and tumor size and extent is difficult to assess after neoadjuvant therapy. The pathologist must also be informed of the clinical trial and histological type of the pretreatment biopsy.

This standard approach include a precise macroscopic description of the surgically resected NSCLC with macroscopic size in two dimensions of the largest cross section, location of the tumor and distance to close margin i.e. pleural surface and bronchial sleeve resection. Extent of macroscopic necrosis for tumor and node must be reported with semi quantitative assessment. Diagram (or images of the sliced resection) is useful to annotate the tissue section. The number of blocks sampled in the tumor area must be reported.

10.3 Surgical snap frozen tissue and paraffin embedded block

Paraffin embedded blocks must be available. Frozen Tissue (one block for tumor and nodes, only collected if there is grossly obvious residual invasive cancer on macroscopic examination), fresh tumor (and if available metastatic lymph nodes) and normal tissue will be collected if available. Local policy must permit the submission of tissue blocks. Tissue blocks from the surgically resected NSCLC must be submitted for:

-pathological examination with at least one slide assessed by cm of tumor in the greatest diameter as well as each removed lymph node

-PD-L1 assessment on paraffin tissue

-Other biomarkers

- Study of the immune reactivity of tumors in response to immunotherapy

11 CRITERIA FOR MEASUREMENT OF STUDY ENDPOINTS

11.1 Definitions

11.1.1 Complete resection

All gross disease must have been removed at the end of surgery. All surgical margins of resection must be negative for tumour.

Margins are classified by the pathologist as:

R0 - no cancerous cells seen microscopically; this is the desired result

R1 - cancerous cells can be seen microscopically

R2 - even gross examination by the naked eye shows tumor tissue on the margin, indicating more remains on the patient.

11.1.2 Pathological response

Extent of sampling is determined by the pre-treatment size in addition to macroscopic pathologic evaluation: at least one slide assessed by cm of tumor in the greatest diameter, i.e. 5 to 30 slides assessed, all surgically removed lymph node should be entirely submitted for histological evaluation, sectioned in two parts.

Pathological response will be assessed by microscopic histological examination on all the sections performed during the macroscopic sampling. The reporting of the 2D size of the largest distance between residual disease tumor cell nests in a cross section of the entire area of the tumor is required with relevant sections recorded on the pathology report.

In absence of tumor cells, the presence of treatment effect may provide additional information to treatment response, in comparison with lymph node pre-treatment status obtained by CT scan and mediastinal evaluation.

The cellularity is assessed across the largest cross section of residual microscopic disease as the average invasive cancer cellularity and compared and calibrated to online cellularity provided by a computer-generated standard to improve reproducibility (AACR Symmans WF) and RCB score MDAnderson.

Major Pathological Response on surgical tissue (tumor + nodes) will be defined as centrally reviewed by a panel of IFCT Pathologists, on both tumor and resected nodes, as semi-quantitative evaluation of less than 10% or remaining tumor cells, in surgical primary tumor and nodes samples (ref).

11.1.3 Evaluable for Toxicity

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

11.1.4 Disease-free Survival

Disease-free survival shall be measured from the day of inclusion until any of the following events: day of documented equivocal or definite relapse (local, regional or distant), diagnosis of a new primary invasive cancer (except squamous cell carcinoma and basal cell carcinoma of skin and any in situ cancer, completely excised and presumed cured), or death from any cause.

NOTE: Patients with Equivocal Changes should be re-imaged at the next planned time or earlier if clinically indicated. If relapse/new primary malignancy is confirmed the date of event is taken from the date that equivocal changes were first described.

Relapse will be categorized as local/regional or distant.

11.1.5 Local or Regional Relapse

Local or regional relapse is defined as relapse in the area of the tumour bed, hilum or mediastinal lymph nodes.

11.1.6 Distant Relapse

Distant relapse is defined as spread of disease beyond the area of the tumour bed, hilum or mediastinal lymph nodes.

11.1.7 Overall Survival

Overall survival is defined as the time from inclusion to the time of death (from any cause) or to the date the patient was last known to be alive.

11.2 Evidence of Disease Relapse

When relapse is first documented at any site, complete restaging is required to identify all sites of relapse.

11.2.1 Local or Regional Relapse

1. Definite - positive cytology, aspiration, or biopsy
2. Suspicious - radiological changes suggesting relapse

11.2.2 Distant Relapse

This is documented only when definite evidence is available but may be dated retrospectively to first onset of a suspicious sign as defined below.

1. Definite (one of 1a or 1b)
    a) Biopsy or cytology proven disease.
    b) New lesions present on clinical examination, ultrasound, CT, or MRI scan that are strongly suggestive of metastatic cancer, that cannot be attributed to other disease, and that cannot reasonably be confirmed by biopsy or cytology. PET imaging can be used to confirm findings from other imaging modalities.
2. Suspicious

a) New lesions present on clinical examination, ultrasound, CT, or MRI scan that are suggestive of metastatic cancer, that are unlikely to be attributable to other disease, and that cannot reasonably be confirmed by biopsy or cytology.

11.3 Dating of First Relapse

This should always be based on the first onset of a sign but never on the onset of a symptom. The date of first detection of a palpable lesion is acceptable only when the diagnosis of tumour involvement is subsequently established. The diagnosis of recurrent disease by radiographs or scans should be dated from the date of the first positive record, even if this is determined in retrospect

11.4 Management Following Relapse

Patient management following local, regional or distant relapse is at the discretion of the investigator.

11.5 New Primary Malignancy

Should be pathologically confirmed and recorded in the case report forms.

Patient management following new primary malignancy is at the discretion of the investigator, but all treatments need to be documented.

12 SERIOUS ADVERSE EVENT REPORTING

12.1 Definition of Serious Adverse Event

A serious adverse event is one that at any dose (including overdose):

- Results in death.
- Is life-threatening.[1]
- Requires inpatient hospitalization or prolongation of existing hospitalization[2].
- Results in persistent or significant disability or incapacity.[3]
- Is a congenital anomaly or birth defect.
- Is an important medical event.[4]

1 “Life-threatening” means that the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

2 The following hospitalizations are not considered SAEs:

- Hospitalization < 24h
- Planned hospitalization required by the protocol (e.g., for study drug administration or to perform an efficacy measurement for the study)
- Hospitalization for a preexisting condition, provided that all of the following criteria are met:
- The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease.
- The patient has not experienced an adverse event.
- Routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

3 “Persistent or significant disability or incapacity” means that there is a substantial disruption of a person’s ability to carry out normal life functions.

4 Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred.

12.1.1 Definition of adverse events of special interest (AESI)

An adverse event of special interest (AESI) is one of scientific and medical interest specific to understanding of the Investigational Product and may require close monitoring and rapid communication by the

investigator to the sponsor. An AESI may be serious or non-serious. The rapid reporting of AESIs allows ongoing surveillance of these events in order to characterize and understand them in association with the use of this investigational product.

AESIs for durvalumab include but are not limited to events with a potential inflammatory or immune-mediated mechanism and which may require more frequent monitoring and/or interventions such as steroids, immunosuppressants and/or hormone replacement therapy. These AESIs are being closely monitored in clinical studies with durvalumab monotherapy and combination therapy. An immune-related adverse event (irAE) is defined as an adverse event that is associated with drug exposure and is consistent with an immune-mediated mechanism of action and where there is no clear alternate aetiology. Serologic, immunologic, and histologic (biopsy) data, as appropriate, should be used to support an irAE diagnosis. Appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of the irAE.

If the Investigator has any questions in regards to an adverse event (AE) being an irAE, the Investigator should promptly contact IFCT.

AESIs observed with durvalumab include:

- Colitis
- Pneumonitis
- ALT/AST increases / hepatitis / hepatotoxicity
- Neuropathy / neuromuscular toxicity (i.e. events of encephalitis, peripheral motor and sensory neuropathies, Guillain-Barré, and myasthenia gravis)
- Endocrinopathy (i.e. events of hypophysitis, adrenal insufficiency, and hyper- and hypothyroidism)
- Dermatitis
- Nephritis
- Pancreatitis (or labs suggestive of pancreatitis - increased serum lipase, increased serum amylase)

Further information on these risks (e.g. presenting symptoms) can be found in the current version of the durvalumab Investigator Brochure.

Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

A diagnosis of cancer during the course of a treatment should be considered as medically important. The List of Critical Terms (1998 adaptation of World Health Organization Adverse Reaction Terminology Critical Terms List, provided in the "Instructions for completing the 'Serious Adverse Event/Expedited Report from a Clinical Trial' form") should be used as guidance for adverse events that may be considered serious because they are medically important.

Clarification of the difference in meaning between "severe" and "serious"

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical

significance (such as severe headache). This is not the same as “serious”, which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

SAEs/AEs will be reported up to one month after surgery, and SAEs notification will be made within 24 hours.

12.2 Period of Observation

For the purposes of this study, the period of observation for collection of adverse events extends from the date of signature of the informed consent until 100 days after the last day of study treatment except for adverse events related to study drug, which must be reported during follow-up period until resolution or initiation of further anti-tumor therapy.

If an Investigator detects a serious adverse event in a study subject after the end of the period of observation, and considers the event possibly related to prior study treatment, he or she should contact the Sponsor to determine how the adverse event should be documented and reported.

At each visit/assessment AEs will be assessed from information collected from the source documents and information transcribed into the CRF.

12.3 Documentation and Reporting of Adverse Events by Investigator

All adverse events that occur during the observation period set in this protocol must be documented on the pages provided in the case report form in accordance with the instructions for the completion of adverse event reports in clinical studies. These instructions are provided in the case report form itself.

The following approach will be taken for documentation:

• All adverse events (whether serious or non-serious, or considered as an alert term) must be documented on the “Adverse Event” page of the case report form.

• If the adverse event is serious, the investigator must complete, in addition to the

“Adverse Event” page in the case report form, a “Serious Adverse Event/Expedited

Report from a Clinical Trial” form at the time the serious adverse event is detected.

This form must be faxed to the IFCT’s Pharmacovigilance department.

Every attempt should be made to describe the adverse event in terms of a diagnosis. If a clear diagnosis has been made, individual signs and symptoms will not be recorded unless they represent atypical or extreme manifestations of the diagnosis, in which case they should be reported as separate events. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

All patients who have adverse events, whether considered associated with the use of the investigational products or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed up according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the investigator considers it medically justifiable to terminate follow-up. All questions on the completion and supply of adverse event report forms and any further forms issued to the investigator at a later date to clarify unresolved issues should be addressed to the IFCT.

12.4 Immediate Reporting by Investigators to IFCT

Serious adverse events and adverse events that fulfil a reason for expedited reporting to Pharmacovigilance must be documented on a "Serious Adverse Event/Expedited Report from a Clinical Trial" form in accordance with the "Instructions for completing the 'Serious Adverse Event/Expedited Report from a Clinical Trial' form". This form must be completed and supplied to the Sponsor within 24 hours or at the latest on the following working day. The "Serious Adverse Event/Expedited Report from a Clinical Trial" form and the instructions are provided in the investigator's study file.

The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s). The report will be completed in the electronic case report form. In case of unavailability of the CRF, a paper report will be sent by e-mail to sae@ifct.fr. An acknowledgment of receipt will be sent to the site.

Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up "Serious Adverse Event/Expedited Report from a Clinical Trial" form.

The "Instructions for completing the 'Serious Adverse Event/Expedited Report from a Clinical Trial' form" give more detailed guidance on the reporting of serious adverse events, adverse events that comply with alert terms, and adverse events initially reported as non-serious that become serious. In the latter situation, when a non-serious event becomes serious, details must be forwarded immediately to the Sponsor on a "Serious Adverse Event/Expedited Report from a Clinical Trial" form.

12.5 Reporting of pregnancy

The investigator is required to report to the sponsor any pregnancy where the embryo/fetus could have been exposed to durvalumab. This means pregnancies occurring in female participants, female partners of male participants, or females exposed through direct contact with the agent during their pregnancy (for example, environmental exposure involving direct contact with the agent). Pregnancies occurring up to 90 days after the completion of durvalumab must also be reported.

12.6 Overdose

An overdose is defined as a subject receiving a dose of durvalumab in excess of that specified in the Investigator's Brochure, unless otherwise specified in this protocol.

Any overdose of a study subject with durvalumab, with or without associated AEs/SAEs, is required to be reported within 24 hours of knowledge of the event to IFCT. Patient Safety or designee using the designated Safety e-mailbox (see Section 10.3.2 for contact information). If the overdose results in an AE, the AE must also be recorded as an AE (see Section 10.3). Overdose does not automatically make an AE serious, but if the consequences of the overdose are serious, for example death or hospitalization, the event is serious and must be recorded and reported as an SAE (see Section 10.1.2 and Section 10.3.2). There is currently no specific treatment in the event of an overdose of durvalumab.

The investigator will use clinical judgment to treat any overdose.

13 PROTOCOL TREATMENT DISCONTINUATION AND THERAPY AFTER STOPPING

13.1 Criteria for Discontinuing Protocol Treatment

Patients may stop protocol treatment in the following instances:

- Intercurrent illness which would, in the judgement of the investigator, affect assessments of clinical status to a significant degree, and require discontinuation of protocol therapy.
- Unacceptable toxicity as defined in Section 7.5 and Appendix II.
- Disease relapse or new primary invasive malignancy as defined in Section 10.
- Request by the patient.
- Pregnancy or intent to become pregnant
- Initiation of alternative anticancer therapy including another investigational agent.
- Completion of therapy as outlined in Section 7. Efforts should be made to maintain the investigations schedule and continue follow-up, even if patients discontinue protocol treatment prematurely and/or no longer attend the participating institution.

