Phase I/II Trial of Intratumoral Administration of Hu14.18-IL2, with Local Radiation, Nivolumab and Ipilimumab in Subjects with Advanced Melanoma

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ABBREVIATIONS USED IN THE TEXT

ACTH	adrenocorticotropin hormone
ADCC	antibody-dependent cellular cytotoxicity
alb	albumin
alk phos	alkaline phosphatase
ALT (SGPT)	alanine aminotransferase (serum glutamate pyruvate transaminase)
AST (SGOT)	aspartate aminotransferase (serum glutamic oxaloacetic transaminase)
bid	twice daily
BUN	blood urea nitrogen
CBC	complete blood count
CDC	complement-dependent cytotoxicity
Ca	calcium
CB	clinical benefit
COG	Children's Oncology Group
CDR	complimentarity-determining region
ch	chimeric
CR	complete response
CRP	C-reactive protein
CT	computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
CXR	chest X-ray
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
diff	white blood cell differential
DLT	dose limiting toxicity
DTH	delayed type hypersensitivity
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
Fc	fragment crystallizable
Glu	glucose
HAMA	human anti-mouse antibody
HBs Ag	hepatitis B surface antigen
Hgb	hemoglobin
HIV	human immunodeficiency virus
IC	immunocytokine
IgG	immunoglobulin G
IgM	immunoglobulin M
IL2	interleukin-2
IND	investigational new drug
INR	international normalization ratio
IV	intravenous
IT	intratumoral
K	potassium

LDH	lactate dehydrogenase
LFT	liver function tests
MAA	Melanoma associated antigen
MAD	maximum administered dose
mAb	monoclonal antibody
Mg	magnesium
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
Na	sodium
NCI	National Cancer Institute
NK	natural killer cells
OS	overall survival
P	pulse
PBL	peripheral blood lymphocyte
PD	progressive disease
PFS	progression free survival
PO	oral
PA	posterior-anterior
PHI	protected health information
PK	Pharmacokinetic
plts	platelets
PR	partial response
PRN	pro re nata (as needed)
PTT	partial thromboplastin time
qd	once daily
RR	respiration rate
SD	stable disease
SIADH	syndrome of inappropriate antidiuretic hormone
SWFI	sterile water for injection
T	temperature
TAA	tumor-associated antigen
T. bili	total bilirubin
tid	three times daily
TIL	tumor infiltrating lymphocytes
TCR	T cell receptor
TSH	Thyroid-stimulating hormone
ULN	upper limit of normal
WBC	white blood cell count
UWCCC	University of Wisconsin Carbone Cancer Center

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STUDY OVERVIEW

Hu14.18-IL2 (APN 301) is a recombinant fusion protein linking the monoclonal antibody (mAb) hu14.18 with interleukin-2 (IL2). The antibody portion specifically binds to the disialoganglioside GD2 antigen, which is strongly expressed in tumors of neuroectodermal origin including melanoma, neuroblastoma, and certain sarcomas[1, 2] Hu14.18 has proven clinical activity when combined with IL2 and GM-CSF[3]. This immunocytokine (IC) is designed to localize to GD2-positive tumor cells and stimulate targeted activation of NK and T cells, leading to tumor cell death via antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity, and tumor-specific T cell response. In phase I and II studies in children with relapsed/refractory neuroblastoma, this IC was found to be safe and induced immune activation with reversible toxicities when administered intravenously[4, 5]. In Phase I/II trials in adults with advanced malignant melanoma, this IC demonstrated biologic activity and was well tolerated when administered intravenously[6-8.]

We recently reported *in vivo* preclinical data in murine melanoma models showing greater efficacy, and involvement of T-cell responses, when this IC is given by intratumoral (IT) injection[9]. In these preclinical models, we have recently shown safety and marked efficacy for a combined modality treatment approach in which hu14.18-IL2 is delivered by IT injection to a site of disease treated with radiation therapy (RT). With this combination treatment, we demonstrated an *in situ vaccination effect*, capable of enhancing the response to systemic T cell checkpoint blockade with anti-CTLA-4 mAb[10]. Most mice become long-term tumor-free survivors and demonstrate tumor-specific protective T cell memory. This triple combination resulted in improvement in local and systemic response as well as improved overall animal survival when compared to dual or single agent combinations of RT, IT- IC and systemic anti-CTLA-4[10]. This triple combination was effective in mice with very large melanomas, and in mice with distant sites of macroscopic disease (where only one of the disease sites received RT and IT- immunocytokine[11]. Given the clinical activity of dual checkpoint blockade using Ipilimumab and Nivolumab, we are eager to test these agents in combination with local RT and IT hu14.18-IL2. This is a phase I/II study of IT delivery of hu14.18-IL2 in combination with local RT and systemic anti-CTLA-4 (ipilimumab) and anti-PD1 (nivolumab) in patients with advanced melanoma to evaluate safety, antitumor activity, and immunologic endpoints. The study is designed to replicate the striking antitumor responses this regimen achieved in our preclinical models.

SCHEMA

Each eligible subject will have at least 1, but preferrably 2, sites of disease that are amendable to safe repeated hu14.18-IL2 injections and two (2) to four (4) biospies (designated Lesions A (index lesion) an B). The index lesion will be injected daily with hu14.18-IL2 for 3 consecutive days in each cycle of treatment. Cycle length will consist of 21 days. All subjects will have their index lesions biopsied pretreatment and at cycle 1 day 5 (with a possible range of day 4-9 to facilitate subject scheduling). Two additional elective biopsies of these same lesions may also be performed on cycles 2 and 4 day 5 (again with a range of day 4-9).

The study will consist of 4 phases (Phase IA, IB, IC and ID):

Phase IA: (hu14.18-IL2 only)

- Hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of each 21 day cycle for 4 cycles if no clinically significant disease progression
- Using the 3+3 dose escalation/de-escalation strategy in Table 5.5, determine the MTD/MAD of IT-hu14.18-IL2; Dose levels (see Schema table):
    - I = 2 mg/m^2/day,
    - ~~II = 4 mg/m2/day,~~ Dose level II removed in protocol amendment 5
    - ~~III= 6 mg/m^2/day,~~ Dose level III removed in protocol amendment 5
    - In the event of excessive toxicity at initial dose level: –I = 1 mg/m^2/day
- As of protocol amendment 5, dose levels II and III have been discontinued. While subjects enrolled in level I did not experience dose limiting toxicities, the frequency and extent of adverse events experienced were greater than expected. Dose escalations were removed for safety purposes.
- Continue with maintenance treatment with hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of each 28 day cycle up for up to a maximum of 13 cycles if updated immune RECIST[12] antitumor response/tumor regression and any residual injectable tumor. Treatment may also be continued for up to a maximum of 13 cycles if stable disease at the discretion of the treating physician.
- Entire treatment will be given as outpatient treatment [Observation in clinical research unit (CRU) for up to ~6 hours post each injection].
- Off treatment for progressive disease or unacceptable toxicity.
- 9-18 subjects will be enrolled in 3 escalating dose levels to determine the Maximum Tolerated Dose (MTD)/Maximum Adminstered Dose (MAD) of hu14.18-IL2 in Phase IA.
- Subjects will continue to be monitored for at least 90 days after completion of the study treatment for late toxicities associated with IT-hu14.18-IL2.
- Off-treatment for progressive disease or unacceptable toxicity.

Phase IB: (RT and hu14.18-IL2 only)

- Palliative RT (dose and fractionation determined at physician discretion) given to any currently or imminently symptomatic tumor sites (to be completed between day -8 to -4, in cycle 1 only), followed by
- Hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of a single radiated tumor each 21 day cycle for 4 cycles if no clinically significant disease progression
    - Continue with maintenance treatment with hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of each 28 day cycle up to a maximum of 13 cycles if immune RECIST antitumor response/tumor regression and any residual injectable tumor. Treatment may also be continued for up to a maximum of 13 cycles if stable disease at the discretion of the treating physician.
- Using the 3+3 dose escalation/de-escalation strategy in Table 5.5, determine the MTD/MAD of IT-hu14.18-IL2 following palliative RT; Dose levels (see Schema table):
    - 1 dose level below the Phase IA determined MTD/MAD of IT-hu14-18-IL2
    - Phase IA determined MTD/MAD of IT-hu14.18-IL2

  - In the event of excessive toxicity at initial dose level: 2 dose levels below the Phase IA determined MTD/MAD of IT-hu14.18-IL2

- Subjects will continue to be monitored for at least 90 days after completion of study treatment for late toxicities associated with any component of therapy (RT, IT-hu14.18-IL2). Potential sub-acute and late local toxicity due to palliative radiation therapy will be monitored for 12 months following the radiation therapy.
- Off treatment for progressive disease or unacceptable toxicity

Phase IC: (RT + hu14.18-IL2 + nivolumab)

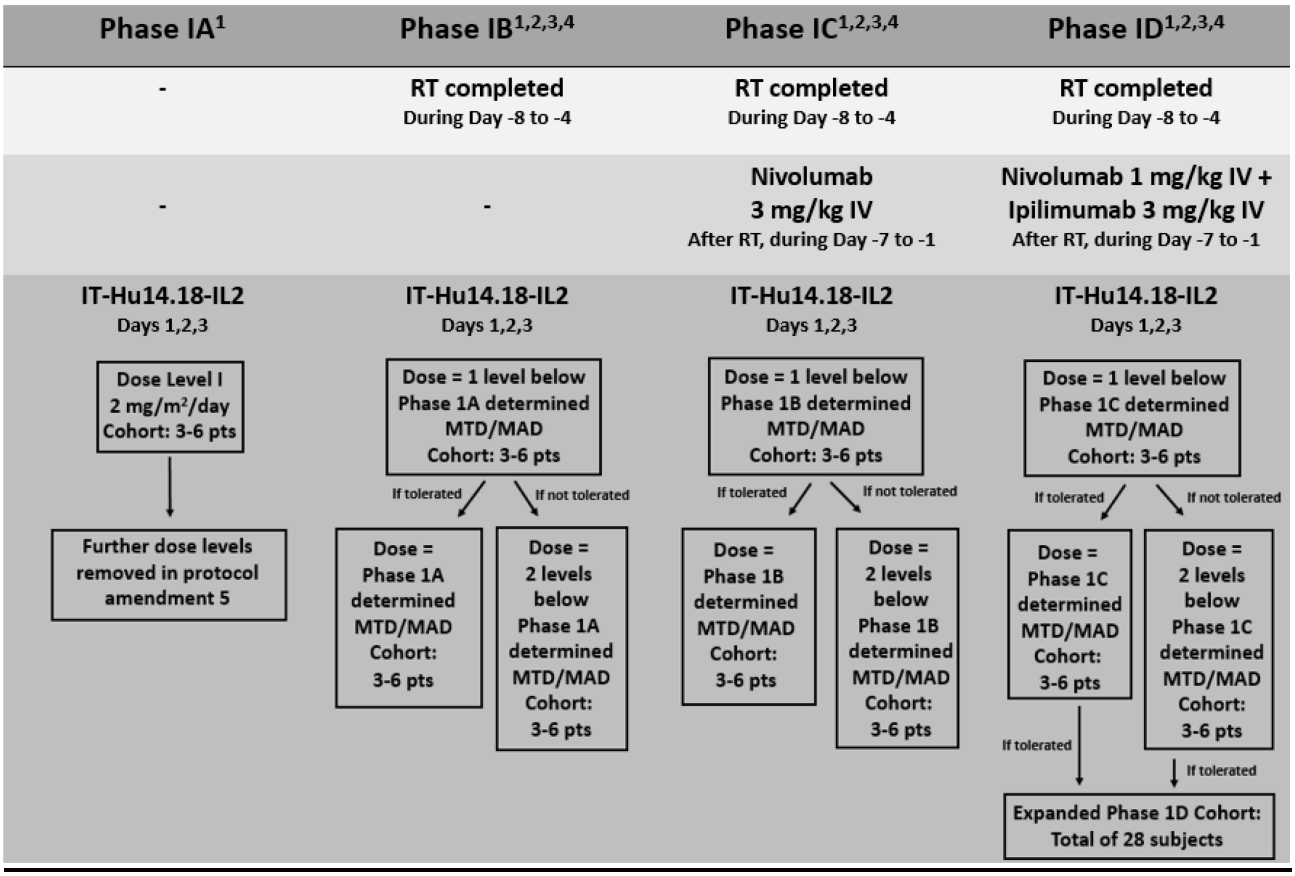
- Palliative RT (dose and fractionation determined at physician discretion) given to any currently or imminently symptomatic tumor sites (to be completed between day -8 to -4, in cycle 1 only), in combination with
- Nivolumab 3 mg/kg every 2 weeks (the initial dose will be between Day -7 to Day -1 of cycle 1), followed by
- Hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of a single radiated tumor each 21 day cycle for 4 cycles if no clinically significant disease progression.
- Using the dose escalation/de-escalation strategy in Table 5.5, determine the MTD/MAD of IT-hu14.18-IL2 following palliative RT in combination with nivolumab; Dose levels (see Schema table):
  - 1 dose level below the Phase IB-determined MTD/MAD of IT-hu14.18-IL2
  - Phase IB determined MTD/MAD for IT-hu14.18-IL2
  - In the event of excessive toxicity at initial dose level: 2 levels below the Phase IB determined MTD/MAD of IT-hu14.18-IL2
- Continue with maintenance treatment with nivolumab at a dose of 3 mg/kg every 2 weeks for up to one year if immune RECIST antitumor response/tumor regression. Treatment may also be continued up to a year if stable disease at the discretion of the treating physician.
- Continue with maintenance treatment with hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of each 28 day cycle up to a maximum of 13 cycles if updated immune RECIST antitumor response/tumor regression and any residual injectable tumor. Treatment may also be continued for up to a maximum of 13 cycles if stable disease at the discretion of the treating physician.
- Subjects will continue to be monitored for at least 90 days after completion of study treatment for late toxicities associated with any component of therapy (RT, IT-hu14.18-IL2, nivolumab). Potential sub-acute and late local toxicity due to palliative radiation therapy will be monitored for 12 months following the radiation therapy.
- Off treatment for progressive disease or unacceptable toxicity.

Phase ID: (RT + hu14.18-IL2 + nivolumab + ipilimumab)

- Palliative RT (dose and fractionation determined at physician discretion) given to any currently or imminently symptomatic tumor sites (to be completed between day -8 to -4, in cycle 1 only), ,in combination with
- Nivolumab 1 mg/kg once every 3 weeks in each 21 day cycle (during Day -7 to Day -1), for 4 cycles, in combination with

- Ipilimumab 3 mg/kg once every 3 weeks in each 21 day cycle (during Day -7 to Day -1), for 4 cycles, in combination with
- Hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of a single radiated tumor each 21 day cycle for 4 cycles if no clinically significant disease progression.
- Using the dose escalation/de-escalation strategy in Table 5.5, determine the MTD/MAD of IT-hu14.18-IL2 following palliative RT in combination with nivolumab and ipilimumab; Dose levels (see Schema table):
    - 1 dose level below the Phase IC determined MTD/MAD of IT-hu14.18-IL2.
    - Phase IC determined MTD/MAD for IT-hu14.18-IL2
    - In the event of excessive toxicity at initial dose level: 2 levels below the Phase IC determined MTD/MAD of IT-hu14.18-IL2
- Enroll a total of 28 subjects at the Phase ID determined MTD/MAD of IT-hu14.18-IL2 to evaluate safety, tolerability, and objective tumor responses of the combination of RT, nivolumab, ipilimumab and IT-hu14.18-IL2.
- Following 4 cycles, no additional ipilimumab will be administered
- Following cycle 4, continue with maintenace treatment with nivolumab at a dose of 3 mg/kg every 2 weeks for up to one year if immune RECIST antitumor response/tumor regression. Treatment may also be continued for up to a year if stable disease at the discretion of the treating physician.
- Continue with maintenance treatment with hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of each 28 day cycle up for up to a maximum of 13 cycles if updated immune RECIST antitumor response/tumor regression and any residual injectable tumor. Treatment may also be continued for up to a maxiumum of 13 cycles if stable disease at the discretion of the treating physician.
- Subjects will continue to be monitored for at least 90 days after completion of study treatment for late toxicities associated with any component of therapy (RT, IT-hu14.18-IL2, nivolumab, ipilimumab). Potential sub-acute and late local toxicity due to palliative radiation therapy will be monitored for 12 months following the radiation therapy.
- Off study for progressive disease or unacceptable toxicity
- Analysis of the 28 subjects treated with the Phase ID determined MTD/MAD of IT-hu14.18-IL2 based on:
    - Prior response to ipilimumab and/or nivolumab.
    - GD2 positivity
    - PD-L1 positivity

Schema Table



1. RT= Radiation Therapy. Nivo=nivolumab. Ipi=ipilimumab. MTD=maximum tolerated dose.
2. Palliative RT to non-injected sites or repeat RT to injected or non-injected sites will be allowed, except in phase IA.
3. In 1B, 1C, and 1D RT to the treatment site is scheduled only in cycle 1.
4. Nivolumab 1 mg/kg IV + ipilimumab 3mg/kg IV every 3 weeks for 4 cycles, followed by nivolumab 3mg/kg IV every 2 weeks for all subsequent treatment cycles starting with treatment cycle #5.