13.2 Withdrawal of study

The Reasons for withdrawal of study are:

- - Withdrawal of consent by the patient,
- - Relapse or 2nd cancer,
- - Death,

Patients have the right to withdraw their consent and ask to leave the study at any time and for whatever reason (they are not required to explain). This should not affect their right to a subsequent management. The investigator must do everything to avoid withdrawal of consent.

13.3 Duration of Protocol Treatment

Treatment will continue for one month from the date of the first infusion.

13.4 Therapy After Protocol Treatment

Anti-cancer therapy after surgery is at the discretion of the investigator. Adjuvant platinum based chemotherapy and adjuvant radiation are allowed, according the current guidelines.

Subsequent anti-cancer therapy will be collected on the CRFs.

13.5 Follow-up Off Protocol Treatment

All patients will be seen at 4 weeks, 6 months and one year after surgery. Thereafter, patients without evidence of disease relapse or new primary malignancy will be followed as per schedule outlined in Section 8.2. Patients that have relapsed disease or new primary malignancy as per definitions in Section 10 will continue to be followed until death as outlined in Section 8.3.

14 CENTRAL REVIEW PROCEDURES AND TISSUE COLLECTION

14.1 Central Radiology Review

Central radiology review of equivocal cases by a review committee may be conducted. A retrospective radiology review of all cases may also be undertaken.

14.2 Central Pathology Review

This central pathological review includes the pre-treatment biopsy with sending of one representative HES section and paraffin bloc. All slides of tumor and nodes must also be sending.

14.3 Tissue Collection

Tissue collection for pre-treatment (one block, frozen tumor if available) and post treatment (fresh tumor and normal tissue, formalin fixed, paraffin embedded and frozen tumor and node tissue if available) is mandatory from existing tumour specimens be submitted to a central reference laboratory for analysis.

The tissue may be used by researchers now or in the future to better understand the nature of non-small cell lung cancer and how patients respond to treatments. Samples will be used for research purposes only and will not be sold. A scientific review process of any proposals to use the tissue will take place and any proposals approved will have undergone ethics approval. Patients will not be identified by name. The only identification of tissue will be by a patient study number assigned at the time of registration to the trial the surgical/ histology number and/or patient initials. Material issued to researchers will be anonymized and only identified by a coded number. This process is applicable to all tissues collected as part of this study, irrespective of the location of the Tissue/Tumour Bank (see Section 13.3.5).

Testing specifically for hereditary genetic defects predisposing to malignant disease will not be carried out without the expressed consent of the patient.

All patients on whom a diagnostic tumour block is collected will be aware of this retrieval and will have given their consent.

14.3.1 Serum and plasma

Serum and plasma will be collected before first infusion and after 3rd infusion of durvalumab.

Anti-tumour immune responses are key contributors to the anti-tumour effects of anti-PD-L1 therapies. Serological responses (cytokines, antibodies and metabolites) provide rich sources of correlative data that can be used to understand the biological consequences of this novel therapy and may provide important biomarkers that will facilitate future patient management.

13.3.2 Feces

Feces will be collected before first infusion of durvalumab.

Currently, only few parameters have been identified as predictive biomarker of response, such as positive expression of PD-L1, and high mutational loads of tumors that appears to be associated with a higher response rate to anti-PD1 mAb. New lines of evidence demonstrated the biological significance of the gut microbiome (diverse range of symbiotic microorganisms) has an important contributor of pulmonary pathologies such as asthma and COPD (see APPENDIX V).

14.3.2 Tumour and Tissue Banks

Blocks and all other samples will be carefully banked at the IFCT Repository.

14.3.3 Translational study

See Appendix V

15 STATISTICAL CONSIDERATIONS

15.1 Objectives and Design

This is a multi-centre, prospective unblinded, single-arm phase II trial of the human monoclonal antibody directed against programmed cell death ligand-1 (PD-L1) durvalumab in neoadjuvant treatment in primary resectable stage IB (only T = 4 cm in greatest dimension, N0), stage IIA (T2b,N0) and some of stage IIB : (T1-2,N1) and (T3 : > 5 cm and ≤ 7 cm in greatest dimension surrounded by lung or associated with separate tumor nodule(s) in the same lobe but **without** mediastinum or chest wall involvements, or superior sulcus tumors, N0) non-small cell lung cancer patients.

The primary objective is surgical resection R0 after maximum 3 cycles of immune therapy.

Secondary objectives will evaluate study treatment effect:

- Response Rate (RECIST 1.1) and metabolic response rate on TEP-FDG

- Tolerance and Post-operative adverse events (30 days after surgery)

- Delay between surgery and start of treatment

- Evaluation of predictive/prognostic significance of PD-1/PD-L1 expression

- Disease Free Survival (DFS)

- Overall survival (OS)

- Evaluation of changes in plasma/serum cytokines and other biomarkers before and after treatment

- Major Pathological Response on surgical tissue

15.2 Sample Size, Power

The maximum sample size (N) is calculated based on α, ß, and the expected effect size, using a test for single binomial proportion for a two-stage design with a 1:1 randomization and O'Brien-Fleming (OF) stopping rules, allowing for early stopping for futility after Stage I. The software East 6.0 was used.

The rate of complete resection equal to or lower than 85% (P0) is considered as unacceptable after immune treatment. On the other hand, we can conclude to a good rate of complete resection if it is 95% (P1). The computation is based on the following assumption, considering the probability of compliance: P0 =85%; P1 = 95%; statistical power of 0.90; type I error rate (one-sided) of 0.05.

The null hypothesis that the rate of complete resection is P0 = 85% will be tested against a one-sided alternative. In the first stage, 39 patients will be accrued. If there are 34 or fewer responses in these 39 patients, the study will be stopped for futility. Otherwise, 38 additional patients will be accrued for a total of 77. The null hypothesis will be rejected if 71 or more responses are observed in 77 patients. To take into account 5% of ineligible patients, 81 patients will be included.

Duration of accrual: 18 months and 1 year follow-up.

15.3 Analysis population

- The population of tolerance is defined as all patients who received one dose of treatment

- The population the intent to treat (ITT) will include all patients included

- The population of eligible patients will consist of ITT patients presenting no deviation judged major on the criteria for inclusion and non-inclusion

The deviations to the protocol will be reviewed by the principal investigators and then classified into minor or major deviations, and listed.

15.4 Study Endpoints and Analysis

The primary endpoint will be performed on all eligible patients based on patient percentage surgical resection R0 after a maximum of 3 cycles of chemotherapy. It will be presented associated with confidence interval of 95%.

DFS is a secondary endpoint, defined as the time from the date of inclusion to the date of first documented disease relapse or the occurrence of a new invasive primary malignancy or death from any cause. Patients who are alive and disease/new invasive primary malignancy-free at the time of each analysis will be censored at their last disease assessment date.

OS is a secondary endpoint, defined as the time from the inclusion to the date of death of any cause, or censored at their last known alive date. All analysis on DFS will be carried out for the OS endpoint.

The analysis of secondary endpoints will be performed on patient eligible.

Although PD-L1 expression as measured by immunohistochemistry is hypothesized to be a potential predictive marker, this is as yet not validated in either the adjuvant or palliative settings. Further, the cut-off defining PD-L1 positivity has not been validated. This trial will also explore the utility of PD-L1 as a predictive biomarker as well as the optimal cut-off for positivity.

15.5 Safety Monitoring

All patients who receive at least one dose of MEDI4376 will be included in the safety analysis. Descriptive summary tables will be presented on safety parameters

Immune adverse events of special interest will be analysed including: pneumonitis, endocrinopathies, dermatologic (rash/pruritus) and hepatic (elevation of transaminases/bilirubin).

The number of post-operative events and a tolerance report will be achieved on the first 15 patients to discuss the pursuit of the trial.

A definitive discontinuation of the study in real time is planned in case of occurrence of a new death (any cause combined) occurring within 90 days of the intervention among the 12 consecutive first patients enrolled after the resuming of the trial (46th patient and the following).

Tolerance data (90-day postoperative follow-up) for these 57 patients will be analyzed by the steering committee.

16 ADMINISTRATIVE SECTION

16.1 Sponsor obligations

16.1.1 Before the trial

The sponsor will:

- Ensure regulatory requirements are met before the trial is implemented
- Ensure that all administrative procedures are in place for each department in the associated establishments
- Provide the complete protocol and its appendices, the adverse event report form, CPP (Independent ethics committee) permission, statement of insurance and authorisation from the French health authority (ANSM).

The sponsor and their representatives will:

- Provide centres with the instructions and documents needed to conduct the trial properly (protocol, data collection form and investigator file)
- Organise an orientation session to train study investigators and coordinators (during this session, all protocol sections will be reviewed and how to fill out case report forms and study procedures will be explained)

16.1.2 During the trial

The sponsor and their representatives will:

- Regularly visit the investigating centres
- Be available at all times for consultation and remain in contact with the personnel of the investigating centre by email, telephone and/or fax
- Examine and evaluate the data in the case report form and look for possible errors in data collection

In cooperation with the principal investigator, the sponsor will provide all of the investigators involved with the study with any new information that may interfere with conducting the trial.

16.1.3 In terms of the trial

It is the sponsor's responsibility to make sure that the procedures are completed at the end of the trial.

16.2 Investigator obligations

The investigator agrees to conduct the study in compliance with the 1974 Declaration of Helsinki, revised in 1975 and 1989, good clinical practice, and effectual French regulations.

In regards to the French law of 9 August 2004, the investigator from each centre agrees to collect the informed written consent from each patient participating in the trial. One copy of the consent form will be given to the patient and the other will be kept in the patient's clinical file. Patients must be able to give their informed consent freely and not be under guardianship or suffer from any type of mental impairment that may affect their judgement.

The investigator also agrees to fill out the case report form needed for study follow-up.

The investigator also agrees to:

- Inform the sponsor of any serious or unexpected adverse event occurring during the trial within the time periods described in chapter 7.1 using the appropriate form.
- Agree to monitoring with access to source documents to validate data from the case report forms and if needed, agree to an internal or external audit by the sponsor or a representative from the regulatory authorities
- Archive trial documents (copies of case report forms, written consent forms) for a period of at least 15 years
- Include at least one patient during the first six months following the trial's implementation
- Make sure that there is no interference with another trial for the same indications
- Respect the confidentiality of the documents provided

16.3 Ethical considerations

16.3.1 Participant information and consent

Before conducting this biomedical research on a person, they must provide their **free, informed** and **express** consent after being informed of the purpose of the research, the study procedures and duration, the benefits, and potential risks and constraints of the study, as well as the type of product being researched and the opinion of the CPP (art. L.1122-1).

The patient and investigator or representing physician will personally date and sign the consent form (original copy archived by the investigator, a copy will be given to the patient or their legal representative).

16.3.2 Request for CNIL authorisation for automated data processing

This biomedical research will produce scientific information. This coded, directly or indirectly personal information is part of the legal framework of file use (Law n° 78-17 from 6 January 1978 and law n° 94-548 from 1 July 1994)

The sponsor (IFCT) has authorisation (*n°1227585*) to process personal data for the purpose of Biomedical Research on medication and diseases under the law of 20 December 1988 modified by law n° 2004-806 of 9 August 2004.

To the extent that this biomedical research is conducted within the strict regulatory and legal requirements (the "Huriet-Sérusclat" law of 20 December 1988 modified by law n° 2004-806 of 9 August 2004) and according to standard methods, the CNIL has adopted a standard method (MR001 according to article 54 from the law of 6 January 1978 modified), which now covers all personal data processing conducted as part of biomedical research - including pharmacogenetic trials - as defined in the public health code, and commitment to comply with said method.

16.3.3 Amendment and additional clauses procedures

The study's principal investigator or sponsor will suggest any substantial change in the protocol, and will then inform the other party. This will be an amendment submitted to the CPP and AFSSAPS. No change can be made without agreement from the committee. The sponsor will inform each investigator and send them the amendment and related statement of intent.

16.4 Quality control and assurance

Regulatory considerations: medical procedures for this trial comply with the most recent recommendations from the Declaration of Helsinki and public health law no. 2004-806 of 9 August 2004 related to the protection and safety of humans.

Confidentiality: the protocol and its appendixes, as well as all of the data, are confidential as noted at the beginning of the protocol.

Data monitoring: The IFCT's Clinical Research Unit will monitor this trial in order to ensure accurate, complete and reliable data collection. It will also provide logistical support to investigating centres. An inspection by an employee obligated to professional secrecy mandated by Regulatory Authorities may be required to ensure that all of the source documents needed are available and that the clinical trial is being conducted according to Good Clinical Practices and the law from 9 August 2004.

16.4.1 Study schedule

The protocol should begin at 2nd quarter of 2016. The trial recruitment period is expected to be 32 months, follow-up is one year. The trial should be ended by mid-June 2020.

16.4.2 Early study termination

The sponsor and coordinating investigator will issue any early termination of the study. The sponsor will provide written notification. This letter will be sent to the Competent Authority (ANSM) and to each investigator, as well as the CPP.