Assessments/Procedures:

Biopsies (2 time points required for 2-4 mm biopsies, 4 time points are preferred, biopsies required for all study phases):

- Pre-treatment: Up to 1 month prior to treatment (required).
- Repeat: During cycles 1, 2, and 4 of hu14.18-IL2, preferably on day 5 of the cycle (approximately 2 days after last injection of hu14.18-IL2; may be completed as early as day 4, or as late as day 9).
    - The cycle 1 biopsy may be omitted at the discretion of the PI, the protocol chair, or their designee if the biopsy is felt to be unsafe.
    - If judged not to be clinically feasible, the biopsies during cycles 2 and 4 may be omitted by the investigator.

Imaging:
- Computed tomography (CT) at baseline.
- Repeat: After 4 cycles (12 weeks from C1D1) and then every 12 weeks.

1.0 BACKGROUND

Immunotherapeutic strategies for melanoma have evolved over the past twenty-five years from systemic activation of the immune system via administration of high-dose IL2 to molecularly-targeted vaccines, adoptive immunotherapy, anti-tumor monoclonal antibodies and several antibodies that help activate the immune system via "checkpoint blockade"[13-18]. One of the potential benefits of immunotherapy is the ability to destroy not only grossly visible disease but also micrometastases, which have been a significant barrier to achieving long-term benefit from locally-driven modalities such as surgery and RT, and have been a challenge for cytotoxic chemotherapy as well. Often these sites of micrometastatic disease are able to withstand initial therapeutic efforts only to progress at a later date with significant morbidity and mortality. An ideal approach would be one that was well-tolerated, tumor-specific, and was able to manage both macro- and microscopic sites of disease.

The immunocytokine (IC) hu14.18-IL2 is a humanized monoclonal antibody (mAb) that is covalently linked to two molecules of IL2 at the Fc region[19, 20]. The hu14.18 mAb recognizes GD2, a cell membrane molecule found in tumors of neuroectodermal origin (including melanoma, neuroblastoma, and certain sarcomas) and a relative paucity of normal tissues (mostly cerebellum and peripheral nerves).[1, 2, 21-29] This molecule was developed as a way to elicit antigen-specific and innate immune responses while mitigating some of the toxicities associated with high-dose IL2. This IC has been studied extensively *in vitro* and in preclinical models of both melanoma and neuroblastoma[30-39] and has undergone both Phase I and II testing in adults with melanoma and children with neuroblastoma using intravenous (IV) delivery[4, 6, 8]. In mice, the antitumor effect of hu14.18-IL2 against melanoma and neuroblastoma can involve T cells and NK cells[31-37]. Far better antitumor effects are seen in mice with smaller amounts of tumor or with minimal residual disease (MRD)[40]. Hu14.18-IL2 given intravenously (IV) to adults with melanoma or children with neuroblastoma is generally well-tolerated and is able to produce demonstrable immune activation *ex vivo* and *in vivo*[4, 6]. For patients with measurable disease, IV hu14.18-IL2 has shown modest anti-tumor effects in Phase II trials: 0 responses of 15 patients with neuroblastoma and 1 transient PR and 0 CR for 14 patients with melanoma in our Phase II trials[5, 8]. In contrast, and consistent with our preclinical data, patients with evaluable but non-bulky disease (neuroblastoma patients with disease evaluated only by ^{123}MIBG scintigraphy or by bone marrow histology) showed reproducible antitumor activity: 5 of 24 evaluable patients with CR[5]. We have recently reported on a separate study of IV hu14.18-IL2, given as 3 courses of treatment to adults with advanced melanoma that achieved CR following surgical resection of all evaluable disease. Several of these treated patients have maintained their CR status without progressive disease[41], and demonstrate that the level of TIL infiltration into the tumors resected after a first course of IC treatment is associated with progression free survival[41] (and unpublished observation, Albertini et al). One potential explanation of this activity for microscopic disease but relative lack of efficacy against measurable disease is that an

inadequate amount of the IC is getting to these sites of disease to achieve a clinically significant effect when administered IV.
We recently tested this hypothesis by administering hu14.18-IL2 directly into established GD2-positive tumors in mice[42]. This IT-IC approach demonstrated significantly greater antitumor effects than IV administration of IC (IV-IC). A minority of mice treated with IV-IC cleared the tumor (n = 3 of 17), while a majority of mice treated with IT-IC showed clearing of tumor (n = 12 of 17; p = 0.002 comparing the two groups). Moreover, we observed a tumor-specific memory response after IT-IC: most mice that became tumor-free after IT-IC were able to specifically reject subsequent re-challenges with tumor cells. Better antitumor effects are seen at distant tumor sites when the IC is injected into the primary tumor, than when the IC is injected subcutaneously into non-involved skin at an equidistant site. The vaccine effect suggested by these experiments provides a proof-of-principle that this approach can control and potentially eradicate both macro- and microscopic disease[42].

Detailed flow cytometry and immunohistochemistry has been performed on murine neuroblastomas treated with IT-IC using the hu14.18-IL2. These studies show that IT-IC induces an augmented accumulation of NK cells and CD8+ T cells at the tumor site, and also induces greater activation of these cells, as detected by greater upregulation of NKG2D on NK cells and CD8+ T cells in the injected tumor site (but not in the spleen). These analyses also show that in the first several hours after the administration of the IT-IC, there is approximately 100-fold more IC bound to the tumor cells in vivo than that seen with comparable dosing of IC given IV[9, 43]. Furthermore, while IT-IC is more potent against macroscopic tumors than is IV-IC, the likelihood of an IT-IC induced antitumor effect on these measurable tumors correlates inversely with tumor size[43].

More recently, we identified a cooperative interaction between local tumor RT, IT injection of hu14.18-IL2 and checkpoint blockade with anti-CTLA-4 mAb[10, 11]. In the 1-tumor model, immunocompetent C57Bl/6 mice were implanted with $2x10^6$ B78 (GD2+) melanoma in one flank (the primary tumor). In the 2-tumor model, C57Bl/6 mice with a primary B78 tumor received $2x10^6$ B78 3 weeks later in the opposite flank. After 5 weeks, the primary (1^{st}) tumor was ~200mm^3, and after 7 weeks it was ~500mm^3. The 2^{nd} tumor was ~50mm^3 14 days after its implantation (corresponding to the 5-week time point for the 1^{st} tumor). At 5 weeks or 7 weeks, mice received single fraction RT (12Gy) to the 1^{st} tumor and 6 days later received 5-daily 50µg IT-IC.

For mice bearing a single 200mm^3 tumor, RT alone or IT-IC alone slowed tumor growth, but the tumors all continued to grow. In contrast, RT+ IT-IC resulted in complete response (CR) in 71% of mice and a tumor-specific memory T cell response. Mice with a single 500mm^3 tumor showed slowing of tumor growth, but only 27% CR after RT + IT-IC. Adding anti-CTLA-4 to RT + IT-IC improved tumor response (73% CR) and survival compared to doublet combinations of these 3 modalities[10]. In contrast, in the 2-tumor model, providing RT + IT-IC to the 1^{st} ~200mm^3 tumor, but not to the distant ~50mm^3 tumor, did not enhance 1^{st} tumor shrinkage compared to RT alone and had no effect on the 2^{nd} 50mm^3 tumor. The presence of the 2^{nd} B78 tumor resulted in systemic immune suppression that prevented the local RT and IT-IC from eliminating the 1^{st} tumor. This was tumor specific, as local RT + IT-IC to the 1^{st} ~200mm^3 B78 tumor was still effective in treating the 1^{st} tumor if the 2^{nd} (~50 mm^3 tumor) was the syngeneic but unrelated Panc02 (pancreatic) tumor. Delivering RT to both the 1^{st} + 2^{nd} B78 tumors eliminated the inhibitory effect of the 2^{nd} tumor, enabling IT-IC

to the 200mm^3 tumor to cause eradication of that tumor in 64% of mice[10, 11]. Immunohistochemistry analyses of tumors for FoxP3 showed Tregs are partially depleted by RT of the 1st tumor only in mice with 1 tumor and not in mice with 2 tumors. In this 2 tumor model we combined RT + IT-IC of the 1st tumor with anti-CTLA-4. The IgG2b anti-CTLA-4 (which doesn't substantially deplete Tregs) had minimal effect on 1st tumor response to RT + IT-IC. In contrast the IgG2a anti-CTLA-4 (that does deplete Tregs) rendered 60% of mice disease-free (durable CR of both the treated 200mm^3 and the untreated 50mm^3 tumors). Specific depletion of Tregs, using DEREG mice (which express diphtheria toxin receptors under control of the FoxP3 promoter, enabling diphtheria toxin to deplete Tregs), showed Treg depletion in the 2 tumor model also enabled eradication of both 1st and 2nd tumors in 60% of mice after RT + IT-IC to only the 1st tumor[44]. These studies confirmed the necessity of Tregs for the suppressive effect of the 2nd non-treated tumor on the response of the 1st tumor to RT + IT-IC.[44] These studies also demonstrate that local RT+ IT-IC can result in an "*in situ* vaccine", providing long-term tumor eradication of macroscopic tumors via adaptive immunity, provided that Treg-associated immune suppression from distant tumor is eliminated by RT or Treg-depletion. Data from the 1-tumor model have recently been published[10], and data from the 2-tumor model have been presented at national meetings, and have been submitted for publication[11].

The preclinical and clinical immunotherapy team at Stanford led by Dr. Ron Levy has been pursuing IT immunotherapy for some time. Their focus has been IT injection of TLR-activators (like CPG)[45, 46] and IT injection of checkpoint-blockade or immunomodulatory mAbs (like anti-CTLA-4 or anti-CD-137 mAb)[47-49]. They are also combining IT immunotherapy with local radiotherapy[46]. Their goal, like ours, is to enable the combination of local (and systemic) therapy to convert an established tumor into an in situ vaccine, able to activate a systemic anti-tumor adaptive immune response[48]. In sum, these data offer additional support to our own preclinical data for the IT immunotherapy/***in situ vaccine*** concept, using IT-IC (in combination with local RT and checkpoint blockade with anti-CTLA-4 mAb).

Recently, Weide *et al*[50] have performed a clinical trial of IT injections of a separate agent that links part of a mAb to IL2. They have given the L19-IL2 fusion protein to patients with melanoma by IT injection. L19-IL2 is a monovalent construct that links a single molecule of IL2 to a single chain fragment of the L19 mAb, which recognizes the alternatively spliced extra domain-B (EDB) of fibronectin that is found on neo-angiogenic blood vessels in tumors. Even though this construct is univalent, does not bind to the tumor cells directly, and does not mediate ADCC, this group found striking local antitumor effects at sites receiving IT injections. They conclude that this approach gave comparable results as their prior approach using IT IL2 itself, but at a more favorable (less frequent) dosing schedule[51, 52]. In other words, Weide *et al.* feel that the benefit of the IT injection of L19-IL2 reflects the ability to keep IL2 at the tumor site longer than with IT-IL2 (or IV-IL2). We have shown that IT-IC with hu14.18-IL2 can also keep IL2 at the tumor at higher levels and longer than IV-IC[9, 43]. Furthermore, our mouse models using IT-IC with hu14.18-IL2 have also shown involvement of both NK and T cells, and induction of memory T cell responses, and also involve ADCC. Thus we feel that several additional mechanisms are involved in generating the superior responses we see when comparing IT-IC (using hu14.18-IL2 for GD2+ tumors) to IV-IC (or to IT-IL2). This trial represents the first-in-human clinical testing of IT-IC using an intact mAb linked to IL2 that binds to tumor cells and mediates ADCC, as well as bringing IL2 to the tumor cell surface.

In addition, we believe it is also the first-in-human testing of this IT-IC approach combined with local, low dose (immunomodulatory) RT and with anti-CTLA-4 mAb.

Based on these promising preclinical and clinical data, we are interested in testing the hypothesis that IT hu14.18-IL2 (designated IT-IC in this protocol) is well tolerated and has antitumor effects in human subjects, especially when combined with local, low dose (immunomodulatory) RT, with nivolumab (anti-PD1 antibody), and with combined immune checkpoint blockade with ipilimumab and nivolumab. We plan to study combined immune checkpoint blockade using nivolumab and ipilimumab with this IT-IC regimen due to the demonstrated clinical activity of this regimen[18]. In this Phase I/II study, we intend to investigate the safety and tolerability of IT-IC in human subjects with advanced melanoma and clinically palpable disease. After determining a maximum tolerated dose (MTD)/maximum administered dose (MAD) for this IT-IC approach **when given on an outpatient basis**, we will then in separate cohorts, evaluate the safety and tolerance of administering hu14.18-IL2 following local RT, with systemic standard dosing of nivolumab (anti-PD1 antibody), and then with systemic standard dosing of nivolumab (anti-PD1 antibody) in combination with ipilimumab (anti-CTLA-4). We will then accrue additional subjects to this combined regimen to better study the potential antitumor effects and the *in vivo* immunobiology (both locally and at distant sites) of this novel strategy.

Our most recent study of patients with Stage III and IV melanoma evaluated tumors resected from patients for GD2 expression. Immunohistochemical analyses showed that 6 of 12 evaluable patients were positive for GD2 on their melanoma[41]. However, there is not a standardized assay for detection of GD2 expression in human tumors. In addition, our preclinical data demonstrate that the IL2 component of this IC could mediate antitumor activity following IT administration (without requiring GD2 recognition) (Morris et al, unpublished). Thus, we will not use GD2 expression as an eligibility criterion in this phase I/II trial. However, we plan to determine GD2 status for all treated patients. For all clinical parameters and lab correlates we will first evaluate all eligible/evaluable patients as a group, and then we will separately examine a subset analysis for the GD2+ patients separate from the GD2- patients.

2.0 OBJECTIVES

2.1 Primary Objectives

2.1.1 Phase IA

2.1.1.1 Determine the maximum tolerated dose (MTD) or maximum administered dose (MAD) of IT-hu14.18-IL2 in subjects with advanced melanoma.

2.1.1.2 Evaluate the safety and tolerability of IT-hu14.18-IL2 when given alone.

2.1.2 Phase IB

2.1.2.1 Determine the maximum tolerated dose (MTD) or maximum administered dose (MAD) of IT-hu14.18-IL2 after receiving palliative RT in subjects with advanced melanoma.

2.1.2.2 Evaluate the safety and tolerability of the combination of palliative RT with IT-hu14.18-IL2.

2.1.3 Phase IC

2.1.3.1 Determine the maximum tolerated dose (MTD) or maximum administered dose (MAD) of IT-hu14.18-IL2 after receiving palliative RT and in combination with nivolumab in subjects with advanced melanoma.

2.1.3.2 Evaluate the safety and tolerability of the combination of palliative RT, nivolumab and IT-hu14.18-IL2

2.1.4 Phase ID

2.1.4.1 Determine the maximum tolerated dose (MTD) or maximum administered dose (MAD) of IT-hu14.18-IL2 after receiving palliative RT and in combination with nivolumab and ipilimumab in subjects with advanced melanoma.

2.1.4.2 Evaluate the safety and tolerability of the combination of palliative RT, nivolumab, ipilimumab and IT-hu14.18-IL2.

2.1.4.3 Evaluate local and systemic objective tumor responses to treatment with IT-hu14.18-IL2 in combination with palliative RT, nivolumab, and ipilimumab.

2.2 Secondary Objectives

2.2.1 Evaluate progression-free survival (PFS), overall survival (OS), clinical benefit (CB, defined as CR + PR + SD) and duration of response to hu14.18-IL2 in combination with RT, nivolumab and ipilimumab.

2.2.2 Evaluate pathologic (tissue) evidence of immune response at the injection site and untreated sites.

2.2.3 Evaluate PFS, CB and duration of response to hu14.18-IL2 in combination with palliative RT, nivolumab and ipilimumab based on resistance to prior treatment with anti-CTLA-4 and/or anti PD1/PD-L1 antibody.

2.2.4 Evaluate serial serum samples to determine the pharmacokinetics of hu14.18-IL2 administered intratumorally.

2.2.5 Evaluate each subject's tumor cells for expression of GD2 and PD-L1, and determine if either antitumor activity or selected treatment-associated biologic effects are more likely for tumor that are GD2+ then GD2- and PD-L1+ than PD-L1-. Also, evaluate whether PD-L1 expression is induced or augmented from baseline following initiation of treatment (by comparing serial biopsies).

2.2.6 Evaluate the immunologic activation induced in vivo by IT-hu14.18-IL2, addressed by in vitro cellular, serologic and flow cytometry immune assays.

2.2.7 Evaluate for histological evidence of antitumor activity based on the presence of necrotic tumor cells, inflammatory infiltrate, cellular phenotype of infiltrate, and presence of hu14.18-IL2 within the tumor at selected post-treatment timepoints.