16.4.3 Statement of commitment

New investigators

Investigators can only participate in the trial after submitting a written request to the sponsor. It should include the following items:

- A statement of commitment indicating the expected number of patients that the investigator can include in the protocol per year
- A recent *CV* with French medical board registration number

Site opening

Before inclusions can begin, a site must be officially opened; in other words, the investigator's name, institution, telephone and fax number, and email address must be duly issued to the sponsor, CPP and Ministry (initial and additional authorisation statement). The investigator must have all of the documents needed to conduct the trial properly (protocol, investigator brochure, case report form). He must have obtained agreement from the facility's pharmacist to distribute treatments and resolve any problems with the study coordinator. He must have informed the director of his facility by letter of his participation. An agreement must be signed between the director of his facility and the sponsor.

16.5 Study organisation

The Steering Committee makes all of the decisions concerning the study's implementation, execution, analysis and reporting. It meets three times per year two times, and periodically sends information on the study's progress to investigators.

It is comprised of members of the editorial board, study statisticians and a sponsor representative.

The Coordination Centre will be the Clinical Research Unit (Unité de Recherche Clinique - URC) of the IFCT, located at 10 rue de la Grange-Batelière, 75009 PARIS. Its purpose is to ensure that the trial is being conducted as intended in the protocol: patient inclusion management, data collection, data management, SAE management, organisation of investigator and committee meetings. It informs the Steering Committee of any issues concerning the trial's progress.

Moreover, the Steering Committee can organise regular investigator meetings during which the files of included patients will be reviewed. The purpose of this review panel will be to jointly verify compliance and

understanding of the eligibility criteria and treatment methods dictated by the protocol. In addition, it will conduct a progress analysis for retrospective validation.

17 FINAL REPORT AND PUBLICATIONS

Once the study is finished, a clinical trial report will be published by the study's principal investigators and statistician. The coordinating investigator will sign the final version of the clinical trial report for this study, and by doing so will indicate his approval of the report's analyses, results and conclusions.

The key participants in a clinical study are the principal investigator, investigators, IFCT-employee team members, and members of the management and scientific boards who all collaborate to various degrees from designing the trial to writing the final report.

1. The coordinator can choose between first and last place. In both cases, he must have participated significantly in designing the trial, inclusions and writing the article.
2. If the investigator chooses the first place, last place goes to the president, one of the secretaries, or one of the other elected members of the Management Board or Scientific Council. The person must be chosen based on their participation in designing the trial, inclusions and writing the article. If there is difficulty in choosing this person, a secret vote by the management board will decide.
3. If the investigator chooses the last place, the next-to-last place goes to the president, one of the secretaries, or another elected member of the Management Board or Scientific Council under the same conditions, unless he/she takes the first position if, in agreement with the principal investigator, he writes the article. If there is difficulty in choosing this person, a secret vote by the management board will decide.
4. Investigators appear by the order in which eligible patients were included. All other investigators must appear in a list in the appendix.
5. Two members of the same team cannot sign the same article unless one of them appears as a coordinating investigator, Management Board member, or Scientific Council member signing as such in first, second, or next-to-last place.
6. Members of the IFCT employee team play a vital role in clinical studies. For this reason, those who were involved in designing the trial, managing it or writing the article must systematically appear in the acknowledgements or as signatories. The director will provide their names to the principal investigator (coordinator). If they appear as signatories, they cannot be included in the first six or last two positions. There can be no more than two if the number of signatories is more than 10 and only one if the number is equal to or less than 10.

7. If a university statistician has worked on developing or processing trial data, he can sign with the approval of the principal investigator (coordinator) in a place on which they both agree (generally 3rd or 4th place).
8. Under no circumstances can someone sign for having provided a patient with routine care that was unrelated to the research.
9. All investigators (one per IFCT investigating centre) will be listed after each article in a table that may also include pathologists, surgeons and radiation therapists from the centre based on the article, so that this citation appears on Medline.
10. For ancillary studies (biological, radiology, or others), if the article or abstract has a maximum of 10 signatures, the principal investigator (coordinator) of the biological study can choose between first and last position. In both cases, he must have participated widely in the study's design, financing, inclusions and writing the article. The rules above apply to last position if he chooses last position. Next-to-last place can go to the research lab director who contributed most to the study. The last but two place can go to the study's principal investigator (coordinator) if it is not taken by the biological study's principal investigator. The first four places can be taken by scientists or physicians, with the IFCT or not, who contributed the most to the ancillary study. This may also include the university statistician if suggested by the coordinating investigator and management board office, and validated by the management board. If there are a maximum of 10 signatures, three to four places in the middle will be reserved for the best clinical contributors (in the sense that they contributed the most in terms of pathology specimens). In the case of articles with 20 signatures, two to three places will be reserved for pathologists who contributed the most to the study (either in collecting specimens or reviewing the IFCT's pathology panel). All of the labs that participated in the study shall be represented by one signatory, the remaining signatory places must be attributed to clinicians according to the rules above, including one permanent member of the IFCT team who contributed the most to the study (decision made by the CI + Director + President), as well as the CI for the clinical study.
11. All articles should include the mention "..on behalf of IFCT" at the end of the signatories list, and include the acronym IFCT-XXYY in the title of the clinical trial to which the ancillary study is related.
12. The IFCT can receive assistance with the English formatting of an article, but can never delegate the writing itself to an agency or industry.

These rules were created and validated by the IFCT Management Board in September 2010.

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APPENDIX I - PATIENT EVALUATION FLOW SHEET

Required investigations	Pre-study	Prior to each infusion	Prior to surgery	4 weeks after surgery	6 months and one year after surgery
History	X	X		X	X
Physical exam	X	X		X	X
Smoking history	X				
ECOG	X	X		X	X
Collection of adverse events	X	X		X	X
CBC, Differential leukocyte, platelet count	X	X		X	As clinically indicated
AST, ALT, alkaline Phosphatase, bilirubin, gamma glutamyltransferase, albumin, serum creatinine, urea, lactate dehydrogenase, uric acid, lipase, amylase (if lipase > 2,5N), magnesium, potassium, bicarbonate sodium, calcium, chloride, total protein, HIV antibody[1], Hepatitis A antibody[1], Hepatitis B surface antigen[1], Hepatitis C antibody[1]	X	X		X	As clinically indicated
TSH[2]	X	X[3]		X	As clinically indicated
CRP	X	X			
INR/APTT	X	As clinically indicated			
CT chest and upper abdomen (must include adrenal glands)[3]	X		X		X
CT or MRI brain	X	As clinically indicated			
PET scan thorax	X		X		
Other imaging	As clinically indicated[4]				
ECG	X	As clinically indicated		X	As clinically indicated
Pregnancy test (if applicable)	X	As clinically indicated			
Lung function tests (FEV1, FVC, TLCO and SaO2 on room air)	X	As clinically indicated			
Urinalysis	X	As clinically indicated			
LVEF	X[5]				
Bio-IFCT : serum, plasma	X	X[6]			

1 Pre-study only

2 If TSH is abnormal, both T3 and T4 should be measured (both are recommended but at least one of T3 or T4 is mandatory if TSH is abnormal).

3 One month after the first infusion only

4 When relapse is first documented at any site, complete restaging is required to identify all site of relapse

5 MUGA or ECHO, only for patients with significant cardiac history.

6 3rd infusion only

APPENDIX II – TOXICITY MANAGEMENT

	Dose Modifications	Toxicity Management
Immune-mediated Adverse Events (imAEs) (Overall Management For toxicities not noted below)	Drug administration modifications of study drug/study regimen will be made to manage potential immune-related AEs based on severity of treatment-emergent toxicities graded per NCI CTCAE v4.03. In addition to the criteria for permanent discontinuation of study drug/regimen based on CTC grade/severity (table below) , permanently discontinue study drug/study regimen for the following conditions: • Inability to reduce corticosteroid to a dose of ≤10 mg of prednisone per day (or equivalent) **within 12 weeks** after last dose of study drug/regimen • Recurrence of a previously experienced Grade 3 treatment-related AE following resumption of dosing.	It is recommended that management of immune-mediated adverse events (imAEs) follows the guidelines presented in this table – It is possible that events with an inflammatory or immune mediated mechanism could occur in nearly all organs, some of them not noted specifically in these guidelines. – Whether specific immune-mediated events (and/or laboratory indicators of such events) are noted in these guidelines or not, patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, concomitant medications, and infections) to a possible immune-mediated event. – In the absence of a clear alternative etiology, all such events should be managed as if they were immune related. General recommendations follow. – . – Symptomatic and topical therapy should be considered for low-grade (Grade 1 or 2, unless otherwise specified) events – For persistent (greater than 3 to 5 days) low-grade (Grade 2) or severe (Grade ≥3) events promptly start prednisone PO 1-2mg/kg/day or IV equivalent – Some events with high likelihood for morbidity and/or mortality – e.g., myo-carditis, or other similar events even if they are not currently noted in the guidelines – should progress rapidly to high dose IV corticosteroids (methylprednisolone at 2 to 4 mg/kg/day) even if the event is Grade 2, and if clinical suspicion is high and/or there has been clinical confirmation. Consider, as necessary, discussing with the study physician, and promptly pursue specialist consultation. – If symptoms recur or worsen during corticosteroid tapering 28 days of taper), increase the corticosteroid dose (prednisone dose [e.g. up to 2-4mg/kg/day or IV equivalent]) until stabilization or improvement of symptoms, then resume corticosteroid tapering at a slower rate (≥ 28 days of taper) – More potent immunosuppressives such as TNF inhibitors (e.g. infliximab) – (also refer to the individual sections of the imAEs for specific type of immunosuppressive) should be considered for events not responding to systemic steroids. Progression to use of more potent immunosuppressives should proceed more rapidly in events with high likelihood for morbidity and/or mortality – e.g., myocarditis, or other similar events even if they are not currently noted in the guidelines – when these events are not responding to systemic steroids. – With long-term steroid and other immunosuppressive use, consider need for Pneumocystis jirovecii pneumonia (PJP, formerly known as Pneumocystis carinii pneumonia) prophylaxis, gastrointestinal protection, and glucose monitoring.
	Grade 1 No dose modification Grade 2 Hold study drug/study regimen dose until grade 2 resolution to ≤ Grade 1 • If toxicity worsens then treat as Grade 3 or Grade 4 • If toxicity improves to baseline then treat at next scheduled treatment date Study drug/study treatment can be resumed at the next scheduled dose once event stabilizes to grade ≤1 and 5-7 days have passed after completion of steroid taper Patients with endocrinopathies who may require prolonged or continued steroid replacement can be retreated with study drug/study regimen on the following conditions: 1) the event stabilizes and is controlled , 2) the patient is clinically stable as per Investigator or treating physician's clinical judgement, and 3) doses of prednisone are at less than or equal to 10mg/day or equivalent.	
	Grade 3 Depending on the individual toxicity, may permanently discontinue study drug/study regimen. Please refer to guidelines below	
	Grade 4 Permanently discontinue study drug/study regimen Note: For Grade ≥3 asymptomatic amylase or lipase levels, hold study drug/study regimen, and if complete work up shows no evidence of pancreatitis, study drug/study regimen may be continued or resumed. Note: Study drug/study regimen should be permanently discontinued in Grade 3 events with high likelihood for morbidity and/or mortality – e.g., myocarditis, or other similar events even if they are not currently noted in the guidelines. Similarly, consider whether study drug/study regimen should be permanently discontinued in Grade 2	Discontinuation of study drug/study regimen is not mandated for Grade 3/Grade 4 inflammatory reactions attributed to local tumor response (e.g.,

	events with high likelihood for morbidity and/or mortality – e.g., myocarditis, or other similar events even if they are not currently noted in the guidelines – when they do not rapidly improve to Grade <1 upon treatment with systemic steroids and following full taper Note: There are some exceptions to permanent discontinuation of study drug for Grade 4 events (i.e., hyperthyroidism, hypothyroidism, Type 1 diabetes mellitus).	inflammatory reaction at sites of metastatic disease and lymph nodes). Continuation of study drug/study regimen in this situation should be based upon a benefit-risk analysis for that patient.