2.2.8 Evaluate circulating tumor cells, exosomes, endogenous antibodies, and/or DNA as explaratory biomarkers associated with clincal response to IT-hu14.18-IL2 in combination with RT, nivolumab and ipilimumab.

2.2.9 Evaluate serial PBMC samples to monitor the induction of T cell responses to melanoma-associated antigens.

2.2.10 Evaluate objective tumor responses, both locally and systemically, (by immune-related response criteria) in Phases IA, IB and IC of this trial (involving IT-hu14.18-IL2 alone and in combinations with palliative RT, and with palliative RT and nivolumab, respectively).

3.0 SELECTION OF SUBJECTS

Use the below checklist to confirm a potential subject's eligibility. For each subject, this checklist must be completed and maintained in the subject chart.

3.1 Inclusion Criteria

_____ **3.1.1** Subjects must have histologically proven, malignant melanoma, that is advanced (stage IV) or is unresectable and therefore considered surgically incurable. _____ _____

_____ **3.1.2** Subject's disease must be measurable by immune-related RECIST criteria using clinical assessments or imaging _____ _____

_____ **3.1.3** Subjects must have at least one (1), but preferably two (2), sites of disease that are amenable to safe repeated hu14.18-IL2 injections and two (2) to four (4) biopsies (designated Lesions A (index lesion) and B). The lesions can be readily-accessible, superficial (i.e. cutaneous, subcutaneous, and/or readily palpable lymphadenopathy) sites of disease or other sites of disease amenable to safe image-guided hu14.18-IL2 injection and safe image-guided biopsies. _____ _____

3.1.3.1 If there are two lesions, one will be injected with hu14.18-IL2 and undergo biopsies. The second will not undergo injections with hu14.18-IL2, but will undergo two biopsies and be observed clinically. It is preferable, but not required, that these lesions have not received prior RT.

_____ **3.1.4** Subjects must be ≥ 18 years old _____ _____

_____ **3.1.5** Subjects must have an ECOG performance status of 0 or 1. _____ _____

Performance Status: _____ Date: _____

_____ **3.1.6** Subjects must have received or declined at least one FDA approved treatment, either in the adjuvant setting or for metastatic disease, demonstrating an impact on survival (i.e: anti-CTLA-4 antibody, anti-PD-1 antibody, IL2, etc). _____ _____

_____ **3.1.7** Subjects with CNS metastases are eligible if the CNS lesions are stable for at least 2 months and if tapered off treatment doses of systemic corticosteroids for at least 2 weeks prior to enrollment on the trial. Management with maintenance physiologic doses of corticosteroids is acceptable. _____ _____

_____ **3.1.8** Subjects to be entered into Phase IB, IC and ID must be evaluated by a radiation oncologist and determined to have a need for palliative RT based on current or imminent symptoms at a tumor site that is also injectable. If palliative RT is needed to one or more disease sites, a separate site of disease that does not require RT must remain to enable assessment of systemic disease response. _____ _____

_____ **3.1.9** Subjects must have adequate bone marrow, liver, and renal function as defined by: _____ _____

3.1.9.1 Total neutrophil count ≥ 1,500/mm^3, platelets ≥ 100,000/mm^3, and hemoglobin ≥ 10 g/dL. Packed red blood cell transfusions are allowed if needed to maintain an appropriate hemoglobin.

WBC: _____ Date: _____

Total neutrophils: _____ Date: _____

Platelets: _____ Date: _____

Hemoglobin: _____ Date: _____

3.1.9.2 AST/ALT ≤ 3 x the upper limit of normal. Total bilirubin ≤ 1.5 x the upper limit of normal (< 3.0 mg/dL for subjects with Gilbert's Syndrome).

AST: _____ ULN: _____ Date: _____

ALT: _____ ULN: _____ Date: _____

Total Bilirubin: _____ ULN: _____ Date: _____

3.1.9.3 Serum creatinine ≤ 1.5 x the upper limit of normal.

Serum Creatinine: _____ ULN: _____ Date: _____

_____ **3.1.10** Subjects with a history of ischemic cardiac disease or congestive heart failure must complete a stress radionuclide scan with results that show no evidence of myocardial ischemia or heart failure. _____ _____

_____ **3.1.11** Subjects must be willing and able to provide informed written consent for the study. _____ _____

_____ **3.1.12** Subjects must have no immediate requirements for palliative surgery. Subjects in Arm 1A must have no immediate requirement for palliative RT. _____ _____

_____ **3.1.13** Subjects must be willing and able to discontinue antihypertensive medications if advised to do so for the days of hu14.18-IL2 administration. _____ _____

_____ **3.1.14** Subjects must have a washout period of at least 28 days between any prior systemic anti-melanoma therapy (including immunotherapies) and the first dose of study drug(s). _____ _____

3.2 Exclusion Criteria

_____ **3.2.1** Subjects currently receiving immunosuppressive therapy for a diagnosis of auto-immune disease. _____ _____

_____ **3.2.2** Phase IB, Phase IC, and Phase ID only: Subjects with known genetic conditions causing pre-disposition to RT toxicity (i.e: Li-Fraumeni, ATM deficiency, active scleroderma, etc). _____ _____

_____ **3.2.3** Subjects who cannot provide independent, legal, informed consent. _____ _____

_____ **3.2.4** Women of childbearing potential will be excluded if they are pregnant, nursing, or not willing to use effective contraception, as discussed with the treating physician, during the treatment period. A negative pregnancy test (serum or urine) is required for women of child bearing potential within 14 days before study registration. _____ _____

A person of childbearing potential is anyone (regardless of sexual orientation, gender identity, having undergone tubal ligation, or remaining celibate by choice) who was born with a uterus and at least one ovary and meets the following criteria:

- Has not undergone a hysterectomy or bilateral oophorectomy; or
- Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had a menses at any time in the preceding 12 consecutive months).

_____ **3.2.5** Subjects with an uncontrolled, symptomatic, cardiac rate related to a cardiac rhythm disturbance. _____ _____

_____ **3.2.6** Subjects with significant psychiatric disabilities or a seizure disorder if considered unsafe for this study intervention in the opinion of the treating physician. _____ _____

_____ **3.2.7** Subjects with symptomatic pleural effusions or ascites. _____ _____

_____ **3.2.8** Subjects with organ allografts. _____ _____

_____ **3.2.9** Subjects who require, or are likely to require, systemic treatment doses of corticosteroids, or other immunosuppressive drugs, or have used them within 2 weeks of registration (clarification: subjects receiving physiologic maintenance or replacement doses of systemic steroids are eligible). _____ _____

_____ **3.2.10** Subjects with significant intercurrent illnesses per physician discretion. _____ _____

_____ **3.2.11** Subjects with active or acute infections or active peptic ulcers, unless these conditions are adequately corrected or controlled, in the opinion of the treating physician. _____ _____

_____ **3.2.12** Subjects with any prior or current malignancy whose natural history or treatment is considered by the investigator to be likely to interfere with the safety or efficacy assessments of this investigational regimen. _____ _____

_____ **3.2.13** Subjects with known human immunodeficiency virus (HIV) infection, active or chronic hepatitis B or hepatitis C infection, or with clinical evidence of hepatitis. _____ _____

_____ **3.2.14** Subjects with objective peripheral neuropathy (Grade ≥3). _____ _____

_____ **3.2.15** Subjects with known hypersensitivity to hu14.18-IL2 or human immunoglobulin (all subjects), or those who experienced significant immune-related adverse events requiring treatment with steroids or other immunosuppressant therapy during prior treatment with ipilimumab, or anti-PD1/PD-L1 checkpoint blockade therapy (this pertains only to subjects in Phase IC and Phase ID). _____ _____

_____ _____
Investigator Signature Date

_____ _____
Coordinator #1 Signature Date

_____ _____
Coordinator #2 Signature Date

4.0 REGISTRATION PROCEDURE

Eligible subjects will be entered on study using the University of Wisconsin Carbone Cancer Center OnCore Database.

To register a subject, the following documents should be completed/obtained by the research staff and maintained in the subject study chart:

- Copy of required eligibility tests
- Copy of eligibility checklist (signed and dated by investigator)
- Signed consent form
- Signed HIPAA authorization form

The research coordinator at the site will then register the subject into the OnCore database prior to starting study treatment. OnCore will assign the unique subject number.

There will not be access to the OnCore database registration until documented IRB approval is obtained and entered into the OnCore database.

5.0 TREATMENT PLAN

Study medication dosing (all phases), including nivolumab and ipilimumab, may be delayed for up to 2 weeks for any adverse event, laboratory abnormality, or intercurrent illness if warranted in the judgement of the investigator.

5.1 Intratumoral Hu14.18-IL2 (All phases)

Hu14.18-IL2 will be given on days 1, 2, and 3 of each cycle of therapy as an IT and/or peritumoral injection. One lesion (the index lesion) will be used for the planned injections with the hu14.18-IL2. Additional lesions, when available, can be used as alternate sites for hu14.18-IL2 injection for reasons including non-DLT toxicity at the index lesion, resolution of the index lesion, or at the discretion of the treating investigator. The time interval between each dose will be 24 hours (+/- 6 hours) Treatment cycles will be repeated every 21 days at the same dose for the first 4 cycles, provided that there is no DLT (see protocol section 6.1.1). Maintenace treatment with hu14.18-IL2 can be given every 28 days for up to a maximum of 13 doses, if there is observed immune RECIST response or tumor regression and evidence of residual injectable tumor. Treatment may also be continued for up to a maximum of 13 doses if disease remains stable, at the discretion of the treating physician. Treatment will be under strict medical supervision and performed in the clinical research unit (CRU). Subjects will remain under close observation in the CRU after treatment and have vital signs monitored hourly for 6 hours post each injection during cycle 1. For cycle 2 and all subsequent cycles, subjects will remain under close observation in the CRU and have vital signs hourly; they will be observed for a minimum of 2 hours post injection if no significant hypotension is observed during cycle 1. If hypotension is observed in cycle 1, monitoring duration for cycle 2 and all subsequent cycles will be based on toxicities observed during cycle 1 and determined at the

discretion of the treating physician. Following each day of treatment with hu14.18-IL2, subjects should stay within driving distance to the UW hospital.

Following day 3 of treatment with hu14.18-IL2, subjects will be required to come in for a CRU visit on day 4 to monitor vital signs and for reporting of any adverse events. Subjects will continue to be monitored for at least 90 days after completion of study treatment for late toxicities associated with IT-hu14.18-IL2. Safety monitoring will include adverse event assessment per Tables 9.1-1 through 9.4-3.

For all treatment cycles beginning with cycle 2, subjects may be retreated if all toxicities have recovered to ≤ Grade 1 or to baseline eligibility criteria, and there is no symptomatic disease progression (Section 10). After the 4th cycle, treatment with IT-hu14.18-IL2 can continue for up to a maximum of 13 cycles if immune RECIST shows stable disease or tumor response as measured by the treating physician. For all cycles, treatment will continue by IT injection, provided that tumor remains (clinically) at the injection site (i.e.: it did not show a localized histological clearance of tumor, and was not completely resected with the biopsy/resection following cycle 1). If no tumor remains at the injection site following cycle 1 of therapy (either due to clinical complete response, or due to complete resection of the lesion), we will inject an alternate injectable lesion, if present, for any remaining cycles of therapy. Subjects without an additional injectable lesion will be followed clinically for toxicity and for response to treatment (see tables in sections 9.1 - 9.4).

5.1.1 Hu14.18-IL2 Injection process

The hu14.18-IL2 will be reconstituted in sterile water for injection, USP, to a concentration of 8 mg/mL (or 16 mg/mL when needed for higher doses), and then diluted as needed to enable each dose to be delivered in at least 1.0 mL but no more than 2.0 mL (See section 11.1 for material preparation and dilution). It will be injected throughout and around the lesion. The hu14.18-IL2 will be injected slowly (approximate rate of 0.5-1 mL over ~5 minutes), with additional breaks allowed during administration, if needed, for puncture site clotting in order to minimize leakage of injected material at the current or prior puncture sites. The gauge of the needle to be used for injection (range: 30 to 26 guage) will be determined by the responsible clinician (either the study chair or a clinician identified by the study chair). The total volume will be administered using multiple subcutaneous needle re-directions into the target lesion to distribute the injected material as uniformly as possible within and surrounding the lesion. The complete injection of hu14.18-IL2 for a given day should occur over 5 to 60 minutes. Injections will be performed daily for 3 days. There will be no intra-subject dose escalation. Systemic non-steroidal analgesics (i.e.: ibuprofen, acetaminophen) and topical or local anesthetics (i.e.: EMLA cream, lidocaine 4% cream) can be used if needed for local pain at the injection site. Ultrasound guidance is permitted but not required for IT injection. Non-target lesions will be observed clinically without receiving direct IT-IC treatment and will not be injected with saline or other matching placebo.

5.1.2 Premedication prior to hu14.18-IL2 injections on days 1-3

Preclinical data suggest that concomitant use of indomethacin may enhance the antitumor effect of immunocytokine treatment in mice (S. Gillies, unpublished), and that indomethacin can ameliorate clinical toxicity of IL2.

- Indomethacin 25 mg PO 1 hour prior to injection, then every 6 hours until 12 hours after completion of the last dose of hu14.18-IL2 in each treatment cycle, then every 6 hours as needed. Indomethacin should not be given if renal function is compromised (serum creatinine > 1.8 mg/dl) or if the platelet count is ≤ 50,000/µl. Other anti-inflammatory drugs, such as ibuprofen, are permitted as a substitute for indomethacin if, in the investigators judgement, indomethacin will not be well tolerated.
- Any subject showing allergic symptoms with hu14.18-IL2 should receive diphenhydramine, acetaminophen, and an H_2 blocker (e.g. ranitidine 150 mg PO) as premedication before all subsequent doses.
- Any subject experiencing pain with a dose should receive premedication with morphine (or an appropriate opiod) before all subsequent hu14.18-IL2 doses.

5.1.3 Severe allergic reaction (anaphylactic precautions should be taken)

Treatment can include:

- Dexamethasone 10 mg IV
- Diphenhydramine 50 mg IV
- Epinephrine 3-5 mL IV [1:10,000]; 0.5 mL SC [1:1000]
- Equipment for assisted ventilation
- A free-flowing IV line must be established at all times

NOTE: Treatment with systemic treatment doses of corticosteroids will result in discontinuation of protocol therapy with hu14.18-IL2 (clarification: physiologic maintenance or replacement doses of systemic steroids and topical steroids are acceptable). If the use of systemic treatment doses of corticosteroids or other immunosuppressive drugs is considered unlikely or not related to hu14.18-IL2, the hu14.18-IL2 treatment may be resumed following discontinuation of systemic steroids with the subsequent cycle at the discretion of the responsible physician.

5.1.4 Premedication and ancillary medications for management of common symptoms related to hu14.18-IL2

Appropriate antibiotics, blood products, antiemetics, fluids, electrolytes, and general supportive care are to be used as necessary.

5.2 Radiation (Phases 1B, 1C, and 1D)

In Phase IB, IC and ID of the study, subjects must be determined to have a need for palliative RT based on current or imminent symptoms at a tumor site that is also injectable. These subjects will receive a course of palliative RT targeting any sites deemed to merit palliative RT treatment and will complete this RT on days -8 to -4 relative to the first day of IT-hu14.18-IL2.

A 12 month safety monitoring period following radiation therapy in which sub-acute and late adverse events related to the RT will be assessed in all phases of the study in which RT is employed. Safety monitoring will include adverse event assessment per Table 9.1-1 through 9.4-3.

5.2.1 Selection of an Index Tumor Site

From the tumor site(s) receiving palliative RT, a targeted (index) tumor (lesion A) will be selected at the discretion of the attending physician based on location, and the ease and safety of readily delivering hu14.18-IL2 injections to that site, as well as being able to obtain pre and post-treatment biopsies from that site. Whenever possible, the targeted region should not have received RT prior to enrollment in this study. Palliative RT may be delivered to other sites of disease if deemed necessary and safe, provided that there is at least one site of disease that is not receiving RT. These other sites will not be injected with hu14.18-IL2. The dose and fractionation of RT to all sites will be left to the discretion of the treating physician and these treatments should conclude on the same day and between days -8 to -4 relative to the first day of IT-hu14.18-IL2 injection of the index lesion.

5.2.2 Radiation Dose

Subjects who enroll in Phase IB, IC, and ID of this protocol will receive a standard course of palliative RT treatment to any tumor sites that are currently or imminently symptomatic. The treating physician will use his or her discretion to determine the appropriate dose, fractionation, and target sites for palliative RT, consistent with current practice standards. Generally, a range of 1 to 10 fractions will be delivered with dose ranging from 8 to 30 Gy.

5.2.3 Radiotherapy Delivery

RT delivery will follow standard practice for palliative RT treatment and will utilize megavoltage photons or electrons. Any number or arrangement of beams may be used at the discretion of the treating physician to achieve target coverage and conformality. Standard quality assurance measures will be taken prior and during RT.

5.2.4 Treatment Simulation, Subject Positioning and Set-up.

Subject will be positioned in a comfortable posture. Any immobilization apparatus may be used at the discretion of the treating physician as long as it does not interfere with the proper functioning of any necessary treatment planning or image-guidance systems. A CT simulation will be performed to facilitate treatment planning, consistent with standard practice approaches for the planning of palliative RT.

5.2.5 Tumor localization and Target Volumes

CT will be the primary image platform for treatment planning. Treatment planning and dosimetry will be performed to confirm adequate target coverage. Tumor localization will be performed by the treating physician. This can be done clinically by visual demarcation for electron treatment and can be aided by placing a wire or other marker at the time of CT simulation. The Gross Tumor Volume (GTV) will be defined by the treating physician using a combination of clinical assessment, available diagnostic imaging, and the planning CT scan. The GTV should encompass the entirety of the tumor volume that will be injected

with hu14.18-IL2. This volume may be expanded to generate a clinical target volume (CTV). The CTV may include any contiguous areas that the treating physician deems likely to harbor microscopic disease. The CTV will be confined by an external skin contour and will exclude uninvolved tissues such as bone, organs, or fascia that the treating physician deems to be anatomic barriers to the spread of disease. The CTV may be expanded as needed to define a planning target volume (PTV) that accounts for potential variations in positioning and set-up, as well as isodose convergence at depth in the case of electron therapy.

5.2.6 Radiation Treatment Planning and Dosimetry

3D-conformal RT planning will generally be utilized to determine target coverage and to evaluate the dose delivered to other organs at risk. Intensity modulated radiation therapy (IMRT) planning and delivery may be utilized, if deemed necessary by the treating physician for the purpose of achieving target coverage, conformality, or other dosimetric parameters. The treating physician will use his or her discretion to determine the appropriate dose, fractionation, and treatment planning/delivery approach for any treatment site. Dose conformality should be maximized, when feasible. The point of maximum dose should be placed within the GTV when practical.

Treatment planning will be carried out respecting any requisite normal tissue constraints, as defined by the treating physician. These constraints will be guided by QUANTEC data (http://individual.utoronto.ca/dtsang/misc/quantec.pdf) with 2 Gy equivalent doses (EQD2) determined as needed using an assumption of a normal tissue alpha:beta equal to 3. Any normal tissue avoidance structures will be contoured and considered as solid organs. Structures not deemed to be at risk due to their distance from the target area need not be contoured. If the targeted lesion is within 10 cm of the following organs it is anticipated they will be contoured: spinal cord, kidneys, bowel, stomach, liver, heart, lungs, esophagus, bladder, rectum, brain, eyes, lenses, cochlea, brainstem, larynx, oral cavity, gonads, genitalia.

5.2.7 Treatment Verification

After the subject is setup on the treatment table, standard imaging approaches (orthogonal x-rays, CT, MRI, and/or light field clinical set-up) will be used to align the subject with the treatment machine geometry based on the treatment plan. After initial alignment, shifts or rotations will be made as necessary using couch motion to achieve precise replication of the planned positioning. After initial localization is performed, all effort should be made to initiate the treatment delivery as quickly as possible. If reproducible positioning is not achieved treatment re-planning will be allowed as deemed necessary by the treating physician.

5.2.8 Sequencing of radiation with other treatments

Following RT, subjects will receive either IT-hu14.18-IL2 (Phase IB) or nivolumab and IT-hu14.18-IL2 (Phase IC) or nivolumab and ipilimumab and IT-hu14.18-IL2 (Phase ID). The timing of these treatments relative to the completion of RT is specified in section 5.0. A minimum of 24 hours should separate completion of RT treatment from nivolumab +/-

ipilimumab administration and at least 4 days should separate completion of RT from the first hu14.18-IL2 injection to avoid added toxicity. A maximum of 8 days should separate RT from first IT-hu14.18-IL2 injection.

5.2.9 Additional Radiation Treatments

For subjects in Phase IB, IC and ID who meet criteria for continuing on trial after completion of cycle 1 of hu14.18-IL2 injections, additional courses of palliative RT may be delivered if deemed necessary for palliative purposes by the treating radiation oncologist. An anticipated range of 1 to 10 fractions will be delivered with dose ranging from 8 to 30 Gy. These courses may target any tumor sites that become currently or imminently symptomatic. One of these secondarily targeted sites may constitute a second index lesion if it is readily injectable and if any one (or more) of the following circumstances exist:

1) The initially treated lesion regresses completely
2) The initially treated lesion regresses to a point that it is felt unlikely to contain viable tumor cells.
3) The initially treated lesion cannot be injected due to small size, local infection, local skin reaction, or pain.

Under these circumstances, such a secondary index sites will be injected with IT-hu14.18-IL2, as specified in section 5.0. This process may be repeated indefinitely under circumstances defined in this section (section 5.2.9) until the subject is not eligible to remain on study or does not have an identifiable and injectable tumor site that requires palliative RT treatment. In all cases RT should be initiated and completed a minimum of 24 hours before or after any treatments with hu14.18-IL2, nivolumab and ipilimumab. If necessary, one cycle of hu14.18-IL2 may be skipped to allow for scheduling and administration of the RT in these scenarios.

5.3 Nivolumab (Phases 1C & 1D)

5.3.1 Phase 1C

Treatment will consist of a standard dose of 3 mg/kg of nivolumab (subject's actual body weight) administered intravenously over 30 minutes, once every 2 weeks. The initial dose will be administered between Day -7 and -1 of cycle 1. Treatment will be administered in a clinic location to optimize emergency care due to the risk of hypersensitivity reaction and subjects will be strictly monitored by capable medical personnel throughout infusion. Nivolumab dose rounding and administration are per institutional standard.

This drug will be administered in the FDA-approved dose and schedule every 2 weeks.

5.3.2 Phase 1D

For the first 4 cycles of treatment (when nivolumab is being administered with ipilimumab, see section 5.4 below), nivolumab will be dosed at 1 mg/kg (subject's actual body weight) and administered intravenously over 30 minutes in the outpatient setting, once every 3 weeks. Doses will be administered between Day -7 and Day -1 of each 21 day cycle.

Treatment will be administered in a location to optimize emergency care due to the risk of hypersensitivity reaction and subjects will be strictly monitored by capable medical personnel throughout infusion. Nivolumab dose rounding and administration are per institutional standard. After completion of the first 4 cycles (and the cessation of ipilimumab treatments), provided the subject is still eligible for continued treatment, the maintenance dose of nivolumab will be increased to 3 mg/kg/dose and administered every 2 weeks.

5.4 Ipilimumab (Phase 1D)

Treatment will consist of a standard dose of 3mg/kg ipilimumab (subject's actual body weight) administered intravenously over 30 minutes in the outpatient setting, once every 3 weeks. Dose recalculation is required when there is a ≥ 10% change in body weight. Doses will be administered between Day -7 and day -1 of each 21 day cycle. Treatment will be administered in a location to optimize emergency care due to the risk of hypersensitivity reaction and subjects will be strictly monitored by capable medical personnel throughout infusion. Ipilimumab dose rounding and administration are per institutional standard.

5.5 Phases of Treatment

Table 5.5-1 Dose escalation/de-escalation plan for analysis of hu14.18-IL2 toxicity

NUMBER OF EVALUABLE SUBJECTS WITH UNACCEPTABLE TOXICITIES	NEXT DOSE LEVEL
0/3	Accrue 3 new subjects for next higher dose level.
1/3	Accrue additional 3 subjects at current dose level.
1/3 + 0/3	Accrue 3 new subjects for next higher dose level.
1/3 + 1/3	Declare the **Previous** dose as MTD.
2/3	De-escalate to previous dose level

5.5.1 Phase IA: Dose escalation treatment plan and design

Three dose levels are scheduled for Phase IA of the study:
Level –I: 1 mg/m^2/day
Level I: 2 mg/m^2/day
~~Level II: 4 mg/m^2/day~~ Dose level II removed in protocol amendment 5
~~Level III: 6 mg/m^2/day~~ Dose level III removed in protocol amendment 5

If IT-hu14.18-IL2 level I dose is not tolerated, the dose will be de-escalated to the level –I dose.

In the Phase IA study, approximately 9 to 12 subjects will be entered in three cohorts of three subjects or until MTD, or MAD if MTD is not reached. Toxicity determinations relating to dose escalation will continue for 3 weeks following the second cycle of hu14.18-IL2 (namely, until the scheduled time to initiate the 3rd cycle of injections). The criteria for proceeding to the next cohort of subjects are based upon the frequency of DLTs related to hu14.18-IL2 until 3 weeks

following the first IT-IC dose of the second cycle of treatment. As of protocol amendment 5, dose levels II and III have been discontinued. While subjects enrolled in level I did not experience dose limiting toxicities, the frequency and extent of the adverse events experienced were greater than expected. Dose escalations were removed for safety purposes.

The index tumor (lesion A) will be injected once daily for 3 consecutive days with the predefined dose of hu14.18-IL2. Escalating doses of hu14.18-IL2 will be administered intratumorally in cohorts of three subjects. If no DLT is observed during cycles 1 and 2 in the first three subjects, the next dose level will be accrued. If one subject out of three experiences DLT, three additional subjects will be accrued at that same level. If no additional DLTs are observed in those next 3 subjects, the next dose level will then be accrued. However, if two or more DLTs out of 6 subjects are observed in a given dose level, dose escalation will be terminated. MTD will be defined as the dose below which ≥ 2 DLTs were observed in the first 2 treatment cycles. Please refer to Table 5.5.1-1, above, regarding the dose escalation and MTD. MAD, or maximum administered dose, will be the highest administered dose for this phase of the study, if MTD is not determined.

Subjects will be monitored for toxicity (see tables in sections 9.1 - 9.4) during all cycles of treatment. The DLT monitoring period for Phase IA (and all other phases) will continue for 3 weeks following the second cycle of hu14.18-IL2 (until the scheduled time to initiate the 3rd cycle of injections). The toxicity criteria for not continuing the study to the next cohort of subjects are based upon the frequency and severity of toxicity related to hu14.18-IL2 during this 6 week DLT observation period.

If there was any DLT then management, retreatment and dose reductions will be done as detailed in section 6.1.1. Subjects are off study if there is any clinically significant symptomatic progressive disease at any time (see section 10.3.5 regarding documentation of progressive disease at the site receiving IT-hu14.18-IL2 treatment).

5.5.2 Phase IB Treatment plan and design

After enrollement into Phase IA is complete, Phase IB will begin. The dose escalation/de-escalation strategy in Table 5.5-1 will be employed. In Phase IB, 3-6 subjects will be enrolled into a cohort one dose level below the Phase 1A determined IT-hu14.18-IL2 MTD/MAD. If 0/3 or 1/6 DLTs are observed at that dose, 3-6 subjects will be enrolled into a cohort at the Phase 1A determined IT-hu14.18-IL2 MTD/MAD. If 0/3 or 1/6 DLTs are observed at the Phase 1A determined IT-hu14.18-IL2 MTD/MAD, that will be the Phase IB determined MTD/MAD. A total of 6-12 subjects are expected to be enrolled in this phase of the study. If the initial IT-hu14.18-IL2 dose level in this phase is not tolerated, the dose will be de-escalated to two dose levels below the Phase IA determined IT-hu14.18-IL2 MTD/MAD.

Treatment Plan

- Palliative RT to any currently or imminently symptomatic tumor sites, delivered using physician-determined appropriate dose and fractionation. All initial RT will be completed between day -8 to -4, in cycle 1 only, if no intercurrent events, RT will be followed by:
- Hu14.18 as an IT injection once daily on Days 1, 2 and 3 of each 21 Day Cycle for 4 Cycles if no clinically significant disease progression.
    - Treatment detailed in section 5.1
    - Continue with maintenance treatment with hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of each 28 day cycle up to a maximum of 13 cycles if immune RECIST antitumor response/tumor regression and any residual injectable tumor. Treatment may also be continued for up to a maximum of 13 cycles if stable disease at the discretion of the treating physician.

After hu14.18-IL2 administration, which will be given in the outpatient setting, subjects will remain under close observation in the CRU for observation and hourly vital signs for a minimum of 6 hours following treatment on all 3 treatment days for cycle 1. The observation period can be reduced to a minimum of 2 hours observation post injection for all subsequent cycles of hu14.18-IL2 administration at the discretion of the treating physician.

5.5.3 Phase IC Treatment plan and design

After enrollment into Phase IB is complete, we will begin Phase IC. The dose escalation/de-escalation strategy in table 5.5-1 will be employed. In Phase IC, we will enroll 3-6 subjects into a cohort one dose level below the Phase 1B determined IT-hu14.18-IL2 MTD/MAD. If 0/3 or 1/6 DLTs are observed at that dose, 3-6 subjects will be enrolled into a cohort at the Phase IB determined IT-hu14.18-IL2 MTD/MAD. If 0/3 or 1/6 DLTs are observed at the Phase IB determined IT-hu14.18-IL2 MTD/MAD, that will be the Phase IC determined MTD/MAD. A total of 6-12 subjects are expected to be enrolled in this part of the study. If the initial IT-hu14.18-IL2 dose level in this phase is not tolerated, the dose will be de-escalated to two dose levels below the Phase IB determined IT-hu14.18-IL2 MTD/MAD.

Treatment Plan

- Palliative RT to any currently or imminently symptomatic tumor sites, delivered using physician-determined appropriate dose and fractionation. All initial RT will be completed between day -8 to -4, in cycle 1 only, if no intercurrent events, RT will be followed by nivolumab and hu14.18-IL2
- Nivolumab 3mg/kg every 2 weeks in each cycle (the initial dose will be between Day -7 to Day -1 of cycle 1),
    - Administration detailed in section 5.3
    - Continue maintenance treatment with nivolumab at a dose of 3 mg/kg every 2 weeks for up to one year if immune RECIST antitumor response/tumor regression. Treatment may also be continued up to a year if stable disease at the discretion of the treating physician.
- Hu14.18/IL2 as an IT injection once daily on Days 1, 2 and 3 of each 21 Day Cycle for 4 Cycles if no clinical significant disease progression
    - Treatment detailed in section 5.1

- Continue with maintenance treatment with hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of each 28 day cycle up to a maximum of 13 cycles if immune RECIST antitumor response/tumor regression and any residual injectable tumor. Treatment may also be continued for up to a maximum of 13 cycles if stable disease at the discretion of the treating physician. Subjects may elect to discontinue hu14.18-IL2 while continuing treatment with nivolumab.
- If treatment with IT-IC needs to be discontinued, due to hu14.18-IL2 related toxicity, and the subject has received at least 3 doses of hu14.18-IL2 and still otherwise meets criteria for continued treatment, nivolumab tratment may continue, per the discretion of the responsible physician, as noted in this section.

5.5.4 Phase ID design

After enrollment into Phase IC is complete, we will begin Phase ID. The dose escalation/de-escalation strategy in table 5.5-1 will be employed. In Phase ID, we will enroll 3-6 subjects into a cohort one dose level below the Phase IC determined IT-hu14.18-IL2 MTD/MAD. If 0/3 or 1/6 DLTs are observed at that dose, 3-6 subjects will be enrolled into a cohort at the Phase IC determined IT-hu14.18-IL2 MTD/MAD. If 0/3 or 1/6 DLTs are observed at the Phase IC determined IT-hu14.18-IL2 MTD/MAD, that will be the Phase ID determined MTD/MAD. A total of 6-12 subjects are expected to be enrolled in the dose-escalation component of Phase ID. If the initial IT-hu14.18-IL2 dose level is not tolerated, the dose will be de-escalated to two dose levels below the Phase IA determined IT-hu14.18-IL2 MTD/MAD.

We plan to expand the cohort that employs the Phase ID determined MTD/MAD of hu14.18-IL2 in combination with RT, nivolumab and ipilimumab in order to enroll a total of 28 subjects at the Phase ID determined MTD or MAD. The total number of subjects enrolled in Phase ID is thus anticipated to be 30-34. Treatment with ipilimumab and nivolumab will be followed by IT-hu14.18-IL2 on days 1, 2 and 3 of the initial 4 treatment cycles, as detailed above. For cycles 5 and beyond subjects will receive only nivolumab, at a dose of 3 mg/kg, followed by IT-hu14.18-IL2 on days 1, 2 and 3 of each cycle. Based on previous experience, we expect approximately half of the subjects to be GD2+ (i.e., 14 GD2+ subjects and 14 GD2- subjects).