	Grade of the Event (NCI CTCAE version 4.03)	**Dose Modifications**	**Toxicity Management**
Pneumonitis/ Interstitial Lung Disease (ILD)	Grade of Pneumonitis (CTCAE version 4.03)	Any Grade	– Monitor patients for signs and symptoms of pneumonitis or ILD (new onset or worsening shortness of breath or cough). Patients should be evaluated with imaging and pulmonary function tests including other diagnostic procedures as described below – Initial work-up may include clinical evaluation, monitoring of oxygenation via pulse oximetry (resting and exertion), laboratory work-up and high-resolution CT scan.
	Grade 1 (Asymptomatic, clinical or diagnostic observations only, intervention not indicated)	No dose modification required. However, consider holding study drug/study regimen dosing as clinically appropriate and during diagnostic work-up for other etiologies	For Grade 1 (Radiographic Changes Only) - Monitor and closely follow up in 2-4 days for clinical symptoms, pulse oximetry (resting and exertion) and laboratory work-up and then as clinically indicated - Consider Pulmonary and Infectious disease consult
	Grade 2 (Symptomatic, medical intervention indicated, limiting instrumental ADL)	Hold study drug/study regimen dose until grade 2 resolution to ≤ Grade 1 • If toxicity worsens then treat as Grade 3 or Grade 4 • If toxicity improves to baseline then the decision to reinitiate study drug/regimen at next scheduled treatment date will be based upon treating physician's clinical judgment. Study drug/study treatment can be resumed at the next scheduled dose once event stabilizes to grade ≤1 and 5-7 days have passed after completion of steroid taper	For Grade 2 (Mild to Moderate New Symptoms) – Monitor symptoms daily and consider hospitalization – Promptly start systemic steroids (e.g., prednisone 1-2mg/kg/day or IV equivalent) – Reimaging as clinically indicated – If no improvement within 3-5 days, additional workup should be considered and prompt treatment with IV methylprednisolone 2-4mg/kg/day started – If still no improvement within 3-5 days despite IV methylprednisolone at 2-4/g/kg/day, promptly start immunosuppressive therapy such as TNF inhibitors (e.g. infliximab at 5mg/kg every 2 weeks). Caution: Important to rule out sepsis and refer to infliximab label for general guidance before using infliximab – Once improving, gradually taper steroids over ≥4 weeks and consider prophylactic antibiotics, antifungal or anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections

			[Category 2B recommendation])[123] – Consider pulmonary and infectious disease consult – Consider as necessary discussing with study physician
	Grade 3 or 4 (Grade 3: Severe symptoms; limiting self-care ADL; oxygen indicated; Grade 4: life threatening respiratory compromise, urgent intervention indicated [e.g. tracheostomy or intubation])	Permanently discontinue study drug/study regimen	For Grade 3 or 4 (severe or new symptoms, new/worsening hypoxia, life threatening): – Promptly initiate empiric IV methylprednisolone 1 to 4 mg/kg/day or equivalent – Obtain Pulmonary and Infectious disease consult; consider, as necessary, discussing with study physician – Hospitalize the patient – Supportive Care (oxygen, etc.) – If no improvement within 3-5 days, additional workup should be considered and prompt treatment with additional immunosuppressive therapy such as TNF inhibitors (e.g. infliximab at 5mg/kg every 2 weeks dose) started. Caution: rule out sepsis and refer to infliximab label for general guidance before using infliximab – Once improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals and in particular, anti-PJPtreatment (please refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation])[1]
Diarrhea/ Colitis	Grade of Diarrhea (CTCAE version 4.03)	Any Grade	– Monitor for symptoms that may be related to diarrhea/enterocolitis (abdominal pain, cramping, or changes in bowel habits such as increased frequency over baseline or blood in stool) or related to bowel perforation (such as sepsis, peritoneal signs and ileus) – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, infections including testing for clostridium difficile toxin, etc.) – Steroids should be considered in the absence of clear alternative etiology, even for low grade events, in order to prevent potential progression to higher grade event – Use analgesics carefully; they can mask symptoms of perforation and peritonitis
	Grade 1 (Diarrhea: stool frequency of <4 over baseline per day) (Colitis: asymptomatic; clinical or diagnostic observations only)	No dose modification	For Grade 1 : - Close monitoring for worsening symptoms - Consider symptomatic treatment including hydration, electrolyte replacement, dietary changes (e.g., American Dietetic Association colitis diet), and loperamide. Use of probiotics as per treating physician's clinical judgment.
	Grade 2 (Diarrhea: stool frequency of 4-6	Hold study drug/study regimen until resolution to ≤ Grade 1 • If toxicity worsens then treat as	For Grade 2 diarrhea: – Consider symptomatic treatment including hydration, electrolyte replacement, dietary

	over baseline per day) (Colitis: abdominal pain; mucus or blood in stool)	Grade 3 or Grade 4 • If toxicity improves to baseline then treat at next scheduled treatment date Study drug/study regimen can be resumed at the next scheduled dose once event stabilizes to grade ≤1 and 5-7 days have passed after completion of steroid taper	changes (e.g., American Dietetic Association colitis diet), and loperamide and/or budesonide – Promptly start prednisone 1 to 2 mg/kg/day or IV equivalent – If event is not responsive within 3-5 days or worsens despite prednisone at 1-2 mg/kg/day or IV equivalent, GI consult should be obtained for consideration of further workup such as imaging and/or colonoscopy to confirm colitis and rule out perforation, and prompt treatment with IV methylprednisolone 2-4mg/kg/day started. – If still no improvement within 3-5 days despite 2-4mg/kg IV methylprednisolone, promptly start immunosuppressives such as (infliximab at 5mg/kg once every 2 weeks)[3]. **Caution**: Important to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab – Consider, as necessary, discussing with study physician if no resolution to ≤ Grade 1 in 3-4 days – Once improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals and anti-PJPtreatment (please refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation])
	Grade 3 or 4(Grade 3 diarrhea: stool frequency of ≥7 over baseline per day;Grade 4 diarrhea: life threatening consequences) (Grade 3 colitis: severe abdominal pain, change in bowel habits, medi-cal intervention indi-cated, peritoneal signs; Grade 4 colitis: life-threatening consequences, urgent intervention indicated)	**<u>Grade 3</u>** Permanently discontinue study drug/study regimen for Grade 3 if toxicity does not improve to Grade ≤1 within 14 days; study drug/study regimen can be resumed after completion of steroid taper. Grade 4 Permanently discontinue study drug/study regimen.	For Grade 3 or 4: – Promptly initiate empiric IV methylprednisolone 2 to 4 mg/kg/day or equivalent – Monitor stool frequency and volume and maintain hydration – Urgent GI consult and imaging and/or colonoscopy as appropriate – If still no improvement within 3-5 days of IV methylprednisolone 2 to 4mg/kg/day or equivalent, promptly start further immunosuppressives (e.g. infliximab at 5mg/kg once every 2 weeks). – Caution: Ensure GI consult to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab. – Once improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals and anti-PJPPCP treatment (please refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation])
Hepatitis (Elevated LFTs) Infliximab should not be used for management of Immune Related Hepatitis	Grade of Liver Function Test Elevation (CTCAE version 4.03) Any Grade	Any Grade	– Monitor and evaluate liver function test: AST, ALT, ALP and total bilirubin – Evaluate for alternative etiologies (e.g., viral hepatitis, disease progression, concomitant medications)
	Grade 1 (AST or ALT >ULN and ≤3.0×ULN and/or TB > ULN	No dose modification If it worsens, treat as Grade 2 event	For Grade 1 AST or ALT and/or TB elevation - Continue LFT monitoring per protocol

PLEASE SEE shaded area immediately below this section to find guidance for management of "Hepatitis (elevated LFTS)" in HCC patients	and ≤1.5×ULN)		
	Grade 2 (AST or ALT > 3 to 5 times ULN and/or TB >1.5-3.0 times ULN)	• Hold Study drug/study regimen dose until grade 2 resolution to ≤ Grade 1 • If toxicity worsens then treat as Grade 3 or Grade 4 •If toxicity improves to Grade ≤1 or baseline, resume study drug/study regimen after completion of steroid taper.	For Grade 2 AST or ALT and or TB elevation : – Regular and frequent checking of LFTs (e.g. every 1-2 days) until elevations of these are improving or resolved. – If no resolution to ≤ Grade 1 in 1-2 days, consider, as necessary, discussing with study physician. – If event is persistent (> 3-5 days) or worsens, promptly start prednisone 1-2mg/kg/day or IV equivalent. – If still no improvement within 3-5 days despite 1-2mg/kg/day of prednisone or IV equivalent, consider additional work up and prompt treatment with IV methylprednisolone 2-4mg/kg/day started. – If still no improvement within 3-5 days despite 2-4mg/kg/day of IV methylprednisolone, promptly start immunosuppressives (i.e. mycophenolate mofetil)[1]. Discuss with study physician if mycophenolate mofetil is not available. **Infliximab should NOT be used**. – Once improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals and anti-PJP treatment (please refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation])
	(Grade 3: AST or ALT >5.0×ULN and ≤20.0×ULN and/or TB >3.0×ULN and ≤10.0×ULN)	For elevations in transaminases ≤ 8 × ULN, or elevations in bilirubin ≤ 5 × ULN: -Hold study drug/study regimen dose until resolution to ≤ Grade 1 or baseline -Resume study drug/study regimen if elevations downgrade to Grade ≤1 or baseline within 14 days and after completion of steroid taper. -Permanently discontinue study drug/study regimen if the elevations do not downgrade to ≤ Grade 1 or baseline within 14 days For elevations in transaminases > 8 × ULN or elevations in bilirubin > 5 × ULN, discontinue study drug/study regimen Permanently discontinue study drug/study regimen for any case meeting Hy's law criteria (AST and/or ALT > 3x ULN + bilirubin > 2x ULN without initial findings of	For Grade 3 or 4 AST or ALT and/or TB elevation: – Promptly initiate empiric IV methylprednisolone at 1 to 4 mg/kg/day or equivalent – If still no improvement within 3-5 days despite 1 to 4 mg/kg/day methylprednisolone IV or equivalent , promptly start treatment with immunosuppressive therapy (mycophenolate mofetil) Discuss with study physician if mycophenolate is not available. **Infliximab should NOT be used.** – Perform hepatology consult, abdominal workup, and imaging as appropriate. – Once improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals and anti-PJP

		cholestasis (i.e. elevated alkaline P04) and in the absence of any alternative cause[4]	treatment (please refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation])
	Grade 4 (AST or ALT >20×ULN and/or TB >10×ULN)	Permanently discontinue study drug/study regimen	
Hepatitis (elevated LFTs) Infliximab should not be used for management of immune-related hepatitis. See instructions at bottom of shaded area if transaminase rise is not isolated but (at any time) occurs in setting of either increasing bilirubin or signs of DILI/liver decompensation **THIS shaded area is guidance *only* for management of "Hepatitis (elevated LFTs)" in HCC patients**	Any Grade	General Guidance	For Any Grade: − Monitor and evaluate liver function test: AST, ALT, ALP, and TB. − Evaluate for alternative etiologies (e.g., viral hepatitis, disease progression, concomitant medications, worsening of liver cirrhosis [e.g., portal vein thrombosis]). − For HBV+ patients: evaluate quantitative HBV viral load, quantitative HBsAg, or HBeAg − For HCV+ patients: evaluate quantitative HCV viral load − Consider consulting hepatologist/Infectious disease specialist regarding change/implementation in/of antiviral medications for any patient with an elevated HBV viral load >2000 IU/ml − Consider consulting hepatologist/Infectious disease specialist regarding change/implementation in/of antiviral HCV medications if HCV viral load increased by ≥2-fold − For HCV+ with HBcAB+: Evaluate for both HBV and HCV as above
	Grade 1 (Isolated AST or ALT >ULN and ≤5.0×ULN, whether normal or elevated at baseline)	• No dose modifications. • If ALT/AST elevations represents significant worsening based on investigator assessment, then treat as Grade 2 event. For all grades, see instructions at bottom of shaded area if transaminase rise is not isolated but (at any time) occurs in setting of either increasing bilirubin or signs of DILI/liver decompensation	
	Grade 2 (Isolated AST or ALT >5.0×ULN and ≤8.0×ULN, if normal at baseline)	• Hold study drug/study regimen dose until Grade 2 resolution to Grade ≤1 or baseline. • If toxicity worsens, then treat as Grade 3 or Grade 4.	For Grade 2: − Regular and frequent checking of LFTs (e.g., every 1 to 3 days) until elevations of these are improving or resolved. − Recommend consult hepatologist; consider abdominal ultrasound, including Doppler

	(Isolated AST or ALT >2.0×baseline and ≤12.5×ULN, if elevated >ULN at baseline)	If toxicity improves to Grade ≤1 or baseline, resume study drug/study regimen after completion of steroid taper.	assessment of liver perfusion. − Consider, as necessary, discussing with study physician. − If event is persistent (>3 to 5 days) or worsens, and investigator suspects toxicity to be immune-mediated AE, recommend to start prednisone 1 to 2 mg/kg/day PO or IV equivalent. − If still no improvement within 3 to 5 days despite 1 to 2 mg/kg/day of prednisone PO or IV equivalent, consider additional workup and treatment with IV methylprednisolone 2 to 4 mg/kg/day. − If still no improvement within 3 to 5 days despite 2 to 4 mg/kg/day of IV methylprednisolone, consider additional abdominal workup (including liver biopsy) and imaging (i.e., liver ultrasound), and consider starting immunosuppressives (i.e., mycophenolate mofetil).a Discuss with study physician if mycophenolate mofetil is not available. Infliximab should NOT be used.
	Grade 3 (Isolated AST or ALT >8.0×ULN and ≤20.0×ULN, if normal at baseline) (Isolated AST or ALT >12.5×ULN and ≤20.0×ULN, if elevated >ULN at baseline)	• Hold study drug/study regimen dose until resolution to Grade ≤1 or baseline • Resume study drug/study regimen if elevations downgrade to Grade ≤1 or baseline within 14 days and after completion of steroid taper. • Permanently discontinue study drug/study regimen if the elevations do not downgrade to Grade ≤1 or baseline within 14 days Permanently discontinue study drug/study regimen for any case meeting Hy's law criteria, in the absence of any alternative cause[4]	For Grade 3: − Regular and frequent checking of LFTs (e.g., every 1-2 days) until elevations of these are improving or resolved. − Consult hepatologist (unless investigator is hepatologist); obtain abdominal ultrasound, including Doppler assessment of liver perfusion; and consider liver biopsy. − Consider, as necessary, discussing with study physician. − If investigator suspects toxicity to be immune-mediated, promptly initiate empiric IV methylprednisolone at 1 to 4 mg/kg/day or equivalent. − If no improvement within 3 to 5 days despite 1 to 4 mg/kg/day methylprednisolone IV or equivalent, obtain liver biopsy (if it has not been done already) and promptly start treatment with immunosuppressive therapy (mycophenolate mofetil). Discuss with study physician if mycophenolate is not available. Infliximab should NOT be used. − Once the patient is improving, gradually taper steroids over ≥ 28 days and consider prophylactic antibiotics, antifungals, and anti-PCP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]).
	Grade 4 (Isolated AST or ALT >20×ULN, whether normal or elevated at baseline)	Permanently discontinue study drug/study regimen.	For Grade 4: Same as above (except would recommend obtaining liver biopsy early)

If transaminase rise is not isolated but (at any time) occurs in setting of either increasing total/direct bilirubin (y (if it has not been done already) and promptly start treatment with immunosuppressive therapy (mycophenolate nd rule out perforation, - Manage dosing for Grade 1 transaminase rise as instructed for Grade 2 transaminase rise - Manage dosing for Grade 2 transaminase rise as instructed for Grade 3 transaminase rise - Grade 3-4: Permanently discontinue study drug/study regimen			
Nephritis or Renal Dysfunction (Elevated Serum Creatinine)	Grade of Elevated Serum Creatinine (CTCAE version 4.03) Any Grade		- Consult with Nephrologist - Monitor for signs and symptoms that may be related to changes in renal function (e.g. routine urinalysis, elevated serum BUN and creatinine, decreased creatinine clearance, electrolyte imbalance, decrease in urine output, proteinuria, etc.) - Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, infections etc.) - Steroids should be considered in the absence of clear alternative etiology even for low grade events (Grade 2) , in order to prevent potential progression to higher grade event
	Grade 1 [Serum Creatinine > 1-1.5X baseline; > ULN to 1.5X ULN]	No dose modification	For Grade 1 elevated creatinine: - Monitor serum creatinine weekly and any accompanying symptom • If creatinine returns to baseline, resume its regular monitoring per study protocol. • If it worsens, depending on the severity , treat as Grade 2 or Grade 3 or 4 - Consider symptomatic treatment including hydration, electrolyte replacement, diuretics, etc.
	Grade 2 [Serum Creatinine>1.5-3.0X baseline; >1.5X-3.0XULN]	Hold study drug/study regimen until resolution to ≤ Grade 1 or baseline • If toxicity worsens then treat as Grade 3 or Grade 4 • If toxicity improves to baseline then treat at next scheduled treatment date Study drug/study regimen can be resumed at the next scheduled dose once event stabilizes to grade ≤1 for 5-7 days have passed after completion of steroid taper	For Grade 2 elevated creatinine: - Consider symptomatic treatment including hydration, electrolyte replacement, diuretics, etc. - Carefully monitor serum creatinine every 2-3 days and as clinically warranted - Consult Nephrologist and consider renal biopsy if clinically indicated - If event is persistent (> 3-5 days) or worsens, promptly start prednisone 1 to 2 mg/kg/day or IV equivalent - If event is not responsive within 3-5 days or worsens despite prednisone at 1-2 mg/kg/day or IV equivalent, additional workup should be considered and prompt treatment with IV methylprednisolone at 2-4mg/kg/day started. - Once improving gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals and anti-PJP treatment (please refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]). - When event returns to baseline, resume study drug/study regimen and routine serum

			creatinine monitoring per study protocol.
	Grade 3 or 4 (Grade 3: Serum Creatinine > 3.0 X baseline; >3.0-6.0 X ULN Grade 4: Serum Creatinine > 6.0 X ULN)	Permanently discontinue study drug/study regimen	- Carefully monitor serum creatinine on daily basis - Consult Nephrologist and consider renal biopsy if clinically indicated - Promptly start prednisone 1 to 2 mg/kg/day or IV equivalent - If event is not responsive within 3-5 days or worsens despite prednisone at 1-2 mg/kg/day or IV equivalent, additional workup should be considered and prompt treatment with IV methylprednisolone 2-4mg/kg/day started. - Once improving, gradually taper steroids over orsens despite prednisone at 1-2 mg/kg/day or IV equivalent, addition-PJP treatment (please refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]
Rash (excluding Bullous skin formations)	Grade of Skin Rash (Please refer to NCICTCAE version 4.03 for definition of severity/grade depending on type of skin rash)	Any Grade	Monitor for signs and symptoms of dermatitis (rash and pruritus) **IF THERE IS ANY BULLOUS FORMATION, THE STUDY PHYSICIAN SHOULD BE CONTACTED **AND STUDY DRUG DISCONTINUED**** For Grade 1: - Consider symptomatic treatment including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy (e.g., urea cream)
	Grade 1	No dose modification	
	Grade 2	For persistent (> 1- 2 weeks) Grade 2 events, hold scheduled study drug/study regimen until resolution to ≤ Grade 1 or baseline • If toxicity worsens then treat as Grade 3 • If toxicity improves then resume administration at next scheduled dose • Study drug/study regimen	For Grade 2 : - Obtain dermatology consult - Consider symptomatic treatment including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy (e.g., urea cream) - Consider moderate-strength topical steroid - If no improvement of rash/skin lesions occurs within 3-5 days or is worsening despite symptomatic treatment and/or use of moderate strength topical steroid, consider,

		can be resumed at the next scheduled dose once event stabilizes to grade ≤1 and 5-7 days have passed after completion of steroid taper	as necessary, discussing with study physician and promptly start systemic steroids prednisone 1-2 mg/kg/day or IV equivalent – Consider skin biopsy if persistent for >1-2 weeks or recurs
	Grade 3	Hold study drug/study regimen until resolution to ≤ Grade 1 or baseline If temporarily holding the study drug/study regimen does not provide improvement of the Grade 3 skin rash to ≤ Grade 1 or baseline within 30 days, then permanently discontinue Study drug/study regimen	For Grade 3 or 4: – Consult dermatology – Promptly initiate empiric IV methylprednisolone 1 to 4 mg/kg/day or equivalent – Consider hospitalization – Monitor extent of rash [Rule of Nines] – Consider skin biopsy (preferably more than 1) as clinically feasible. – Once improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals and anti-PJPtreatment (please refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]) – Consider, as necessary, discussing with Study Physician
	Grade 4	Permanently discontinue study drug/study regimen	
Endocrinopathy (e.g., hyperthyroidism, hypothyroidism, Type 1 diabetes mellitus, hypophysitis, hypopituitarism, adrenal insufficiency; exocrine event of amylase/lipase increased also included in this section)	Any Grade (Depending on the type of endocrinopathy, refer to NCI CTCAE version 4.03 for defining the CTC grade/severity)		– Consider consulting endocrinologist for endocrine eventsConsider, as necessary, discussing with study physician – Monitor patients for signs and symptoms of endocrinopathies. Non-specific symptoms include headache, fatigue, behavior changes, changed mental status, vertigo, abdominal pain, unusual bowel habits, polydipsia, polyuria, hypotension and weakness. – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression including brain metastases, infections, etc.) – Depending on the suspected endocrinopathy, monitor and evaluate thyroid function tests: TSH, free T_3 and free T_4 and other relevant endocrine and related labs (e.g., blood glucose and ketone levels, HgA1c) – For modest asymptomatic elevations in serum amylase and lipase, corticosteroid treatment is not indicated as long as there are no other signs or symptoms of pancreatic inflammation – If a patient experiences an AE that is thought to be possibly of autoimmune nature (e.g., thyroiditis, pancreatitis, hypophysitis, diabetes insipidus), the investigator should send a blood sample for appropriate autoimmune antibody testing

	Grade 1 (Depending on the type of endocrinopathy, refer to NCI CTCAE version 4.03 for defining the CTC grade 1)	No dose modification	For Grade 1: (including those with asymptomatic TSH elevation) - Monitor patient with appropriate endocrine function tests - For suspected hypophysitis/hypopituitarism, consider consultation of an endocrinologist to guide assessment of early-morning ACTH, cortisol, TSH and free T4; also consider gonadotropins, sex hormones, and prolactin levels, as well as cosyntropin stimulation test (though it may not be useful in diagnosing early secondary adrenal insufficiency) - If TSH < 0.5X LLN, or TSH >2X ULN or consistently out of range in 2 subsequent measurements, include FT4 at subsequent cycles as clinically indicated and consider consultation of an endocrinologist
	Grade 2 (Depending on the type of endocrinopathy, refer to NCI CTCAE version 4.03 for defining the CTC grade/severity 2)	For Grade 2 endocrinopathy other than hypothyroidism and Type 1 diabetes mellitus, hold study drug/study regimen dose until subject is clinically stable • If toxicity worsens then treat as Grade 3 or Grade 4 • If toxicity improves to baseline then treat at next scheduled treatment date Study drug/study regimen can be resumed at the next scheduled dose once event stabilizes to grade ≤1 and 5-7 days have passed after completion of steroid taper Patients with endocrinopathies who may require prolonged or continued steroid replacement (e.g., adrenal insufficiency) can be retreated with study drug/study regimen on the following conditions: 1) the event stabilizes and is controlled ,2) the patient is clinically stable as per Investigator or treating physician's clinical judgement, and 3) doses of prednisone are at less than or equal to 10mg/day or equivalent.	For Grade 2: (including those with symptomatic endocrinopathy) - Consult endocrinologist to guide evaluation of endocrine function and, as indicated by suspected endocrinopathy and as clinically indicated, consider pituitary scan - For all patients with abnormal endocrine work up, except for those with isolated hypothyroidism or Type 1 DM, and as guided by an endocrinologist, consider short-term, corticosteroids (e.g., 1-2mg/kg/day methylprednisolone or IV equivalent) and prompt initiation of treatment with relevant hormone replacement (e.g., hydrocortisone, sex hormones). - Isolated hypothyroidism may be treated with replacement therapy, without study drug/study regimen interruption, and without corticosteroids - Isolated Type 1 diabetes mellitus (DM) may be treated with appropriate diabetic therapy, without study drug/study regimen interruption, and without corticosteroids - Once patients on steroids are improving, gradually taper immunosuppressive steroids (as appropriate and with guidance of endocrinologist) over ≥28 days and consider prophylactic antibiotics, antifungals and anti-PJP treatment (please refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]) - For patients with normal endocrine work up (lab or MRI scans), repeat labs/MRI as clinically indicated.

	Grade 3 or 4 (Depending on the type of endocrinopathy, refer to NCI CTCAE version 4.03 for defining the CTC grade/severity 3 or 4)	For Grade 3 or 4 endocrinopathy other than hypothyroidism and Type 1 diabetes mellitus, hold study drug/study regimen dose until endocrinopathy symptom(s) are controlled. Study drug/study regimen can be resumed once event stabilizes and after completion of steroid taper. Patients with endocrinopathies who may require prolonged or continued steroid replacement (e.g., adrenal insufficiency) can be retreated with study drug/study regimen on the following conditions: 1. The event stabilizes and is controlled. 2. The patient is clinically stable as per investigator or treating physician's clinical judgement. 3. Doses of prednisone are ≤10 mg/day or equivalent	For Grade 3 or 4: – Consult endocrinologist to guide evaluation of endocrine function and, as indicated by suspected endocrinopathy and as clinically indicated, consider pituitary scan. Hospitalization recommended – For all patients with abnormal endocrine work up, except those with isolated hypothyroidism or Type 1 DM, and as guided by an endocrinologist, promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent, as well as relevant hormone replacement (e.g., hydrocortisone, sex hormones) – Isolated hypothyroidism may be treated with replacement therapy, without treatment interruption and without study drug/study regimen interruption, and without corticosteroids – Isolated Type 1 diabetes mellitus may be treated with appropriate diabetic therapy, without study drug/study regimen interruption, and without corticosteroids For adrenal crisis, severe dehydration, hypotension, or shock: immediately initiate intravenous corticosteroids with mineralocorticoid activity – Once patients on steroids are improving, gradually taper immunosuppressive steroids (as appropriate and with guidance of endocrionologist) over ≥4 weeks and consider prophylactic antibiotics, antifungals and anti-PJP treatment (please refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation]) –

Immune mediated Neurotoxicity (to include but not limited to limbic encephalitis . autonomic neuropathy, excluding Myasthenia Gravis and Guillain-Barre)	Grade of Neurotoxicity Depending on the type of neurotoxicity , refer to NCI CTCAE version 4.03 for defining the CTC grade/severity Any Grade		Patients should be evaluated to rule out any alternative etiology (e.g., disease progression, infections, metabolic syndromes and medications, etc.) Monitor patient for general symptoms (headache, nausea, vertigo, behavior change, or weakness) Consider appropriate diagnostic testing (e.g. electromyogram and nerve conduction investigations) Symptomatic treatment with neurological consult as appropriate
	Grade 1	No dose modifications	See "Any Grade" recommendations above.
	Grade 2	• For acute motor neuropathies or neurotoxicity, hold study drug/study regimen dose until resolution to ≤ Grade 1 • For sensory neuropathy/neuropathic pain, consider holding study drug/study regimen dose until resolution to ≤ Grade 1. o If toxicity worsens then treat as Grade 3 or Grade 4 o If toxicity improves to baseline then treat at next scheduled treatment date • Study drug/study regimen can be resumed once event improves to Grade ≤1 and after completion of steroid taper.	Consider, as necessary, discussing with the study physician Obtain Neurology Consult Sensory neuropathy/neuropathic pain may be managed by appropriate medications (e.g., gabapentin, duloxetine, etc.) Promptly start systemic steroids prednisone 1-2mg/kg/day or IV equivalent If no improvement within 3-5 days despite 1-2mg/kg/day prednisone or IV equivalent consider additional workup and promptly treat with additional immunosuppressive therapy (e.g. IVIG)
	Grade 3	• Hold Study drug/study regimen dose until resolution to ≤ Grade 1 • Permanently discontinue Study drug/study regimen if Grade 3 imAE does not resolve to ≤ Grade 1 within 30 days.	For Grade 3 or 4: – Consider, as necessary, discussing with study physician – Obtain Neurology Consult – Consider hospitalization – Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent – If no improvement within 3-5 days despite IV corticosteroids, consider additional workup and promptly treat with additional immunosuppressants (e.g. IVIG) – Once stable, gradually taper steroids over ≥4 weeks
	Grade 4	• Permanently discontinue study drug/study regimen	