Treatment Plan

- Palliative RT to any currently or imminently symptomatic tumor sites, delivered using physician-determined appropriate dose and fractionation. All initial RT will be completed between day -8 to -4, in cycle 1 only, followed by nivolumab, ipilimumab and hu14.18-IL2
- Nivolumab 1 mg/kg once every 3 weeks in each cycle (during Day -7 to Day -1), for 4 cycles
  - Administration detailed in section 5.3
  - Nivolumab may be continued beyond Cycle 4 at a dose of 3 mg/kg every 2 weeks per criteria in section 5.3
- Ipilimumab 3 mg/kg once every 3 weeks in each cycle (during Day -7 to Day -1), for 4 cycles
  - Administration detailed in section 5.4

  - Ipilimumab is not continued beyond Cycle 4
- Hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of each 21 day cycle for 4 cycles if no clinically evident disease progression
  - Treatment detailed in section 5.1
  - Continue with maintenance treatment with hu14.18-IL2 as an IT injection once daily on Days 1, 2 and 3 of each 28 day cycle for up to a maximum of 13 cycles if immune RECIST antitumor response/tumor regression and any residual injectable tumor. Treatment may also be continued for up to a maximum of 13 cycles if stable disease at the discretion of the treating physician. Subjects may elect to discontinue hu14.18-IL2 while continuing treatment with nivolumab +/- ipilimumab.
  - If treatment of IT-IC has needed to be discontinued, due to hu14.18-IL2 related toxicity, but the subject has received at least 3 doses of hu14.18-IL2 and still otherwise meets criteria for continued treatment, nivolumab treatment (and ipilimumab treatment, if any of courses 2-4 remain) may continue, per the discretion of the responsible physician, as noted in this section.

5.6 Staggering of Subjects

5.6.1 Intra-cohort Stagger

For the first three subjects in each cohort, a safety monitoring period of 48 hours will occur between the initiation of therapy on Day 1 of Cycle 1 for a subject and the initiation of therapy on Day 1 of Cycle 1 for the next subject.

5.6.2 Inter-cohort Stagger for All Phases

The DLT monitoring period for the final subject in a cohort must be complete (42 days from the first hu14.18-IL2 intratumoral injection) prior to treating the first subject in the next cohort with IT-hu14.18-IL2.

5.7 Duration of Therapy

Subjects will receive protocol therapy until progressive disease, unacceptable toxicity, non-protocol therapy, or up to one year (maximum of 13 cycles of hu14.18-IL2), unless subject meets criteria for removal from study (See section 6.6).

5.8 Duration of Follow-up

Every attempt will be made to continue to monitor subjects for: 1) at least 90 days after completion of study treatment for late toxicities associated with any component of therapy (RT, IT-hu14.18-IL2, nivolumab, ipilimumab), and 2) twelve months following radiation therapy for any late local toxicity due to palliative radiation, in those subject who receive RT. Whenever possible, this follow up monitoring will occur at the UWCCC. However, it is recognized that returning to UWCCC for follow-up may be unrealistic, particularly for subjects who live considerable distances away from the UWCCC or who have progressive disease, and have moved on to other treatments or to palliative care. In such cases, we will: 1) attempt to make phone contact with the subject and/or the subject's local health care provider,

2) attempt to obtain results of laboratory tests and clinic notes from the local provider, and 3) request a detailed description (or photo when possible) of the local/radiation treatment site. This information will be kept in the subject's case report file.

After completion of therapy, subjects will be followed on this protocol every 12 weeks (+/- 2 weeks) for response to treatment until disease progression occurs or until 2 years after receiving therapy. After such time, subjects will be followed every 6 months for survival.

6.0 EVALUATION AND MANAGEMENT OF TOXICITY AND SUPPORTIVE CARE

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

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

Related: An AE will be considered related if, in the opinion of the investigator, the AE is possibly, probably, or definitely related to at least one of the study agents or procedures.

Not related: An AE will be considered not related if, in the opinion of the investigator, the AE is unlikely to be related or is unrelated to all study agents or procedures.

Grading of toxicities on this study will be done using the NCI CTCAE 5.0. **Subjects will be monitored for at least 90 days after completion of study treatment for late toxicities associated with any component of therapy (RT, IT-hu14.18-IL2, nivolumab, ipilimumab). Potential sub-acute and late toxicities due to palliate radiation therapy will be monitored for 12 months following the radiation therapy. Following the required monitoring periods all adverse events will be followed until they resolve to baseline or < Grade 2, or are deemed irreversible.**

Management plans are provided separately for toxicity related to hu14.18-IL2 and for toxicity related to either nivolumab or to ipilimumab. Since the management guidelines for a given toxicity may be different for hu14.18-IL2 and for nivolumab and ipilimumab, the management guidelines separately indicated for each agent should be followed for that agent for any toxicity that is possibly, probably, or definitely related to hu14.18-IL2 or to either ipilimumab or nivolumab.

Reactions at hu14.18-IL2 injection sites will be evaluated using Appendix 3.

6.1 Definition of DLT and management of hu14.18-IL2 related toxicity

6.1.1 Dose limiting toxicities (All Phases, Cycles 1 – 2 only)

6.1.1.1 Definition of DLT:

The definition for hu14.18-IL2 DLT in this study differs from the standard definition. Dose-limiting toxicity (DLT) will be defined as Grade 3 or 4 toxicity that is possibly, probably or definitely related to IT-hu14.18-IL2, using the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, except as detailed in section 6.1.1.3. In addition, a Hy's Law case will be considered a DLT (three-fold or greater elevations above the upper limit of normal of ALT or AST, with elevation of the serum total bilirubin greater than two times the upper limit of normal, in the absence of other etiologies such as progression of hepatic metastasis, viral hepatitis, pre-existing or acute liver disease, or another drug or clinical event capable of causing the observed injury).

6.1.1.2 Clarification of need for exceptions for DLT determination

A secondary objective of this study is to evaluate safety and tolerability of the hu14.18-IL2 fusion protein administered intratumorally as a single agent, as well as in combination with RT, therefore, careful documentation of toxicity, according to the NCI CTCAE version 5.0, is essential. The hu14.18-IL2 fusion protein has had substantial Phase I and Phase II clinical testing. It is derived from the human IL2 molecule and the humanized form of the ch14.18 chimeric antibody, both of which are FDA approved and have had extensive clinical testing. Over the past 30 years of IL2 testing and nearly 25 years of ch14.18 testing, certain side effects have been noted to occur frequently. These toxicities are generally transient, can be well controlled clinically, and have not, therefore, been used to determine "dose limiting toxicity" for IL2 or ch14.18. Some of these well-controlled transient side effects do actually score as Grade 3 or 4 toxicities using the NCI CTCAE 5.0 scale. In order to evaluate the toxicity as well as clinical antitumor activity in this trial of hu14.18-IL2, the list of toxicities below will **NOT** be considered as dose limiting for the purposes of IC drug discontinuation, dose modification, or DLT determination for this study. These exceptions have been used in our most recent Phase I and pilot studies of IV hu14.18-IL2 in subjects with advanced melanoma. Specific issues regarding management of known toxicities related to ipilimumab and nivolumab are detailed in section 6.2.

6.1.1.3 Detailed listing of hu14.18-IL2 DLT exceptions

The following toxicities will be graded and recorded, but will not be used as criteria for determining hu14.18-IL2 DLT:

a) Grade 3 pain, requiring intravenous narcotics, provided that the narcotics are controlling the pain, and that IV narcotics for pain are not required > 48 hours after completion of hu14.18-IL2 on Day 3 of any treatment cycle;
b) Grade 3 nausea and vomiting that resolves within 48 hours after completion of hu14.18-IL2 on Day 3 of any treatment cycle;
c) Grade 3 fever (i.e.: T > 40° C) lasting < 6 hours and controllable with antipyretics;

d) Grade 3 systemic skin toxicity (rash, erythema) that does not require management with steroids and improves with non-steroidal treatment (e.g., IV diphenhydramine) within 24 hours;
e) Grade 3 metabolic/laboratory toxicity of hyponatremia, hyperglycemia, or hypophosphatemia, in the absence of CNS symptoms and/or sequelae, that improve with or without treatment within 48 hours;
f) Grade 3 hematologic toxicity (or grade 4 lymphopenia – a known transient marker of immune activation by IL2) which improves to at least Grade 2 or pre-therapy baseline values before the subsequent hu14.18-IL2 treatment cycle.
g) Grade 3 infusion reactions lasting less than 24 h, readily controlled with supportive (non-steroidal) treatments (i.e., Benadryl or subcutaneous epinephrine).
h) Grade 3 fatigue or decrease of ECOG performance status to an ECOG performance status of 3 that resolves to pre-treatment, baseline values in $\leq$ 1 week.
i) Grade 3 infection that resolves in $\leq$ 1 week either with or without antibiotic therapy.
j) Grade 3 skin toxicities possibly, probably, or definitely related to RT and no more than unlikely related to hu14.18-IL2.
k) Grade 3 neutropenic fever within the first seven days after the first IT-hu14.18-IL2 injection in the absence of any other signs or symptoms of infection. Fever is an anticipated side effect of hu14.18-IL2 and grade 4 neutropenia may occur as a transient marker of immune activation by IL2. Grade 3 neutropenic fever on or after Day 8 will be considered a DLT.
l) In addition: Local skin reactions at the injection site are anticipated and will be recorded. Local skin site reactions will also be photographed when possible. Localized erythema and induration are expected. Localized ulceration at the treatment site may be indicative of antitumor effect. (Per CTCAE 5.0, grade 4 skin ulceration will be considered as DLT for that site, unless the responsible physician feels it is due to a rapid antitumor effect or due to tumor itself). In this case, no additional IT-IC administration will be delivered to that site, but the subject may continue in subsequent courses of treatment, if eligible, as described in sections 5.5.1, 5.5.2, 5.5.3 and 5.5.4).

The above dose limiting toxicity exceptions are based on the known, published, transient, reversible toxicities of IL2 and ch14.18 and hu14.18-IL2. These toxicities may be expected, based on observations of hu14.18-IL2 given to 70 adults with melanoma, administered IV by a similar schedule at similar doses to that being tested in this trial, and will not require interruption, modification, or discontinuation of hu14.18-IL2 treatment [8]. All grade 4 toxicities (except for transient lymphopenia or localized ulceration at the treatment site) are considered as DLT and require stopping treatment with hu14.18-IL2. Following resolution of a DLT, and if subsequent hu14.18-IL2 treatment is given, it must be given at a reduced dose**.**

6.1.1.4 Dose Limiting Toxicity Monitoring Periods for Each Phase of Study

Phase of Study	DLT Monitoring Period
IA	42 days* from first hu14.18-IL2 injection
IB	42 days* from first hu14.18-IL2 injection
IC	42 days* from first hu14.18-IL2 injection
ID	42 days* from first hu14.18-IL2 injection

*Monitoring period from hu14.18-IL2 injection on Day 1 of Cycle 1 through the time point when hu14.18-IL2 injection is to be initiated on Day 1 of Cycle 3

6.1.1.5 Hu14.18-IL2 Treatment Cessation/Dose Modification for DLT or significant non-DLT Toxicity from hu14.18-IL2

In the event of DLT or significant non-DLT toxicity that is considered unrelated or unlikely related to hu14.18-IL2, the hu14.18-IL2 will continue per protocol guidelines. However, study medication dosing may be delayed (or skipped) for any adverse event, laboratory abnormality, or intercurrent illness if warranted in the judgement of the investigator.

In all phases of the study, any subject demonstrating a DLT or significant non-DLT toxicity that is possibly, probably, or definitely attributed to IT-hu14.18-IL2 must have treatment with hu14.18-IL2 discontinued (see section 6.1.1.3 for DLT exceptions). If this toxicity occurs on Day 1 or 2 of any cycle, treatment can be resumed during that same cycle (i.e., on Day 2 or 3) at 50% of the starting dose of hu14.18-IL2 only if the toxicity has resolved to pre-treatment, baseline values in time to give the Day 2 or Day 3 dose of hu14.18-IL2 on schedule. Any planned drug administration that is withheld due to DLT will not be given at a later date.

If pre-treatment baseline values are not reached in time to receive additional therapy during the cycle in which the toxicity occurred, but are met in time to start the next cycle (i.e., Day 22 of the cycle associated with DLT +/- 7 days), treatment will resume at 50% of the dose of hu14.18-IL2 that caused the DLT. If toxicity again requires treatment cessation, treatment can be restarted with an additional 50% reduction of the dose of hu14.18-IL2 at the next cycle. If treatment at this reduced dose (second dose reduction) results in recurrence of DLT or significant non-DLT toxicity, treatment with hu14.18-IL2 will be permanently discontinued. Maximum of two dose reductions allowed. If the toxicity doesn't resolve to pre-treatment baseline no further hu14.18-IL2 will be administered and the subject will be followed per protocol. In Phases IC and ID, toxicities that are unrelated or unlikely to be attributed to IT-hu14.18-IL2, but possibly, probably or definitely related to treatment with nivolumab and/or ipilimumab, please see Section 6.2.2 for guidance on treatment cessation/delay of nivolumab and ipilimumab. If the guidelines recommend cessation of Nivolumab and/or ipilimumab, stop all therapy including hu14.18-IL2. If after one full cycle, the subject is eligibile for hu14.18-IL2, then resume treatment with IT-hu14.18-IL2. Guidance is provided in Table 6.2.2-2 for treatment cessation, modification and resumption for DLT toxicities or significant non-DLT toxicities in phase IC and ID, in which treatment with hu14.18-IL2 is combined with treatment with nivolumab (phase IC) or with nivolumab and ipilimumab (phase ID).

6.1.2 Hu14.18-IL2 Toxicity Management

Management guidelines to be used only for adverse events deemed possibly, probably, or definitely related to hu14.18-IL2.

As indicated in protocol section 6.6.1, treatment related toxicities that have not recovered to ≤ grade 2 in ≤ 2 weeks following day 3 of any treatment cycle require discontinuation of hu14.18-IL2.

Please refer to APPENDIX 4 for a decision guide to assist with toxicity management for subjects in Phase IC and Phase ID

Table 6.1.2-1: Hu14.18-IL2 Toxicity Management		
Toxicity	**Management**	**Further Instructions**
Hematologic Toxicity		
• Grade 3 Hgb OR • Grade 3 PLT	• Transfusions are at MD discretion	See section 6.1.2.1 for further instructions.
Hepatic Toxicity (AST, ALT or total bilirubin)		
• Transient hepatic toxicity ≤ Grade 2	• No dose interruption or dose modification required	
• Grade 3 hepatic toxicity	• Hold hu14.18-IL2 for current cycle and resume at next cycle at 50% dose if recovered to < 2 x the ULN or < 2 x baseline if baseline was abnormal.	
Cardiac Toxicity and/or Dyspnea		
• Any evidence of cardiac abnormality	• Requires immediate ECG evaluation	• Evidence of ischemia will require immediate discontinuation of therapy. • Evidence of asymptomatic atrial irregularities related to elevated temperature, without evidence of ischemia or clinically significant hypotension, will be monitored but continue therapy.
• Fluid Overload	• Subjects with clinical problems related to fluid overload can be treated with furosemide (20 mg IV) if they	

	are not also hypotensive (i.e. if they have a < 40 mmHg decrease in systolic BP from day 1 of cycle, obtained prior to initiation of protocol therapy, and a systolic BP >90 mmHg)	

• Hypotension (Mild hypotension with systolic BP 10-15% below baseline is common in patients receiving IL2)		
○ Asymptomatic/mild hypotension	• No intervention required	
• Symptomatic hypotension **AND/OR** • Systolic BP < 85mmHg or < 100mmHg if >20% systolic decrease from baseline on Day 1 of treatment cycle	• Give 500 mL IV bolus of normal saline over 15 minutes	• If unresponsive, see section 6.1.2.2.1 for further management instruction
• Grade 3 Acute vascular leak syndrome	• Subject will be admitted to hospital for management. If resolves to baseline by the start of the next cycle, dose reduce 50%	See section 6.1.2.2.2 for further details
• Dyspnea with oxygen saturation <90%	• Hospitalized for close monitoring and may receive brief oxygen supplementation • Discontinue hu14.18-IL2 until oxygen saturation is >90% without supplementation. ○ If recovered in less than 1 hour, may reinitiate hu14.18-IL2 the following day (if scheduled) at 100% dose. ○ If recovery took > 1 hour, reinitiate hu14.18-IL2 at the next scheduled dose at 50%. • If dyspnea with oxygen saturation < 90% is recurrent,	See section 6.1.2.2.3 for further instruction • If subject is fluid overloaded, hypoxic and hypotensive, treatment should be stopped and vasopressor support initiated, as needed. Once toxicities resolve, subsequent treatment will be at the 50% dose level. • If dyspnea with oxygen saturation <90% is recurrent at 25% of the initial

	stop treatment, treat as above and reduce dose an additional 50%	hu14.18-IL2 dose, treatment will be permanently discontinued (maximum of 2 dose reductions allowed prior to permanent discontinuation being required).
• Arrhythmias	• Any evidence of arrhythmia will require an immediate ECG. o Evidence of ischemia will require hospital admission for management and immediate discontinuation of hu14.18-IL2 o Subjects with evidence of asymptomatic atrial irregularities related to elevated temperature, but no evidence of ischemia or clinically significant hypotension will be monitored but continue therapy	
Neurotoxicity		
• Any ≥ grade 3 neurotoxicity for > 3 days (except confusion)	• Subjects with objective peripheral neuropathy or motor weakness will have treatment held. • Treatment with hu14.18-IL2 may resume at 50% dose once toxicity recovers to ≤ Grade 1.	
• Confusion	• Confusion related to temperature elevations will be managed by aggressive use of antipyretics and cooling blankets, and will not require dose reduction or drug stoppage, if the confusion resolves. • Persistent confusion (>6 hours) and/or confusion not related to temperature	

	elevation or supportive care medicines (Benedryl, morphine, etc), will require admission to the hospital for additional management. Treatment with hu14.18-IL2 will be held. Subsequent re-initiation of hu14.18-IL2 at 50% of the prior dose is allowed at the next scheduled dose upon reversal of this toxicity.	
Metabolic/Laboratory		
• Hyponatremia (Na < 125 nmol/L, > 48 hours) OR • Hyponatremia without symptoms (Na <120 nmol/L)	• Treatment with hu14.18-IL2 will be stopped. The hu14.18-IL2 can be resumed at 50% of the prior dose upon reversal of this toxicity to pre-treatment baseline.	
• Symptomatic hypophosphatemia OR • Asymptomatic hypophosphatemia (PO4 $\leq$ 2.0 mg/dL)	• Treat with oral or IV phosphate supplementation.	
• Glucose > 250 mg/dL persisting for > 12 hours	• May be treated with parenteral insulin	
Fatigue & Performance Status		
• Grade 4 fatigue	• Treatment with hu14.18-IL2 will be stopped. If the fatigue improves to $\leq$ Grade 2 fatigue in < 1 week, hu14.18-IL2 can be resumed at 50% of dose	
• ECOG Performance Status = 4	• Treatment with hu14.18-IL2 will be stopped. If improves to ECOG $\leq$ 2 in < 1 week, hu14.18-IL2 can be resumed at 50% of dose	
Temperature Elevations		
• Any fever (>37.5° C)	• Management depends on grade.	• See section 6.1.2.2.4 for detailed management.

Pain		
• Pain due to hu14.18-IL2	• Management depends on grade.	• See section 6.1.2.2.5 for detailed management.
Nephrotoxicity		
• Serum Creatinine > 1.5 x ULN	• Avoid nephrotoxic drugs • Hold Hu14-18-IL2 if Grade 3 toxicity	

Injection-Site Complications		• See section 6.1.2.2.6 for detailed management.
All Other		
Grade 3 toxicity (not listed in section 6.1.1.3) or Grade 4 toxicity (excluding Grade 4 lymphopenia or localized ulceration at the treatment site)	First Occurrence: Hold dose; may restart at next scheduled dose timepoint (Day 2 or Day 3, even if day 2 is skipped), or Day 1 of next cycle, with 50% dose reduction if relevant test/value returns to pre-treatment baseline	
	Second Occurrence: Hold dose; may restart at next scheduled dose (Day 2 or Day 3, even if day 2 is skipped), or Day 1 of next cycle at 25% of initial dose if relevant test/value returns to pre-treatment baseline (no further reductions allowed) If toxicity doesn't resolve to pre-treatment, baseline, no further treatment –follow per protocol.	

6.1.2.1 Hematologic Toxicity

All subjects may be transfused as needed to maintain an adequate Hgb level (≥ 8.0 g/dL) and platelet count (≥ 50,000/mm^3) during Days 1-3 of treatment each cycle. Hematologic DLT criteria are detailed in protocol section 6.1.1. As there may be an association of vascular leak syndrome with co-administration of monoclonal antibody conjugated IL2 and transfusions, it is recommended to avoid transfusions during the 3 days of hu14.18-IL2 administration. If transfusions are needed, it is recommended that that they be given at least 12 hours before starting treatment on Day 1 of each cycle and at least 12 hours after finishing the Day 3 injection. If transfusions must be given on Days 1 or 2 to maintain Hgb > 8.0 g/dL and PLT > 50,000/mm^3, these should be given 8-12 hours after IT injection of hu14.18-IL2 and require admission to the hospital for management. If the subject experiences neutropenia

(ANC < 1000) while receiving IC, treatment would not be interrupted since neutropenia is common and rapidly reversible upon stopping IL2 treatment. For Days 4-21 of each cycle of therapy, transfusions may be given at the discretion of the treating physician to maintain a platelet count of > 50,000/mm^3, and a Hgb of > 8.0 g/dL.

6.1.2.2 Cardiac Toxicity and/or Dyspnea

6.1.2.2.1 Hypotension

Mild hypotension with systolic blood pressure 10-15% below baseline is common in subjects receiving IL2.

a. Asymptomatic subjects with mild hypotension should be monitored accordingly to protocol guidelines.

b. Symptomatic subjects and/or those with systolic blood pressure < 85 mmHg or < 100 mmHg if representing a decrease in systolic blood pressure > 20% from that obtained pretreatment on day 1 of treatment cycle should be given a 500 mL IV bolus of normal saline over 15 minutes. If responsive, (i.e., blood pressure increases to a systolic blood pressure over 100 mm Hg or to the subject's blood pressure prior to administration of hu14.18-IL2 for that day if the baseline systolic blood pressure was less than 100 mm Hg), hu14.18-IL2 treatment may be reinitiated on the following day (if scheduled) at 50% reduced dose. If unresponsive:
   - Discontinue the hu14.18-IL2 IT injection.
   - Repeat the fluid bolus: 500 mL IV bolus of normal saline over 15 minutes. Fluid bolus may be repeated once or twice as needed. This may be limited by symptomatic fluid overload (i.e. pulmonary edema, tense ascites).
   - If unresponsive to fluid bolus, albumin (25%) 50 gm IV may be given and repeated q6-12 h PRN if serum albumin ≤ 3.0.
   - Give RBC transfusions to maintain Hgb ≥ 8.0 g/dL.
   - If responsive, (i.e., blood pressure increases to a systolic blood pressure over 100 mm Hg or to the subject's blood pressure prior to administration of hu14.18-IL2 for that day if the baseline systolic blood pressure was less than 100 mm Hg), hu14.18-IL2 treatment may be reinitiated on the following day (if scheduled) at 50% reduced dose. If hypotension is recurrent, treatment will be stopped and subjects managed as outlined above in this section. Treatment can be restarted (at their next scheduled infusion day) at 25% of the initial dose, if systolic blood pressure is responsive to the fluid management described above. If hypotension is recurrent at this 25% dose, hu14.18-IL2 treatment will be permanently discontinued and the subject will be followed, per protocol.

c. Symptomatic hypotension that does not respond to these measures requires admission to the hospital for management of hypotension as an in-patient. Guidelines for additional management include the following:
   - IV vasopressors (i.e. phenylephrine, dopamine, etc.).

- If responsive (i.e. blood pressure increases to a systolic blood pressure over 100 mm Hg or to the subject's blood pressure prior to administration of hu14.18-IL2 for that day if the baseline systolic blood pressure was less than 100 mm Hg and no longer requiring vasopressor support), hu14.18-IL2 may be reinitiated the following day (if scheduled) at 50% reduced dose. If hypotension is recurrent, treatment will be stopped and subjects managed as outlined above in this section. Treatment can be restarted (at their next scheduled infusion day) at 25% of the initial dose, if systolic blood pressure is responsive to the fluid management described above. If hypotension is recurrent at this 25% dose, treatment will be permanently discontinued and the subject will be followed per protocol.
- For grade 3 hypotension, see dose modification guidelines in section 6.1.1.6.
- Additional boluses of 500 mL IV normal saline can be administered as clinically indicated.

6.1.2.2.2 Acute vascular leak syndrome

High dose IL2 can induce a capillary leak syndrome and sepsis-like physiology (decreased systemic vascular resistance, increased cardiac output and some degree of peripheral or pulmonary interstitial edema and/or ascites). Cardiovascular toxicity may be additive when IL2 is combined with hu14.18. Subjects will be admitted to the hospital for management of grade 3 acute vascular leak syndrome. Dose modification guidelines are provided in section 6.1.1.6.

6.1.2.2.3 Dyspnea

Subjects experiencing dyspnea and whose oxygen saturation is less than 90% will be admitted to the hospital for close monitoring and may receive brief oxygen supplementation. Subjects who experience this toxicity are often fluid overloaded and will need furosemide, provided that they are not hypotensive (see management of hypotension, above). Therapy will be discontinued and not restarted until the oxygen saturation is above 90% without oxygen supplementation. Once the oxygen saturation is above 90% without oxygen supplementation, treatment with hu14.18-IL2 may be reinitiated on the following day (if scheduled) at 100% dose (if recovery occurred in less than 1 hour) or at 50% reduced dose (if recovery took longer than 1 hour to resolve). If they are fluid overloaded, hypoxic and hypotensive, treatment should be stopped and vasopressor support initiated, as needed. Once toxicities resolve, subsequent treatment will be at the 50% dose level.

If dyspnea with oxygen saturation below 90% is recurrent, treatment will be stopped and subjects managed as outlined in this section. Treatment can be restarted (at their next scheduled infusion day) at 50% of the prior dose, if oxygenation is responsive to the management described in this section. If dyspnea with oxygen saturation below 90% is recurrent at 25% of the initial dose, treatment will be permanently discontinued and the subject will be followed per protocol.

6.1.2.2.4 Temperature Elevations

Fusion protein treatment will be held for persistent temperature elevations (6 hours) of 40º C or greater not responding to symptomatic treatment with antipyretics. Provided that renal function remains stable (i.e. creatinine does not increase more than 50% over baseline), all subjects will receive indomethacin 25 mg/dose every 6 hours, starting 1 hour before the first dose of hu14.18-IL2 and continuing 12 hours after the last dose on Day 3 (see premedication, section 6.1.3). Temperature elevations > 38º C will then be treated with addition of acetaminophen every 4 hours. As most subjects experience fevers, it is recommended to begin acetaminophen (325-650 mg) as soon as any fever (>37.5° C) develops after the first dose of hu14.18-IL2 and to continue it on a scheduled basis for the next 60-72 hours. Rigors, which may accompany temperature changes, can be treated with meperidine IV. Persistent temperature elevations > 39° C which are not well controlled with acetaminophen and indomethacin may also be treated with a cooling blanket. No dose modifications will be made for temperature elevations (unless the temperature persists > 40º C for > 6 hours) despite treatment with the above antipyretic approaches. If fever does persist for > 6 hours despite antipyretics, then hu14.18-IL2 treatment will be stopped. Treatment can be restarted within a 3 day cycle of treatment once the temperature is ≤ 38º C. Treatment will then be at 50% of the dose which caused this toxicity.

Use of prophylactic antibiotics for fever:

Although the IL2 component of hu14.18-IL2 is known and expected to cause fever, IL2 is also known to cause some neutrophil dysfunction and predispose to bacterial infections. Thus, for fever > 38.5° C, it is recommended that blood cultures be drawn, and a broad spectrum antibiotic be considered. Subjects will be admitted to the hospital for additional management if fever persists or the subject is clinically unstable.

6.1.2.2.5 Pain

Subjects experiencing pain due to hu14.18-IL2 will be treated with morphine or similar analgesics as needed and have their pain graded according to the CTCAE. It is recommended to have morphine available, but not given as a premedication, on the first day of treatment. If pain is noted and morphine is needed on that first day, then including morphine as a premedication for all subsequent doses may be helpful. Subjects with pain that is not controlled with IV opiods or that requires IV opiods > 48 hours after completion of hu14.18-IL2 on day 3 of any treatment cycle will not receive additional protocol treatment at that dose. If the pain resolves and returns to pre-treatment/baseline values, subsequent cycles would be given at 50% of the prior dose.

6.1.2.2.6 Injection-Site Complications

Erythema and induration are anticipated local complications from IT administration of hu14.18-IL2. Systemic analgesics (acetaminophen or ibuprofen) and topical analgesics (topical lidocaine) can be used to management symptoms. Small ulcerations may develop, particularly if tumor necrosis is developing. Topical antibiotic cream or ointment can be used to prevent secondary infection. Major ulceration requiring operative intervention is considered a DLT unless the responsible physician feels it is

due to a rapid antitumor effect. In this case, no additional IT-IC administration will be delivered to that site, but the subject may continue in subsequent courses of treatment, if eligible, as described in sections 5.5.1, 5.5.2, 5.5.3 and 5.5.4. Subjects who experience Grade 3 allergic reactions that are considered true allergic reactions (i.e. IgE-mediated and usually worsen with re-exposure) will not be retreated.

6.1.3 Medications acceptable for hu14.18-IL2 symptom management*

- Acetaminophen 650 mg PO q4 hrs PRN (maximum of 3250 mg daily) for fever
- Diphenhydramine 25-50 mg PO/IV q6 hrs PRN, pruritis, urticaria
- Hydroxyzine 50-100 mg PO/IM q6 hrs PRN, pruritis, urticaria, or anxiety
- Loperamide 2 mg tablets, 4 mg PO initially followed by 2 mg after each unformed stool; maximum of 16 mg qd PRN
- Lorazepam 0.5-1 mg PO/IV q2-4 hrs PRN, anxiety
- Meperidine 25 mg IV q3-4 hrs PRN for chills/rigors; subjects developing chills may be treated for possible infection with broad spectrum antibiotics
- Morphine sulfate 1-4 mg IV q2-4 hrs PRN, pain
- Naproxen 250-500 mg PO bid PRN**
- Ondansetron 8 mg PO/IV q12 hours PRN, nausea/vomiting
- Ondansetron 24 mg IV prior to treatment/8 mg PO tid PRN, nausea/vomiting
- Oxycodone 5-10 mg PO q4 hrs PRN, pain
- Prochlorperazine 10 mg PO q6 hrs PRN or 25 mg PR q12 hrs PRN, nausea
- Pseudoephedrine 60 mg PO q6 hrs PRN, nasal congestion
- Ranitidine 150 mg PO bid PRN, peptic ulcers

* Systemic treatment doses of corticosteroids or other immunosuppressive drugs will result in discontinuation of protocol therapy with hu14.18-IL2 (clarification: physiologic maintenance or replacement doses of systemic steroids is acceptable). If the use of systemic treatment doses of corticosteroids or other immunosuppressive drugs is considered unlikely or not related to hu14.18-IL2, the hu14.18-IL2 treatment may be resumed with the subsequent cycle at the discretion of the responsible physician.

** Care should be taken when using non-steroidal, anti-inflammatory drugs (NSAIDs) secondary to potential nephrotoxicity.

6.2 Management of Nivolumab and Ipilimumab related toxicity

6.2.1 Description of immune-related adverse reactions (Adapted from the EMA – "Yervoy: EPAR - Product Information" [53])

Nivolumab and ipilimumab are associated with inflammatory adverse reactions resulting from increased or excessive immune activity (immune-related adverse reactions), likely to be related to its mechanism of action.

Immune-related adverse reactions, which can be severe or life-threatening, may involve the gastrointestinal, liver, skin, nervous, endocrine, or other organ systems. While most immune-related adverse reactions occurred during the induction period, onset months after the last dose

of ipilimumab or nivolumab has also been reported. Unless an alternate etiology has been identified, diarrhea, increased stool frequency, bloody stool, LFT elevations, rash and endocrinopathy must be considered as potential immune-related reactions. Early diagnosis and appropriate management are essential to minimise lifethreatening complications.

Systemic high-dose corticosteroid therapy is often required for management of severe immune-related adverse reactions. Additional immunosuppressive agents, such as a single dose of infliximab 5 mg/kg, may also be required for severe immune-related adverse reactions that do not improve with corticosteroid therapy unless contraindicated. Infliximab must not be used if gastrointestinal perforation or sepsis is suspected.

6.2.2 Treatment Cessation/Dose Modifications for toxicity from Nivolumab and Ipilimumab

The following guidelines apply to treatment with nivolumab alone, or nivolumab in combination with ipilimumab. Regardless of whether the event is attributed to either nivolumab, ipilimumab, or both, both study drugs (if applicable) must be delayed or discontinued. **The below criteria only apply if the adverse event is considered possibly, probably, or definitely related to either nivolumab or ipilimumab**. Tumor assessments for all subjects should continue as per protocol even if dosing is delayed.

Dose reductions or dose escalations are not permitted.

Phase 1D only: Subjects experiencing grade ≥ 3 toxicities that require holding of therapy during the ipilimumab/nivolumab induction period (first 12 weeks) that meet re-treatment criteria per the below table by week 16 may resume nivolumab monotherapy at the earliest convenient opportunity, but not less than at the time of the next scheduled dose. In this situation, ipilimumab may not be resumed. Dose discontinuations still apply for all adverse events as indicated below.

Any event that leads to a delay in dosing lasting > 6 weeks from the previous dose requires discontinuation, with the following exceptions:

- Dosing delays to allow for prolonged steroid tapers to manage drug-related adverse events are allowed
- Dosing delays lasting > 6 weeks from the previous dose that occur for non-drug-related reasons may be allowed if approved by the Principle Investigator.

Periodic study visits to assess safety and laboratory studies should also continue every 6 weeks or more frequently if clinically indicated during dosing delays.

Please refer to APPENDIX 2 for Ipilimumab and Nivolumab Toxicity Management Algorithms. The toxicity management algorithms are recommendations only, and the above dose modification should always be followed. Not following the management algorithms, as long as the above dose modification requirements are adhered to, will not be considered a protocol deviation.