Immune-mediated peripheral neuromotor syndromes, such as Guillain-Barre and Myasthenia Gravis		Any Grade	– The prompt diagnosis of immune-mediated peripheral neuromotor syndromes is important, since certain patients may unpredictably experience acute decompensations which can result in substantial morbidity or in the worst case, death. Special care should be taken for certain sentinel symptoms which may predict a more severe outcome, such as prominent dysphagia, rapidly progressive weakness, and signs of respiratory insufficiency or autonomic instability – Patients should be evaluated to rule out any alternative etiology (e.g., disease progression, infections, metabolic syndromes and medications, etc.). It should be noted that the diagnosis of immune-mediated peripheral neuromotor syndromes can be particularly challenging in patients with underlying cancer, due to the multiple potential confounding effects of cancer (and its treatments) throughout the neuraxis. Given the importance of prompt and accurate diagnosis, it is essential to have a low threshold to obtain a neurological consult – Neurophysiologic diagnostic testing (e.g., electromyogram and nerve conduction investigations, and "repetitive stimulation" if myasthenia is suspected) are routinely indicated upon suspicion of such conditions and may be best facilitated by means of a neurology consultation – Important to consider that the use of steroids as the primary treatment of Guillain-Barre is not typically considered effective. Patients requiring treatment should be started with IVIG and followed by plasmapheresis if not responsive to IVIG
	Grade 1	No dose modification	Consider, as necessary, discussing with the study physician Care should be taken to monitor patients for sentinel symptoms of a potential decompensation as described above Obtain a neurology consult
	Grade 2	Hold study drug/study regimen dose until resolution to ≤ Grade 1 Permanently discontinue study drug/study regimen if it does not resolve to ≤ Grade 1 within 30 days or if there are signs of respiratory insufficiency or autonomic instability	**Grade 2**Discuss with the study physician Care should be taken to monitor patients for sentinel symptoms of a potential decompensation as described aboveObtain a Neurology Consult Sensory neuropathy/neuropathic pain may be managed by appropriate medications (e.g., gabapentin, duloxetine, etc.) *MYASTHENIA GRAVIS* o Steroids may be successfully used to treat Myasthenia Gravis. Important to consider that steroid therapy (especially with high doses) may result in transient worsening of myasthenia

			and should typically be administered in a monitored setting under supervision of a consulting neurologist. o Patients unable to tolerate steroids may be candidates for treatment with plasmapheresis or IVIG. Such decisions are best made in consultation with a neurologist, taking into account the unique needs of each patient. o If Myasthenia Gravis-like neurotoxicity present, consider starting acetylcholine esterase (AChE) inhibitor therapy in addition to steroids. Such therapy, if successful, can also serve to reinforce the diagnosis. *GUILLAIN-BARRE:* o Important to consider here that the use of steroids as the primary treatment of Guillain-Barre is not typically considered effective. o Patients requiring treatment should be started with IVIG and followed by plasmapheresis if not responsive to IVIG.
	Grade 3	Hold study drug/study regimen dose until resolution to ≤ Grade 1 Permanently discontinue Study drug/study regimen if Grade 3 imAE does not resolve to ≤ Grade 1 within 30 days or if there are signs of respiratory insufficiency or autonomic instability	For severe or life threatening (Grade 3 or 4) events: - Consider, as necessary, discussing with study physician - Recommend hospitalization - Monitor symptoms and obtain neurological consult *MYASTHENIA GRAVIS* o Steroids may be successfully used to treat Myasthenia Gravis. It should typically be administered in a monitored setting under supervision of a consulting neurologist. o Patients unable to tolerate steroids may be candidates for treatment with plasmapheresis or IVIG. o If Myasthenia Gravis-like neurotoxicity present, consider starting acetylcholine esterase (AChE) inhibitor therapy in addition to steroids. Such therapy, if successful, can also serve to reinforce the diagnosis. *GUILLAIN-BARRE*: o Important to consider here that the use of steroids as the primary treatment of Guillain-Barre is not typically considered effective. o Patients requiring treatment should be started with IVIG and followed by plasmapheresis if not responsive to IV IG
	Grade 4	Permanently discontinue study drug/study regimen	

Myocarditis	**Any Grade**	General Guidance Discontinue drug permanently if biopsy-proven immune-mediated myocarditis.	For Any Grade: − The prompt diagnosis of immune-mediated myocarditis is important, particularly in patients with baseline cardiopulmonary disease and reduced cardiac function. − Consider, as necessary, discussing with the study physician. − Monitor patients for signs and symptoms of myocarditis (new onset or worsening chest pain, arrhythmia, shortness of breath, peripheral edema). As some symptoms can overlap with lung toxicities, simultaneously evaluate for and rule out pulmonary toxicity as well as other causes (e.g., pulmonary embolism, congestive heart failure, malignant pericardial effusion). A Cardiology consultation should be obtained early, with prompt assessment of whether and when to complete a cardiac biopsy, including any other diagnostic procedures. − Initial work-up should include clinical evaluation, BNP, cardiac enzymes, ECG, echocardiogram (ECHO), monitoring of oxygenation via pulse oximetry (resting and exertion), and additional laboratory work-up as indicated. Spiral CT or cardiac MRI can complement ECHO to assess wall motion abnormalities when needed. − Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections)
	Grade 1 (asymptomatic with laboratory (e.g., BNP) or cardiac imaging abnormalities)	No dose modifications required unless clinical suspicion is high, in which case hold study drug/study regimen dose during diagnostic work-up for other etiologies. If study drug/study regimen is held, resume after complete resolution to Grade 0.	**For Grade 1 (no definitive findings):** - Monitor and closely follow up in 2 to 4 days for clinical symptoms, BNP, cardiac enzymes, ECG, ECHO, pulse oximetry (resting and exertion), and laboratory work-up as clinically indicated. - Consider using steroids if clinical suspicion is high.
	Grade 2, 3 or 4 (Grade 2: Symptoms with mild to moderate activity or exertion) (Grade 3: Severe with symptoms at rest or with minimal activity or exertion; intervention indicated) (Grade 4: Life-threatening consequences; urgent intervention	- If Grade 2 -- Hold study drug/study regimen dose until resolution to Grade 0. If toxicity rapidly improves to Grade 0, then the decision to reinitiate study drug/study regimen will be based upon treating physician's clinical judgment and after completion of steroid taper. If toxicity does not rapidly improve, permanently. discontinue study drug/study regimen. - If Grade 3-4, permanently discontinue study drug/study	**For Grade 2-4:** − Monitor symptoms daily, hospitalize. − Promptly start IV methylprednisolone 2 to 4 mg/kg/day or equivalent after Cardiology consultation has determined whether and when to complete diagnostic procedures including a cardiac biopsy. − Supportive care (e.g., oxygen). − If no improvement within 3 to 5 days despite IV methylprednisolone at 2 to 4 mg/kg/day, promptly start immunosuppressive therapy such as TNF inhibitors (e.g., infliximab at 5 mg/kg every 2 weeks). Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. − Once the patient is improving, gradually taper steroids over ≥28 days and

	indicated (e.g., continuous IV therapy or mechanical hemodynamic support))	regimen.	consider prophylactic antibiotics, antifungals, or anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation])
Myositis /Polymyositis ("Poly/myositis")	**Any Grade**	**General Guidance**	**For Any Grade:** – Monitor patients for signs and symptoms of poly/myositis. Typically, muscle weakness/pain occurs in proximal muscles including upper arms, thighs, shoulders, hips, neck and back, but rarely affects the extremities including hands and fingers; also difficulty breathing and/or trouble swallowing can occur and progress rapidly. Increased general feelings of tiredness and fatigue may occur, and there can be new-onset falling, difficulty getting up from a fall, and trouble climbing stairs, standing up from a seated position, and/or reaching up. – If poly/myositis is suspected, a Neurology consultation should be obtained early, with prompt guidance on diagnostic procedures. Myocarditis may co-occur with poly/myositis; refer to guidance under Myocarditis. Given breathing complications, refer to guidance under Pneumonitis/ILD. Given possibility of an existent (but previously unknown) autoimmune disorder, consider Rheumatology consultation. – Consider, as necessary, discussing with the study physician. – Initial work-up should include clinical evaluation, creatine kinase, aldolase, LDH, BUN/creatinine, erythrocyte sedimentation rate or C-reactive protein level, urine myoglobin, and additional laboratory work-up as indicated, including a number of possible rheumatological/antibody tests (i.e., consider whether a rheumatologist consultation is indicated and could guide need for rheumatoid factor, antinuclear antibody, anti-smooth muscle, antisynthetase [such as anti-Jo-1], and/or signal-recognition particle antibodies). Confirmatory testing may include electromyography, nerve conduction studies, MRI of the muscles, and/or a muscle biopsy. Consider Barium swallow for evaluation of dysphagia or dysphonia. Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease

			progression, other medications, or infections).
	Grade 1 (mild pain)	No dose modifications	**For Grade 1:** – Monitor and closely follow up in 2 to 4 days for clinical symptoms and initiate evaluation as clinically indicated – Consider Neurology consult – Consider, as necessary, discussing with the study physician
	Grade 2 (moderate pain associated with weakness; pain limiting instrumental activities of daily living [ADLs])	Hold study drug/study regimen dose until resolution to Grade ≤1. – Permanently discontinue study drug/study regimen if it does not resolve to Grade ≤1 within 30 days or if there are signs of respiratory insufficiency	**For Grade 2:** – Monitor symptoms daily and consider hospitalization. – Obtain Neurology consult, and initiate evaluation. – Consider, as necessary, discussing with the study physician. – If clinical course is rapidly progressive (particularly if difficulty breathing and/or trouble swallowing), promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant – If clinical course is *not* rapidly progressive, start systemic steroids (e.g., prednisone 1 to 2 mg/kg/day PO or IV equivalent); if no improvement within 3 to 5 days, continue additional work up and start treatment with IV methylprednisolone 2 to 4 mg/kg/day – If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 3 to 5 days, consider start of immunosuppressive therapy such as TNF inhibitors (e.g., infliximab at 5 mg/kg every 2 weeks). Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, or anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation])

	Grade 3 or 4 (pain associated with severe weakness; limiting self-care ADLs)	**For Grade 3:** Hold study drug/study regimen dose until resolution to Grade ≤1. Permanently discontinue study drug/study regimen if Grade 3 imAE does not resolve to Grade ≤1 within 30 days or if there are signs of respiratory insufficiency. **For Grade 4:** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4 (severe or life-threatening events):** – Monitor symptoms closely; recommend hospitalization. – Obtain Neurology consult, and complete full evaluation. – Consider, as necessary, discussing with the study physician. – Promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant. – If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 3 to 5 days, consider start of immunosuppressive therapy such as TNF inhibitors (e.g., infliximab at 5 mg/kg every 2 weeks). Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. – Consider whether patient may require IV IG, plasmapheresis. – Once the patient is improving, gradually taper steroids over ≥28 days and consider prophylactic antibiotics, antifungals, or anti-PJP treatment (refer to current NCCN guidelines for treatment of cancer-related infections [Category 2B recommendation])[1]

Infusion-Related Reactions		
Severity Grade	**Dose Modifications**	**Toxicity Management**
Any Grade		– Management per institutional standard at the discretion of investigator – Monitor patients for signs and symptoms of infusion-related reactions (e.g., fever and/or shaking chills, flushing and/or itching, alterations in heart rate and blood pressure, dyspnea or chest discomfort, skin rashes etc.) and anaphylaxis (e.g., generalized urticaria, angioedema, wheezing, hypotension, tachycardia, etc.)
Grade 1	The infusion rate of study drug/study regimen may be decreased by 50% or temporarily interrupted until resolution of the event	For Grade 1 or Grade 2: – Acetaminophen and/or antihistamines may be administered per institutional standard at the discretion of the investigator – Consider premedication per institutional standard prior to subsequent doses
Grade 2	The infusion rate of study drug/study regimen may be decreased 50% or temporarily interrupted until resolution of the event Subsequent infusions may be given at 50% of the initial infusion rate	
Grade 3/4	Permanently discontinue study drug/study regimen	For Grade 3 or 4: Manage severe infusion-related reactions per institutional standards (e.g., IM epinephrine, followed by IV diphenhydramine and ranitidine, and IV glucocorticoid)

Non-Immune-Mediated Reactions

(Note: As applicable, for early phase studies, the following sentence may be added: "Any event greater than or equal to Grade 2, please discuss with Study Physician"

CTC Grade/Severity	**Dose Modification**	**Toxicity Management**
Any Grade	Note: dose modifications are not required for adverse events not deemed to be related to study treatment (i.e. events due to underlying disease) or for laboratory abnormalities not deemed to be clinically significant.	Treat accordingly as per institutional standard
1	No dose adjustment	Treat accordingly as per institutional standard
2	Hold study drug/study regimen until resolution to ≤ Grade 1 or baseline	Treat accordingly as per institutional standard
3	Hold study drug/study regimen until resolution to ≤ Grade 1 or baseline For AEs that downgrade to ≤ Grade 2 within 7 days or resolve to ≤ Grade 1 or baseline within 14 days, resume study drug/study regimen administration at next scheduled dose. Otherwise, discontinue study drug/study regimen	Treat accordingly as per institutional standard

Non-Immune-Mediated Reactions **(Note: As applicable, for early phase studies, the following sentence may be added: "Any event greater than or equal to Grade 2, please discuss with Study Physician"**		
CTC Grade/Severity	**Dose Modification**	**Toxicity Management**
4	Discontinue Study drug/study regimen (Note for Grade 4 labs, decision to discontinue would be based on accompanying clinical signs/symptoms and as per Investigator's clinical judgment and in consultation with the sponsor)	Treat accordingly as per institutional standard

Abbreviations:

AChE = acetylcholine esterase; ADA = American Dietetic Association; ADL = Activities of daily living ; AE = adverse event; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CT = computed tomography; GI = gastrointestinal; IDS=Infectious Disease Service; ILD = interstitial lung disease; IM = intramuscular; imAE = immune-mediated adverse event; IV = intravenous; NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events; NCCN = National Comprehensive Cancer Network ; PJP = *Pneumocystis jirovecii* pneumonia (formerly known as *Pneumocystis*pneumocystis *carinii* pneumonia); PO = by mouth; TNF = tumor necrosis factor; TSH = thyroid stimulating hormone; ULN = upper limit of normal.

1 ASCO Educational Book 2015 "Managing Immune Checkpoint Blocking Antibody Side Effects" by Michael Postow MD

2 NCI CTCAE version 4.03

3 ASCO Educational Book 2015 "Managing Immune Checkpoint Blocking Antibody Side Effects" by Michael Postow MD

4 FDA Liver Guidance Document 2009 Guidance for Industry: Drug Induced Liver Injury – Premarketing Clinical Evaluation

The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for Adverse Event (AE) reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

The 8th Edition of the TNM Classification of Malignant Tumours has recently been released. To facilitate this process, educational resources have been made available to promote the use of staging (visit http://www.cancerstaging.org). These staging criteria should be used for new trials.

A. Exploration of the tumor microenvironment and characterization of tumor genotype

1 BACKGROUND

Up until now, there are no biomarkers which are routinely used for the selection of patients or as early surrogate markers of clinical responses.

PD-L1 expression has been correlated with clinical response to anti-PD-1 in adenocarcinoma (1), but not in squamous cell carcinoma of the lung (2). Surprinsgly, patient with lung cancer not expressing PD-L1 could respond to the blockade of the PD-1-PD-L1 pathway. Other studies showed that pre-existing infiltrating $CD8^+$T cells before therapy and their *in situ* amplification also correlated with the efficacy of anti-PD-1/PD-L1 (3). However, in other trials it was rather the activation status of intratumoral $CD8^+$T cells and not only their simple presence which characterized responsive tumors to immunotherapy (4). In line with these results, various groups showed that parameters (IFNg, PD-1, Perforin, granzyme..) reflecting the presence of activated T cells secondary to the recognition of tumors provide complementary clues to identify potential clinical responders (5, 6).

Recent work also reported that the phenotype of tumors has to be taken into account for the classification of tumors. Indeed, the most responsive tumors to anti-PD-1/PD-L1 – i.e. melanoma and lung cancer - where those with the highest frequency of tumor mutations (7). These tumor mutations generate neoepitopes not recognized as self antigen and result in the amplification of anti-tumor $CD8^+$T cells. A linear relationship was shown between the mutational load of the tumors and the levels of infiltrating $CD8^+$T cells (8) (9). Nevertheless, some exceptions exist such as renal cell carcinomas, which are not considered as tumor with a high mutation rate and yet respond to anti-PD-1 (10). In preclinical models, activation of the WNT/b-catenin signaling pathway resulted in the inhibition of T cell infiltration (11). These data emphasizes the link between the phenotype of tumors and the shaping of tumor microenvironment. A composite biomarker integrating the phenotype of the tumor (mutational load) and an immune signature from the tumor microenvironment may be considered as the most relevant to identify robust biomarkers.

This ancillary study with thus focus on the analysis of the tumor microenvironement using new automated multiparametric immunofluorescence technique combined to the analysis of the genotype of the tumors by transcriptomic analysis and NGS technology.

2 PROJECT AIMS

2.1 Exploring the tumor microenvironment

2.1.1 Basic analysis of PD-L1 expression by tumor cells and its interaction on $CD8^+$T cells

From the literature, the routine biomarker which could be detected on paraffine section on monoparametric immunohistochemistry could be PD-L1, CD8 and PD-1. The simultaneous presence of PD-L1 on tumor cells with the interpretation issue (see below) and the tumor infiltration by $PD\text{-}1^+CD8^+$T cells may represent a good composite biomarker to predict clinical response to anti-PDL1 (3)

2.1.2 Revisiting the predictive role of PD-L1 and PD-L2

Usually PD-L1 expression is performed by Immunohistochemistry using various antibodies with various criteria of positivity in term of cut-off (1% to 50%) or the location of the staining (tumor cells, stroma…)

(12). Since PD-1 expressed by T cells could receive a negative signal by PD-L1 likely expressed on various cells from the tumor microenvironment, we will detect and characterize the cells expressing PD-L1 and PD-L2. We will use the Immunofluorescence technique to simultaneously analyze the PD-L1 expression on various cells including tumor cells, macrophages, dendritic cells, endothelial cell, T cells. PD-L1 staining has already been published by E Tartour's group (13). We have also developed an original staining for PD-L2 (unpublished results).

2.1.3 Characterization of effector T cells in the tumor microenvironment before anti-PD-L1 therapy.

Pre-existing anti-tumor $CD8^+$T cells appear to be a prerequisite for the clinical success of PD-1-PD-L1 blockade. The simple presence of $CD8^+$T cells may be insufficient to be sure that these cells are really directed against the tumor. The location of these cells at the invasive margin and inside tumours will also be determined. Various additional markers could be measured to better endow these cells with anti-tumor properties. Indeed, we could *in situ* address their activation and proliferation status (HLA-DR, CD38, Ki67). Recently it has been shown that resident memory T cells may represent the specific T cells which persist in the tumor inflammation site (14). The levels of these intratumor T cells could represent a good surrogate marker of pre-existing anti-tumor T cells (15). These cells are characterized by the expression of CD103 and CD49a and could be detected by double or triple immunofluorescence studies as already published by our group.

2.1.4 Profile of immune checkpoint on T cells to assess the levels of exhaustion of T cells

In various models the more the T cells express checkpoint inhibitors, the more they are exhausted and difficult to reactivate (16). From these observations, we think that it is relevant to characterize the profile of inhibitory costimulatory molecules expressed by T cells to evaluate possible resistance mechanisms to anti-PD-1/PD-L1 therapy. PD-1, Tim-3, Lag-3, CTLA-4, Icos stainings have been developed in E Tartour's lab (Ms submitted). An interesting study also showed that high co-expression of Eomes and PD-1 also characterized the more exhausted T cells (17).

2.1.5 Characterization of the proliferative immune cells after anti-PD-L1 therapy.

Many cells in the tumor microenvironment could express PD-1 such as $CD4^+$T cells, NK cell, B cells, macrophages, innate lymphoid cells. Most studies have focused on the role of $CD8^+$T cells as the major effectors during PD-1-PD-L1 blockade. Thanks to this neoadjuvant protocol, we could assess if the anti-PD-L1 mAb leads to an activation or amplification of other cells in the tumor microenvironment by comparing their levels and their activating status before and after therapy. The location of these cells inside and outside of the core of the tumor will also be compared. This unique study may identity new targets of anti-PD-L1 with some potential value as predictive biomarker of response. This study could also decipher early surrogate markers.

2.1.5 Assessing other immunosuppressive mechanisms in the tumor microenvironment and in the periphery.

Inhibitory receptors such as PD-1 represent one mechanism likely explaining the anergy of T cells in the tumor. However immunosuppression in the tumor microenvironment could also be related to suppressive cells such as regulatory T cell or myeloid derived suppressive cells (MDSC), as well as molecules inhibiting immune effector cells (IDO, IL-10, TGFb). In some cases, the levels of inflammatory molecules such as IL-6

correlate with the immunosuppressive state (18). We have previously shown that in other immunotherapies with IL-2, inflammatory status correlate with resistance to this therapy (19). We thus propose to monitor regulatory T cells ($CD4^+Foxp3^+CD127^-$) and Myeloid derived suppressive cells both granulocytic and monocytic in the tumor microenvironment and in the blood. Indeed, the level of expression of immune checkpoint on T cells is very high in the tumor microenvironment and very low in the blood for a same patient, precluding the use of blood for immune checkpoint profile (20) (13). In contrast, high levels of regulatory T cells and MDSC could be detected in the blood of lung cancer patients (21) (22) (23) (24) .

Profile of inflammatory and immunosuppressive cytokines by Luminex assay will also be performed.

High levels of immunosuppression could explained some resistance to PD1/PD-L1 blockade and the ratio between effector T cells and an immunosuppression score to be determined may represent a clue to better understand resistance mechanism.

2.2 Characterization of tumor genotype

Various studies reported that the presence of T cells in cancer lesions correlates with high mutational burden (8)(9). A strong correlation between mutational load and clinical response was shown in patients with non-small cell lung cancer and colon cancer with micro-satellite instability treated with anti-PD1 antibody (25).

Whole-exome sequencing (100x coverage) will be performed on tumor samples and their corresponding germline DNA obtained from autologous PBMC of included patients. Somatic mutations will be identified by comparing the nucleic acid sequences between tumors and PBMC using well-established bionformatic pipelines. The presence of mRNA encoding this mutation will be confirmed by RNAseq analysis. The presence of neoantigens will then be compared between responders and non-responders to sort out a signature associated with response to the treatment.

After exome sequencing and identification of the most represented neoantigens among anti-PD1-treated patients who derived a long-term or a minimal clinical benefit, we will select mutated CD8 T-cell peptides using HLA Peptide Binding predictive programs and test their ability to bind to specific HLA molecules.

3 METHODS

3.1 PD-L1 expression measurement in tumor tissue

At least SP263 antibody will be used for PD-L1 expression measurement in paraffin tumor tissue.

3.2 In situ multiparametric analysis of immune infiltrates

We have developed since several years a fluorescent multiparametric platform to allow multiparametric *in situ* analysis of tissue cell infiltration. We have acquired a fluorescent spectral scanner (Perkins*) coupled to a software with the ability to automatically identify various cellular profile characterized by their size, shape, morphology. These cellular results combined with the capacity to integrate and process multiple fluorescent signals represent a unique opportunity to accurately characterize the phenotype of immune cells in the tumor microenvironment. The use of immunofluorescence is especially adapted to address the clinical significance of the co-expression of various biomarkers at the cellular levels and to explore the interaction between the different immune cells present in the TME.

3.3 Measurement of immunosuppressive cells in the blood before and after therapy

After Ficoll (Sigma-Aldrich) separation, human Peripheral Blood Mononuclear Cells (PBMC) will be stained with combinations of the following monoclonal antibodies and corresponding isotype controls (eBioscience, USA): APC- anti-human CD4, FITC-anti-human CD25. Intra-cytoplasmic Foxp3 staining will be performed using the PE-anti-human Foxp3 staining kit according to the manufacturer's instructions. Regulatory T cells will be identified as $CD3^{+}CD4^{+}CD25^{hi}$ $Foxp3^{+}$ T cells. $CD25^{hi}$ gate will be adjusted to contain $CD4^{+}$ T cells that express CD25 more brightly than $CD4^{-}CD25^{+}$ cells as previously described (26). This technique is already standardized in the immunological lab of HEGP (27) .

MDSC analysis will be performed on whole blood after Ficoll-Paque density gradients to eliminate neutrophils. The following labeled anti-human monoclonal antibodies were used for staining : anti-lineage (LIN) FITC including anti-CD3, -CD19, -CD56, -CD14 (Becton Dickinson, Pont de Claix, France), PE labeled anti-CD33 (Biolegend-Ozyme, Saint-Quentin Yvelines, France), APC labeled anti-HLA-DR (Beckman Coulter, Villepinte, France). The % of granulocytic MDSC will be defined as the percent of

$CD33^{+}$Linneg (including CD14neg cells) HLA-DRneg in total PBMC as proposed by Fricke (28). For the measurement of $CD14^{+}$HLA-DRlow/- also called monocytic MDSC (29), whole blood were stained with the following monoclonal antibodies: FITC labeled Anti-CD14 (BDBioscience), APC labeled anti-HLA-DR (Beckman Coulter). Measurement of MDSC has already been reported by our team (30).