Please refer to APPENDIX 4 for a decision guide to assist with toxicity management for subjects in Phase IC and Phase ID

Table 6.2.2-1: Drug-related Nivolumab and Ipilimumab Toxicity Management[a]		
Toxicity	**Management**	**Further Instructions**
Hematologic Toxicity		
Lymphocyte count decreased Grade 4	Dose hold	Resume when resolved to ≤ grade 1
Platelet count decreased Grade 3	Dose hold. Discontinuation required if > 7 days or associated with bleeding	Resume when resolved to ≤ grade 1
Neutrophil count decreased Grade 4	Discontinue treatment if >7 days	Resume when resolved to ≤ grade 1
White blood cell decreased Grade 4	Dose hold	Resume when resolved to ≤ grade 1
Hepatic Toxicity (AST, ALT or total bilirubin)		
Grade 2 AST/ALT OR total bilirubin	Dose hold	Resume when resolved to ≤ grade 1
Grade ≥ 2 AST/ALT AND total bilirubin	Discontinue treatment	
Grade ≥ 3	Discontinue treatment	
Refer to Appendix 2 Hepatic Adverse Event Management Algorithm for recommendations		
Creatinine Increased		
Grade 1	Dose hold not required	
Grade ≥ 2	Dose hold	
Refer to Appendix 2 Renal Adverse Event Management Algorithm for recommendations		
Amylase & Lipase Grade ≥ 3	Dose hold required if associated with symptoms or clinical manifestations of pancreatitis	
Electrolyte imbalances/abnormalities, grade 4	Discontinue treatment unless not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of onset	
All Other Laboratory Based Toxicities[b]		
Grade 3	Dose hold	Resume when resolved to ≤ grade 1
Grade 4	Discontinue treatment	
Skin toxicity		
Grade 1-2	Dose hold not required.	Refer to skin AE management algorithm for

		recommendations if toxicity persists > 1 week or recurs
Any Grade ≥ 3 drug-related skin adverse event	Dose hold	
Refer to Appendix 2 Skin Adverse Event Management Algorithm for recommendations		
Fatigue		
Grade ≥ 3	Dose hold	Resume when resolved to grade ≤ 2
Pneumonitis		
Grade 1	Dose hold not required	
Persistent Grade 1	Dose hold	Subjects with persistent grade 1 pneumonitis after completion of a steroid taper over at least 1 month may be eligible for retreatment following pulmonary consultation and with approval of the pulmonary consultant
Grade 2	Dose hold	Must resolve to grade 0 to resume treatment
Grade 3	Discontinue treatment	
Refer to Appendix 2 Adverse Event Management Algorithm for recommendations		
Diarrhea & Colitis		
Grade 1	Dose hold not required	
Grade 2	Dose hold	May resume when resolved to ≤ grade 1. May hold until baseline at the discretion of the treating physician
Grade ≥ 3	Discontinue treatment	
Refer to Appendix 2 GI Adverse Event Management Algorithm for recommendations		
Endocrinopathies		
Grade ≥ 2	Dose hold	Endocrinopathies controlled with only a physiologic hormone replacement may resume treatment after consultation with an endocrinologist.
Refer to Appendix 2 Endocrinopathy Management Algorithm for recommendations		
Uveitis		
Grade 2	Dose hold	Resume when resolved to ≤ grade 1
Grade ≥ 3	Discontinue Treatment	

Bronchospasm		
Grade 2	Dose hold	Resume when resolved to ≤ grade 1
Grade ≥ 3	Discontinue Treatment	
Neurologic Toxicity		
Grade 2	Dose hold	Resume when resolved to ≤ grade 1
Grade ≥ 3	Discontinue Treatment	
Hypersensitivity Reaction		
Grade 2	Dose hold	Resume per MD discretion
Grade ≥ 3	Discontinue Treatment	
Infusion Reaction		
Grade 2	Dose hold	Resume when resolved, per MD discretion
Grade ≥ 3	Discontinue Treatment	
Other Non-Laboratory Adverse Events[b]		
Grade 2	Dose hold	
Grade 3	• Dose hold if event lasts ≤ 7 days • Discontinue Treatment if event lasts > 7 days or recurs	
Grade 4	Discontinue Treatment	
a) Subjects requiring dose delay should be re-evaluated weekly or more frequently if clinically indicated and resume dosing when re-treatment criteria are met b) Study medication dosing may be delayed for any adverse event, laboratory abnormality, or intercurrent illness if warranted in the judgement of the investigator		

6.3 Toxicities Associated with Palliative Radiation Therapy

Table 6.3-1: Potential Risks of Radiation Therapy

Acute Common	Acute Less common	Subacute/late Common	Subacute/late Less common	Late/Less common
• Fatigue • Skin reaction (erythema, desquamation)	• Nausea • Diarrhea • Pain flare • Mucositis	• Fibrotic and hyperpigmentation changes in skin/subcutaneous tissue	• Injury/damage to normal tissues in the radiation field	• Injury/damage to normal tissues in the radiation field

In the very unlikely event that the initial course of radiation therapy would need to be stopped due to radiation-related toxicity, holding or potentially delaying initiation or resumption of radiation therapy may occur at the discretion of the responsible treating radiation oncologist.

If acute, subacute or delayed unexpected radiation toxicity occurred at the initial palliative radiation site which is considered to be made more severe by the concomitant hu14.18-IL2, and considered clinically unacceptable by the responsible radiation oncologist, this will

preclude administration of hu14.18-IL2 together with radiation for this subject if they are to later require palliative radiation (section 5.2.9).

Palliative radiation therapy is now commonly administered with dual checkpoint blockade in clinic practice. As such, subjects in Phases IC or ID who require additional palliative radiation after an unacceptable toxicitiy at the radiated and hu14.18-IL2 injected site may receive it. This would be given with concurrent checkpoint blockade per protocol guidelines, and with a temporary dose hold for hu14.18-IL2, at the discretion of the treating medical and radiation oncologists."

Subjects receiving radiation therapy will continue safety monitoring for a period of at least 12 months after RT for potential sub-acute and late effects of the RT.

6.4 DLT-Level Toxicities Occuring Outside the DLT Monitoring Period

6.4.1 Monitoring

DLT-level toxicities (section 6.1.1.5) and unexpected severe (grade 3 or greater) non-DLT level toxicities that occur outside the DLT monitoring period will be tablulated and reviewed at least every 8 weeks at UWCCC melanoma immunotherapy clinical research team conferences. These toxicities will be reported quarterly to the UWCCC-DSMC. This applies only to those adverse events which are considered possibly, probably, or definitely related to protocol therapy (including late radiation toxicities). In the event that the protocol chair or responsible medical oncologist feels any clinical event or toxicity requires more timely/urgent discussion, the PI and/or protocol chair will be alerted. They will call an emergency protocol-team meeting to take place within 2 weeks to discuss the issue and consider all appropriate options, including potential suspension of accrual, if warranted.

6.4.2 Course of Action

- For Phases IA, IB and IC, if ≥2 out of 3 or ≥ 3 out of 6 subjects in a given cohort have delayed toxicity probably, or definitely related to hu14.18-IL2 (or if the PI or protocol chair determine a need to suspend accrual for less frequent but concerning toxicity):
    1. Accrual to the protocol will be suspended with notification to the IRB and FDA. This will allow time for consideration by the PI, investigators, study statistician, and UWCCC DSMC, of protocol closure vs. amendment. Investigation of the accumulated toxicity events will inform the decision whether to continue the trial or to terminate for patient safety concerns. Approval of an amended protocol by the IRB and FDA is required prior to re-opening accrual, if that route is pursued.
    2. In the event of suspension of protocol accrual, as defined above, subjects who are still on protocol treatment, and subjects who have come off of treatment, will be notified of the need to suspend accrual and the details requiring such suspension. Details of notification will be included in the subject's medical record.
    3. In the event of suspension of protocol accrual, subjects who are still on protocol treatment, will be presented with the option of continuing on treatment, versus cessation of all protocol treatment, if their clinical course is consistent with staying

on treatment, as assessed by their responsible physician. Documentation of this conversation and decision will be placed in the medical record.

- For the phase ID expanded cohort (anticipated accrual 28 subjects), the approach for determining excessive delayed toxicity takes into account the clinical experience with combination nivolumab + ipilimumab therapy for advanced melanoma, which is associated with treatment-related adverse events of grade 3 or 4 in 59% of patients[54]. This adverse event incidence is expected with this standard of care treatment and notably, many of the patients who required cessation of treatment still demonstrated lasting benefit from the treatment. Thus, an adverse event incidence (grade 3 or 4) of 59% that is possibly, probably or definitely associated with the combination of nivolumab and ipilimumab is expected. To ensure that subjects are not exposed to an unacceptably high incidence of toxicity (i.e., > 59%), adverse events (grade 3-4, other than those defined in protocol section 6.1.1.3 as hu14.18-IL2 DLT exceptions) in subjects treated with the combination therapy of hu14.18-IL2 with local radiation, nivolumab and ipilimumab will be monitored continuously based on sequential probability ratio tests (SPRT; for details see section 12.3.4). If there is an unacceptably high incidence of the aforementioned adverse events (probably or definitely related to hu14.18-IL2) as evidenced by crossing the SPRT boundary, follow the course of action described in 1-4 above.

- Upon completion of accrual for this study, the data used to consider the MTD/MAD of this regimen (as developed in phase ID, or for the regimens developed in phases IA, IB, or IC) for future potential use in subsequent trials, will take into consideration not only the MTD/MAD determined by the 42 day DLT window, but also the entire spectrum of acute and delayed toxicities observed for all subjects throught the monitoring period.

6.5 Concomitant Therapy

6.5.1 No other cancer chemotherapy, growth factors, or therapeutic doses of corticosteroids can be used unless required to manage immune-related toxicity. Management with maintenance physiologic doses of corticosteroids is acceptable.

6.5.2 RT to any currently or imminently symptomatic lesion is acceptable. No radiated lesion may be used to assess overall tumor response to protocol therapy except for index tumor lesions that also receive IT therapy. Subjects should be evaluated prior to RT with appropriate tumor imaging. If any tumor site becomes symptomatic or imminently symptomatic while a subject is enrolled on study, then systemic or topical medication may be used to treat these symptoms and additional palliative RT can be delivered to such a lesion if deemed appropriate by the treating radiation oncologist. If such a lesion requiring palliative RT is an index lesion that also has been receiving IT therapy (i.e. Lesion A) and the subject remains otherwise eligible for continued treatment, then this lesion may be re-treated with palliative RT. Subsequent cycles of IT-therapy in this case should be given into an alternate injectable lesion for any remaining cycles of IT therapy (i.e. Lesion B for

subjects with 2 injectable lesions and Lesion C for subjects with >3 injectable lesions). If this situation arises in a subject enrolled on protocol arms IB, IC, or ID, this alternate injectable lesion should preferentially be chosen from any injectable tumor site that has also received palliative RT during the study enrollment period. If no site other than the index lesion has received or requires palliative RT in such individuals, then an injectable non-radiated tumor site may be selected for continuing IT therapy.

6.5.3 Other supportive medicines

Standard symptom management treatments may be continued per the discretion of the treating physician, provided that they are not growth factors, steroids, or myelosuppressive anti-neoplastic drugs.

6.6 Removal from Protocol Therapy and Off Study Criteria

6.6.1 Criteria for Removal From Protocol Therapy

1) Progressive disease.
    a. Symptomatic progression: Subjects with symptomatic disease progression that, in the opinion of the investigator, is significant enough to warrant a treatment plan change. Where appropriate palliative RT may be used to attempt to control symptoms of progression and do not require removal from protocol therapy. If the only site of progression is the lesion being injected, a biopsy of the lesion can be done at any time to clarify the status of the lesion's response vs. progression (see section 10 for definition of progressive vs. locally responsive disease) or
    b. Clinical or radiological evidence of disease progression after 4 cycles of hu14.18-IL2 treatment have been completed (the first scheduled disease evaluation). Subjects who are determined to have progressive disease, per irRECIST, prior to or at the time of their week 12 disease assessment, will be permitted to continue treatment at the discretion of the treating physician. This is permitted in the absence of a decline in performance status, and as long as there is less than a 50% increase in tumor burden from beginning of treatment. Those subjects may continue to be treated through the week 24 disease assessment timepoint per investigator discretion unless there is a decline in performance status, a need for additional treatment, including new CNS lesions that require immediate treatment.
2) Drug intolerance as described in section 6.
3) Hu14.18-IL2 treatment related toxicities (definitely, probably, or possibly related) that have not recovered to $\leq$ Grade 2 in $\leq$ 2 weeks following day 3 of any treatment cycle require discontinuation of hu14.18-IL2.
4) Refusal of further protocol therapy by subject.
5) Completion of the maximum allowable cycles of therapy.
6) Physician determines that removal from protocol therapy is in subject's best interest.

Subjects who are off protocol therapy are to be followed until they meet the criteria for Off Study (see section 6.6.2). After completion of therapy, subjects still on study will be followed every 3 months for response to treatment until disease progression occurs or until 2 years after

receiving therapy. After such time, subjects will be followed every 6 months for survival. Follow-up data will be required unless consent is withdrawn.

6.6.2 Off Study Criteria

1) Death
2) Lost to follow-up (defined as 3 attempts to contact the subject, the third attempt to be via certified letter)
3) Withdrawal of consent for any further data submission

7.0 DATA AND SAFETY MONITORING PLAN

7.1 Oversight and Monitoring Plan

The UWCCC Data and Safety Monitoring Committee (DSMC) is responsible for the regular review and monitoring of all ongoing clinical research in the UWCCC. A summary of DSMC activities are as follows:

- Reviews all clinical trials conducted at the UWCCC for subject safety, protocol compliance, and data integrity.
- Reviews all Serious Adverse Events (SAE) requiring expedited reporting, as defined in the protocol, for all clinical trials conducted at the UWCCC, and studies conducted at external sites for which UWCCC acts as an oversight body.
- Reviews all reports generated through the UWCCC DSMP elements (Internal Audits, Quality Assurance Reviews, Response Reviews, Compliance Reviews, and Protocol Summary Reports) described in section 7.2 of this document.
- Notifies the protocol chair and Principal Investigator of DSMC decisions and, if applicable, any requirements for corrective action related to data or safety issues.
- Notifies the CRC of DSMC decisions and any correspondence from the DSMC to the protocol Principal Investigator.
- Works in conjunction with the UW Health Sciences IRB in the review of relevant safety information as well as protocol deviations, non-compliance, and unanticipated problems reported by the UWCCC research staff.
- Ensures that notification is of SAEs requiring expedited reporting is provided to external sites participating in multi-institutional clinical trials coordinated by the UWCCC.

7.2 Monitoring and Reporting Guidelines

UWCCC quality assurance and monitoring activities are determined by study sponsorship and risk level of the protocol as determined by the PRMC. All protocols (including Intervention Trials, Non-Intervention Trials, Behavioral and Nutritional Studies, and trials conducted under a Training Grant) are evaluated by the PRMC at the time of committee review. UWCC monitoring requirements for trials without an acceptable external DSMB are as follows:

Protocols subject to intermediate monitoring generally include UW Institutional Phase I/II and Phase II Trials. These protocols undergo review of subject safety at regularly

scheduled DOT meetings where the results of each subject's treatment are discussed and the discussion is documented in the DOT meeting minutes. The discussion includes the number of subjects enrolled, significant toxicities, dose adjustments, and responses observed. Protocol Summary Reports are submitted on a quarterly basis by the study team for review by the DSMC.

7.3 Review and Oversight Requirements

7.3.1 Serious Adverse Event – Reported within 24 Hours

Serious Adverse Events requiring reporting within 24 hours (as described in the protocol) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu within one business day. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (NCI AdEERS form or FDA Medwatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if immediate action is required. Within 10 working days, all available subsequent SAE documentation must be submitted electronically along with a 24 hour follow-up SAE Details Report and a completed UWCCC SAE Routing Form to saenotify@uwcarbone.wisc.edu. All information is entered and tracked in the UWCCC database.

The Protocol Chair notifies all investigators involved with the study at the UWCCC, the IRB, the sponsor, and the funding agency and provides documentation of these notifications to the DSMC. The Protocol chair reviews the event to determine whether the SAE requires reporting to the FDA and other participating investigators.

See Section 8.0 for detailed instructions on SAE reporting.

7.3.2 Serious Adverse Event – Reported within 10 Days

Serious Adverse Events requiring reporting within 10 days (as described in the protocol) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (NCI AdEERS form or FDA Medwatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if further action is required. All information is entered and tracked in the UWCCC database.

The Protocol chair notifies all investigators involved with the study at the UWCCC, the IRB, the sponsor, and the funding agency and provides documentation of these notifications to the DSMC. The Protocol chair reviews the event to determine whether the SAE requires reporting to the FDA and other participating investigators.

See Section 8.0 for detailed instructions on SAE reporting.