3.4 Mutational analysis and neoepitopes identification

The laboratory of Biochemistry of HEGP has acquired an expertise to whole exome sequencing and RNAseq, with more than 100 patients analyzed per year. From DNA extracted of fresh frozen or paraffin embedded tissues, whole exome sequencing will be performed accordingly to the following process.

Generated exome and transcriptome libraries are pooled in order to be sequenced either, on an Illumina NextSeq500 system, or an Illumina HiSeq4000 system. Runs are performed using 75 bases paired-end reads (PE reads). Libraries pooling is made such as the minimum amount of data per library type is the following: (i) Tumor DNA exome library, 80 million PE reads, 12 Gb, in order to achieve 115X minimum of depth. (ii) Constitutional DNA exome library, 50 million PE reads, 7.5 Gb in order to achieve 75X minimum of depth (iii) RNASeq, 66 million PE reads, 10 Gb.

With these specification mean performances obtained on clinical research samples in 2015 are the following: Tumor DNA exome : 135X; Constitutional DNA exome : 88X; RNASeq : 11 Gb.

After standard bioinformatics analysis, an in-house algorithm compares normal and tumor genotypes from exome sequencing data to determine the somatic nature of the variation. A somatic score is calculated for each variant ranging from 1 to 30, a score of 30 translating the highest confidence index. We will then examined the landscape of neoantigens using previously described methods (30) Briefly, this approach identifies mutant nonamers with ≤500 nM binding affinity for patient-specific class I human lymphocyte antigen (HLA) alleles, which are considered candidate neoantigens. The quantity of neoantigens per tumor will be correlated with mutation burden and with response.

4 RESSOURCES REQUIRED FOR THIS ANCILLARY STUDY

- Paraffin tissue section before and after anti-PD-L1 therapy

- Frozen tissue before (optional) and after anti-PD-L1 therapy
- Whole Blood : 42 ml before and after anti-PD-L1 therapy

5 ORIGINALITY OF THIS STUDY

- It integrates a comprehensive analysis of tumor microenvironment by a new automated multiparametric technology.

- It will measure immune biomarkers selected on their role in the mechanisms of action of anti-PD-L1 mAb

- It combines analysis of the tumor microenvironment with the phenotype of tumor cells, especially neoepitopes characterization.

- It could provide new innovative composite biomarker to predict clinical response to anti-PD-L1.

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B. Immune reactivity of tumors in response to immunotherapy

1 BACKGROUND

Immunotherapy leads to significant responses in patients with advanced lung tumors but its benefit is observed in a small fraction of patients (1,2). In this context, predictors of response/resistance represent unmet clinical needs and new immunomodulators, targeting other immune pathways, are developed in order to circumvent the resistance mechanisms of first-in-class immunomodulators. It is likely that the use of combination strategies will increase the number of cancer patients that might benefit from immunotherapy.

Mass cytometry allows a simultaneous single cell analysis of 40 parameters, giving the opportunity to deeply characterize the tumor microenvironment establishing an immune atlas of human cancer. It helps to clarify immune subsets that could functionally be explored by RNA sequencing analysis. This type of analysis was recently conducted in stage I lung adenocarcinoma lesions compared to health paired tissue and revealed significantly altered myeloids cell and lymphoid compartments. Changes in antigen cross-presenting cell subsets and immunosuppressive macrophages were observed compared to healthy tissues, that likely compromise anti-tumor T cell immunity during the priming (3) and probably prevent immune check-point inhibitor activity. Mass cytometry analyses can be performed on FFPE and fresh tumor tissue.

In parallel, in order to guide immunomodulator utilization in a personalized manner, we developed an “In Sitro” assay to perform immunological investigations on the tumor itself (Fig.1). This assay consists in stimulating freshly dissociated tumors with immunomodulators in vitro. Using flow cytometry and ELISA, it allows to delineate at the individual level, for each agent used, the specific pattern of immune parameters of the tumor microenvironment that might dictate the functional outcome (4).

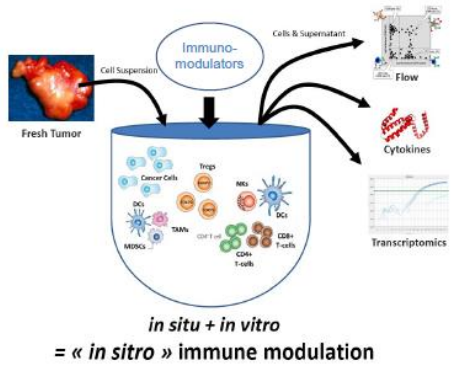

Figure 1: The In Sitro Assay. This method is based on mechanical and enzymatic dissociation of fresh tumors that are stimulated with immunomodulators in vitro. Then cells and supernatants are harvested at various time points to analyze the dynamic immunological and functional changes operating with the assessed immunomodulators.

2 PROJECT AIMS

The research aims are: (i) Define the atlas of immune cells infiltrating lung cancer using mass cytometry. Here, we are going to use tumorectomie pieces (lung and nodes, if present) post-anti-PD-1 treatment. We will compare paired tissue (healthy and tumor) in order to highlight tissue-specific changes. Thanks to multiparametric immuno-histochemistry, we will analyse the geodistribution of the main populations of interest before and after treatment using FFPE slices (or paraffin embedded tissue). The difficulty lies on the acquisition of new technological and scientific assests using mass cytometry. We went over this point by harnessing a preexisting collaboration with experts in the field (Merad’s lab, Mount Sinai).

(ii) Identify mass cytometry-based hallmarks of response and resistance to neoadjuvant PD-1 blockade in lung carcinoma.
An unsupervised hierarchical clustering of immune populations according to patients’ response (pathological response) will be performed attempting to dissect hallmarks and identified immune(s) population(s) related to treatment response. This work will be performed in collaboration with clinicians and bioinformaticians in our lab. The difficulties are in bioinformatics analyses. In order to optimize this key step, we benefit from Velerio IEBBA’s help (PhD and bioinformatician) in our lab and ensure the well processing of the datas with frequent meeting and discussion about the project and immunology.

(iii) Comprehensive understanding of at least one main mechanism of resistance to PD-1 blockade.
Depending on the most striking and exciting results, we will proceed by cell sorting the subset of cells of interest, and perform single-cell transcriptomic analyses to identify new targets of resistance.

(iv) In sitro functional assays to circumvent resistance to PD-1 blockade in lung carcinoma.

The description of the composition and activity of the immune infiltrate in lung cancer post-PD-1 blockade might aid in identifying new immune targets and new combination of treatments. The idea is to revert resistance to anti-PD1 monoclonal antibody on freshly operated tumors using a unique or a combination of reagents targeting other molecular pathways ex vivo, in the in sitro approach described above (4). Based on preexisting collaborations with several pharmaceutical partners, we have access to a variety of innovative immuno-oncological compounds that could be tested on surgically removed anti-PD1-resistant bladder tumors after mechanical and enzymatic dissociation. These compounds belong to several categories of products (immune checkpoint inhibitors such as Lag3, Tim3, 4ABB, OX40, TIGIT, activating receptors such as ICOS, enzymatic checkpoints such as CD73, ADA, IDO, and epigenetic modifiers to ameliorate T cell functions such as Lysine-specific demethylase...). If our library of compounds harbors a molecule which fits with our novel mechanism of resistance, we will test it by the in sitro approach (early and late functional assays). The main milestone is to not find/possess a good candidate for compensating PD-1 resistance. But we would describe the cancer immune atlas of lung cancer in responding and non-responding patients which might permit the discovery of new drug targets, help in understanding the dynamic of immune response during PD-1 treatment and providing a robust basis for pharmaceutical and diagnosis improvement.

3 METHODS

In order to define the immunological atlas of lung cancer exposed to the anti-PD1 monoclonal antibody, the post-treatment surgical specimens (tumor tissue, tissues healthy as well as invaded and normal ganglia if available) will be analyse by time-of-flight cytometry (CytoF) (Helios, FLUIDIGM). This will allow to define the immune changes related to lung tissue (healthy vs tumor) and changes in tumor progression (primary tumor vs. lymph node). After the establishment of a detailed map of intratumoral immune populations, a study on the dynamics of the immune populations undergoing treatment will be carried out. This second study from slides or paraffin blocks will be performed by immunohistochemical analysis in multiplex.

4 RESSOURCES REQUIRED FOR THIS ANCILLARY STUDY

Only samples from patients that accepted to participate to this optional ancillary study will be collected for further analyses.

Of note, only samples that are useless for patient diagnosis will be collected for this ancillary study.

Four FFPE tissue slices from pretreatment biopsy

Fresh tumor, healthy tissue and if available metastatic lymph nodes from resected patients treated with neoadjuvant immunomodulators. To get enough CD45+ cells, ideally a surgically resected piece of $1cm^3$ is needed.

5 ORIGINALITY OF THIS STUDY

The study of the immune microenvironmental environment is necessary for the discovery of relevant biomarkers and the understanding of the mechanism of action of the anti-PD-L1 antibody.

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C. Analysis of the gut microbiome in the context of anticancer immunotherapy

1 BACKGROUND AND PROJECT AIMS

Prospective collection of stool samples from patients enrolled in the Ionesco study before the instauration of immunotehrapy treatment to perform metagenomic analyses in order to (i) compare fecal dysbiosis in NSCLC patients at the diagnosis with matched healthy volunteers from the MetaHIT study results, (ii) identify metagenomics signatures that predict treatment response/resistance and immune related toxicities and (iii) correlate gut microbiome composition with data obtained in the Immune atlas part.

Anti-PD1/anti-PD-L1 mAbs represents a major paradigm shift in cancer therapy. However, despite the unprecedented efficacy of these innovative and treatments determination of novel biomarker is of paramount interest.

Currently, only few parameters have been identified as predictive biomarker of response, such as positive expression of PD-L1, and high mutational loads of tumors that appears to be associated with a higher response rate to anti-PD1 mAb. New lines of evidence demonstrated the biological significance of the gut microbiome (diverse range of symbiotic microorganisms) has an important contributor of pulmonary pathologies such as asthma and COPD (1). The gut-lung axis interaction plays a key role in immune set tone and ultimately could be an important factor in lung cancer carcinogenesis and further studies are warranted.

Beyond the direct interactions between host immune system and microbiome, an additional layer of complexity exists associated to cancer therapies.

Our group and co-investigators have paved the way by demonstrating the pivotal role of microbiome on cyclophosphamide (CTX) and platinum based chemotherapy (2,3). We have extended these findings to immune checkpoint blockade (ICB) by the anti-CTLA4 Ab (Ipilimumab) and identified Bacteroides spp. responsible for IL-12 production increasing systemic Th1, which improves anti-CTLA response in mice (4). Recently, three independent groups, including ours, highlighted that gut microbiome composition at baseline predicts response to PD1/PDL1 blockade in RCC, NSCLC and melanoma (5–7). Furthermore, the cause effect-relationship between gut commensals and treatment response was demonstrated using, avatar murine model. Oral gavage of dysbiotic mice with immunogenic bacteria enriched in responder patients restored anti-PD1 anti-cancer activity.

In this study we plan to collect samples at baseline.

3 METHODS

The sample used will be the stool sample at the diagnosis (or, failing that, the one made before the 3rd injection of durvalumab). An analysis of its composition will be performed by metagenomics to establish the composition of the intestinal microbiota of the patient before treatment.

4 RESSOURCES REQUIRED FOR THIS ANCILLARY STUDY

The collection kit (provided by gustave roussy cancer campus) is given to the patient at the screening visit. Patients bring back their sample at the hospital the day of the first administration of immunotherapy. This corresponds to a single sample, collected at baseline, prior immunotherapy.

According to our previous data, if the baseline sample couldn't have been collected, it is possible to collect feces until before the 3rd administration of durvalumab, without observing significant variation of the gut microbiome.

Depending on the center location, two protocols of collection are available.

In ile de france fresh samples are transferred from the centre to grcc by a courier. Samples are aliquoted and stored at -80°c at grcc (procedure).

Outside of ile de france, patient aliquots themselves their samples in tubes containing a stabilization solution. Samples are stored on site at -80°c (procedure bis).

5 ORIGINALITY OF THIS STUDY

This project offers a unique opportunity to study the role of the gut microbiota in predicting the neoadjuvant response to durvalumab in lung cancer and to study the covariation of its composition with the intraluminal immune environment post-treatment.

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The durvalumab dosing should be done depending on subject weight (if subject is < 30kg)):

1. Cohort dose: X mg/kg

2. Subject weight: Y kg

3. Dose for subject: XY mg = X (mg/kg) × Y (kg)

4. Dose to be added into infusion bag:

Dose (mL) = XY mg / 50 (mg/mL)

where 50 mg/mL is durvalumab nominal concentration

The corresponding volume of durvalumab should be rounded to the nearest tenth mL (0.1 mL). Dose adjustments for each cycle only needed for greater than 10% change in weight.

5. The theoretical number of vials required for dose preparation is the next greatest whole number of vials from the following formula:

Number of vials = Dose (mL) / 10 (mL/vial)

Example:

1. Cohort dose: 10 mg/kg

2. Subject weight: 30 kg

3. Dose for subject: 300 mg = 10 (mg/kg) × 30 (kg)

4. Dose to be added into infusion bag:

Dose (mL) = 300 mg / 50 (mg/mL) = 6.0 mL

5. The theoretical number of vials required for dose preparation:

Number of vials = 6.0 (mL) / 10.0 (mL/vial) = 1 vials