7.3.3 Sponsor-Investigator Responsibilities for SAE Review

As the UWCCC Principal Investigator is acting as the Sponsor-Investigator (i.e., the PI holds the IND), the PI assumes responsibilities of the study sponsor in accordance with FDA 21 CFR 312.32. In this capacity, the UWCCC PI interacts with the study chair to review all reports of serious adverse events occurring on the study at the UWCCC and makes a determination of 1) **suspectedness** (i.e., whether there is a reasonable possibility that the drug caused the AE); and 2) **unexpectedness** (the event is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed) in the context of this study. SAE with suspected causality to study drug and deemed unexpected are reported as IND Safety Reports by the UWCCC PI to the FDA, all participating investigators on the study, and the study supporters (namely BMS and Apeiron Biologics) within 15 calendar days. All fatal or life-threatening SAE that are unexpected and have suspected causality to the study drug will be reported by the UWCCC PI to the FDA, all participating investigators on the study, and the study supporters (namely BMS and Apeiron Biologics) within 7 calendar days.

Refer to Section 8.3 for UWCCC PI instructions for reporting to the FDA.

7.3.4 Study Progress Review

Protocol Summary Reports (PSR) are required to be submitted to the DSMC in the timeframe determined by the risk level of the study (quarterly). The PSR provides a cumulative report of SAEs, as well as instances of non-compliance, protocol deviations, and unanticipated problems, toxicities and responses that have occurred on the protocol in the timeframe specified. PSRs for those protocols scheduled for review are reviewed at each DSMC meeting.

Protocol Summary Reports enable DSMC committee members to assess whether significant benefits or risks are occurring that would warrant study suspension or closure. This information is evaluated by the DSMC in conjunction with other reports of quality assurance activities (e.g., reports from Internal Audits, Quality Assurance Reviews, etc.) occurring since the prior review of the protocol by the DSMC. Additionally, the DSMC requires the study team to submit external DSMB or DSMC reports, external monitoring findings for industry-sponsored studies, and any other pertinent study-related information.

In the event that there is significant risk warranting study suspension or closure, the DSMC will notify the PI of the DSMC findings and ensure the appropriate action is taken for the protocol (e.g., suspension or closure). The DSMC ensures that the PI reports any temporary or permanent suspension of a clinical trial to the sponsor (e.g., NCI Program Director, Industry Sponsor Medical Monitor, Cooperative Group Study Chair, etc.), industry collaborators and other appropriate agencies. DSMC findings and requirements for follow-up action are submitted to the CRC.

8.0 EXPEDITED REPORTING OF ADVERSE EVENTS

Depending on the nature, severity, and attribution of the serious adverse event an SAE report will be phoned in, submitted in writing, or both according to Table 8.0-1 below. All serious adverse events must also be reported to the UWCCC Data and Safety Monitoring Committee Chair. All serious adverse events must also be reported to the UW IRB (if applicable), and any sponsor/funding agency not already included in the list.

Determine the reporting time line for the SAE in question by using the following table.

Table 8.0-1: Expedited Reporting

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1,2]

FDA Reporting Requirements for Serious Adverse Events (21 CRF Part 312)

NOTE: Investigators **MUST** immediately report to the *UWCCC, Apeiron's designated third party FGK (Email: safety@fgk-cro.com; Facsimile: 011-49-89 89 31 19-180),* and any other parties outlined in the protocol ANY Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64).

An adverse event is considered serious if it results in ANY of the following outcomes:

1) Death.
2) A life-threatening adverse event.
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria* **MUST** be immediately reported to the UWCCC within the timeframes detailed in the table below:

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in hospitalization ≥ 24 hrs	10 Calendar Days	24 Hour; 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

Exceptions to Reporting Requirements: The adverse events listed below do not require reporting

- Grade 3 pain, requiring intravenous narcotics, provided that the narcotics are controlling the pain, and that IV narcotics for pain are not required >48 hours after completion of the hu14.18-IL2 on Day 3 of any treatment cycle
- Grade 3 nausea and vomiting that resolves within 48 hours after completion of hu14.18-IL2 on Day 3 of any treatment cycle
- Grade 3 fever (i.e. T > 40° C) lasting less than 6 hours and controllable with antipyretics

- Grade 3 skin toxicity that improves with treatment (e.g. IV Benadryl) within 24 hours.
- Grade 3 metabolic/laboratorytoxicity of hyponatremia, hyperglycemia, or hypophasphatemia, in the absence of CNS symptoms and sequelae, that improve with or without treatment within 48 hrs (See Section 6.1.2 for reporting requirements of symptomatic or severe hyponatremia).
- Grade 3 hematologic toxicity (or grade 4 lymphopenia) which improves to at least Grade 2 or pre-therapy baseline values before the subsequent hu14.18-IL2 treatment cycle.
- Grade 3 infusion reactions lasting less than 24 hours, readily controlled with supportive (non-steroidal) treatments (i.e. Benadryl, subcutaneous epinephrine).
- Grade 3 fatigue or decrease of ECOG performance status to 3 that resolves to pre-treatment, baseline values in ≤ 1 week.
- Grade 3 infection that resolves in ≤ 1 week either with or without antibiotic therapy.
- Grade 3 skin toxicities possibly, probably, or definitely related to RT and no more than unlikely related to hu14.18-IL2.
- Grade 3 neutropenic fever within the first seven days after the first IT-hu14.18-IL2 injection in the absence of any other signs or symptoms of infections.
- Grade 4 skin ulceration if the responsible physician feels it is due to a rapid antitumor effect or due to tumor itself.

These toxicities are exempt from reporting requirements as outlined in the above Expedited Reporting table as they represent known, published, transient, reversible, and non-dose limiting toxicities of IL2 and ch14.18, and of hu14.18-IL2, based on observations of 51 adults with melanoma and 67 children with neuroblastoma or melanoma given similar doses of hu14.18-IL2 by the same schedule tested in this trial, and these toxicities may be expected [23, 26-28, 30].

Expedited AE reporting timelines are defined as:

- **24-Hour; 5 Calendar Days** – The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.

- **10 Calendar Days** – A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE

[1] Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

8.1 SAE Requiring [24] Hour Reporting Occurs at UWCCC:

8.1.1 Report to the UWCCC:

Reference the **SAE SOP** (Standard Operating Procedure) and the **SAE Reporting Workflow for disease oriented teams (DOTs)** on the UWCCC website (http://www.uwccc.wisc.edu) for specific instructions on how and what to report to the UWCCC for *[24]* hour initial and follow-up reports. **A follow-up report is required to be submitted within 10 days of the initial *[24]* hour report.**

For this protocol, the following UWCCC entities are required to be notified:

a) saenotify@uwcarbone.wisc.edu
b) Paul M. Sondel, MD, PhD – Sponsor-Investigator
c) Mark R. Albertini, MD – Study Chair
d) Zachary S. Morris, MD, PhD – Co-Investigator (Phases IB, IC, and ID only)
e) Tamara Koehn – Breast/Melanoma DOT Clinical Team Manager
f) Any other appropriate parties listed on the SAE Routing Form

8.1.2 Report to the Study Supporters:

a) Apeiron Biologics' designated third party FGK:

SAE Email Address: safety@fgk-cro.com
SAE Facsimile Number: 011-49-89 89 31 19-180

SAEs, whether related or not to study drug must be reported to Apeiron's designee FGK within 24 hours. SAEs must be recorded on a FGK form or an approved form (MedWatch 3500A).

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to Apeiron's designated third party FGK using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization.

b) Bristol Myers Squibb, product safety desk

SAE Email Address: Worldwide.Safety@BMS.com
SAE Facsimile Number: 609-818-3804

SAEs, whether related or not to study drug, and pregnancies must be reported to BMS within 24 hours. SAEs must be recorded on a BMS form or an approved form (MedWatch 3500A); pregnancies must be reported on a Pregnancy Surveillance Form.

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to BMS (or designee) using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization.

8.1.3 Report to the IRB:

Consult the UW-IRB website for reporting guidelines.

8.2 SAE Requiring [10] Day Reporting Occurs at UWCCC:

8.2.1 Report to the UWCCC:

Reference the **SAE SOP** and the **SAE Reporting Workflow for DOTs** on the UWCCC website (http://www.uwccc.wisc.edu) for specific instructions on how and what to report to the UWCCC for *[10]* day reports.

For this protocol, the following UWCCC entities are required to be notified:

a) saenotify@uwcarbone.wisc.edu
b) Paul M. Sondel, MD, PhD – Sponsor-Investigator
c) Mark R. Albertini, MD – Study Chair
d) Zachary S. Morris, MD, PhD (Phases IB, IC, and ID only)
e) Tamara Koehn - Breast/Melanoma DOT Clinical Team Manager
f) Any other appropriate parties listed on the SAE Routing Form

8.2.2 Report to the Study Supporters:

a) Apeiron Biologics' designated third party FGK:

   SAE Email Address: safety@fgk-cro.com
   SAE Facsimile Number: 011-49-89 89 31 19-180

   Please refer to Section 8.1.2.a for FGK's (Apeiron's designated third party) reporting requirements.

b) Bristol Myers Squibb, product safety desk

   SAE Email Address: Worldwide.Safety@BMS.com
   SAE Facsimile Number: 609-818-3804

   Please refer to Section 8.1.2.b for BMS SAE reporting requirements.

8.2.3 Report to the IRB:

Consult the UW-IRB website for reporting guidelines.

8.3 Other Reporting Requirements

Reporting to the FDA
Serious Adverse Events occurring on studies on which a UW PI is acting as sponsor-investigator must be reported to the FDA within the appropriate time frame. Mandatory and voluntary reporting guidelines and instructions are outlined on the FDA website: http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm

9.0 STUDY PARAMETERS

9.1 Phase IA Schedule of Events

Table 9.1-1: Phase IA Schedule of Events Through Cycle 1								
(cycle = 21 days)			**Cycle 1**					
DAY	**Screening** [B,C]	**Post-Registration**[D]	**1**	**2**	**3**	**4**	**5**	**8**[N]
Radiotherapy								
Hu14.18-IL2[M]			**X**[A]	**X**	**X**			
Complete history/PE	**X**							
Physical Exam	**X**		**X**					
Adverse event assessment[R] & conmed review			**X**	**X**	**X**	**X**		**X**
Weight	**X**		**X**	**X**	**X**	**X**		**X**
Performance Status	**X**		**X**	**X**	**X**	**X**		**X**
Pregnancy Test (serum or urine, subjects of childbearing potential only)	**X**		**X**[C]					
ECG[Q]	**X**							
Biopsy of Lesions (treated and not treated)[L]		**X**					**X**	
Tumor measurement[G]	**X**							
Skin Photography[G]	**X**							
Evaluation of injection site[G]			**X**	**X**	**X**	**X**		**X**
Vital signs (T, BP, P, RR)[H]	**X**		**X**	**X**	**X**	**X**		**X**
CBC, diff, plts	**X**		**X**[C]		**X**			**X**
CMP[E]	**X**		**X**[C]		**X**			**X**
Phosphate, LDH, Mg, CRP	**X**		**X**[C]		**X**			**X**
INR[L]		**X**					**X**	
Urinalysis[I]	**X**							
TSH			**X**					
HIV, HBsAg, HepC Ab	**X**							
Research Blood Samples	**See table 9.5-1**							

Table 9.1-2: Phase IA Schedule of Events, Cycle 2 through Follow-Up								
(Cycles 2-4 = 21 days, Cycles 5+ = 28 days)	**Cycle 2 +**						**End of Treatment[J]**	**Follow-up[K]**
DAY	**1[F]**	**2[O]**	**3[O]**	**4[O]**	**5**	**8[N,O]**		
Radiotherapy								
Hu14.18-IL2[M]	**X**	**X**	**X**					
Physical Exam	**X[F]**						**X**	
Adverse event assessment[R] & Conmed review	**X**	**X**	**X**	**X**		**X**	**X**	
Weight	**X**	**X**	**X**	**X**		**X**	**X**	
Performance Status	**X**	**X**	**X**	**X**		**X**	**X**	
Biopsy of Lesions (treated and not treated)[L]					**X[L]**			
Tumor measurement[G]	**X**							**X**
Skin Photography[G]	**X**							**X**
Evaluation of injection site[G]	**X**	**X**	**X**	**X**		**X**	**X**	
Vital signs (T, BP, P, RR)[H]	**X**	**X**	**X**	**X**		**X**	**X**	
CBC, diff, plts	**X[F]**		**X**			**X**	**X**	
CMP[E]	**X[F]**		**X**			**X**	**X**	
Phosphate, LDH, Mg, CRP	**X[F]**		**X**			**X**	**X**	
INR[L]					**X**			
Urinalysis[I]	**X[F]**						**X**	
Survival & subsequent anti-cancer therapy								**X**
Research Blood Samples	**See table 9.5-1**							

A. Subjects to have first study treatment (hu14.18-IL2) within 14 days from registration.
B. All screening procedures, except pregnancy test, to be completed within 28 days prior to registration. Pregnancy test for women of child bearing potential to be completed within 14 days prior to registration.
C. Labs (CBC, diff, platelets, CMP, phosphate, LDH, Mg, CRP, pregnancy test), physical exam, vitals, weight, and performance status should be repeated prior to first cycle of IT-IC if not completed within previous 14 days.
D. Post-registration procedures to be completed after registration and prior to start of any study treatment.
E. CMP – AST, ALT, total bilirubin, alkaline phosphatase, albumin, total protein, sodium, potassium, calcium, creatinine, BUN, glucose.

F. Day 1 of cycles 2+ may be delayed up to one week to allow for scheduling around holidays, subject vacations, and clinic scheduling. Safety labs (all labs not located in the research immune assessments section of the calendar) do not need to be repeated on day 1 of each cycle if performed in the previous 7 days. Physical exam, AEs and conmed review can be completed up to 3 days prior to day 1 of cycles 2+.
G. Imaging: Head MRI or CT with contrast at screening. CT Chest with or without contrast and CT Abdomen/Pelvis with contrast to be completed at screening and every 12 weeks (+/- 14 days) from cycle 1 day 1. Imaging can be delayed up to an additional 14 days if treatment and/or cycle delays occurred since the prior imaging. If contrast allergy, may substitute CT chest w/out contrast + MRI abdomen/pelvis. Skin photography for measurement of superficial lesions recommended. Subjects who go off treatment for reasons other than progression should continue to have scans every 12 weeks (+/- 14 days) until progression, withdrawal of patient consent, start of new anti-cancer therapy, or until 2 years from end of study therapy.

   Evaluation of the injection site must include measurement of the injected lesions (caliper, tape measure, etc) on all cycles day 1, when clinically feasible per treating physician. Evaluation of the injection site on all indicated days to include evaluation of reactions at the injection site.
H. Vital signs (temperature, blood pressure, pulse, respiration rate): Vital signs must always be obtained pre-treatment for all study drugs. On hu14.18-IL2 administration days during cycle 1, vital signs will be obtained prior to treatment, and then hourly until six hours post each injection. This can be reduced to a minimum of 2 hours post injection for all subsequent hu14.18-IL2 administrations at the discretion of the treating physician.
I. Urinalysis with microscopy, culture if > 5 WBCs/hpf
J. End of treatment visit to occur 30 days (+/- 3 days) from last protocol therapy. New AEs to be collected through end of treatment visit. Existing AEs to be collected through resolution or until deemed permanent.
K. Follow-up: After completion of therapy, subjects will be followed every 12 weeks (+/- 14 days) from C1D1, until disease progression, or until 2 years from end of study treatment. After 2 years, or disease progression, subjects to be followed every 6 months for survival and subsequent anti-cancer therapy.
L. Biopsies to be completed at baseline, cycle 1 day 5, and day 5 of cycles 2 & 4. Day 5 biopsies may be completed on days 4-9 of the applicable cycle. The cycle 1 biopsy may be omitted at the discretion of the PI, the protocol chair or their clinical designee if the biopsy is felt to be unsafe. INR to be drawn up to four days prior to biopsies.
M. The time interval between day 1, 2, and 3 doses of hu14.18-IL2 is 24 hours +/- 6 hours
N. Day 8 visit window is +/- 3 days. If felt appropriate by the investigator, the day 8 visit can occur remotely. In the case of remote visits lab tests would be completed at a local lab. The day 8 c-reactive protein test may be skipped if the visit is completed remotely, but may be added to a subsequent visit if requested by the treating investigator. Day 8 research samples that are missed due to a remote visit may also be added to the following visit if requested by the treating investigator. Collection of vital signs and weight may be skipped; AEs, conmeds, and performance status can be reviewed and evaluated by phone or telemed visit.

A discussion of the status of the injection site with the subject will be an acceptable alternative to the standard injection site evaluation when remote visits are used.

O. Procedures, labs, and assessments scheduled for days 2, 3, 4, and 8 of each cycle need only occur if the subject will be receiving hu14.18-IL2 during that cycle. Exceptions: Subjects must still have biopsies performed, have research immune labs drawn at the scheduled time-points, and tumor measurements must still be done per the specified imaging schedule.
P. This footnote removed in protocol Amendment 4
Q. ECG required at screening, then as clinically indicated.
R. In all cases, subjects will be monitored for adverse events for a minimum of 90 days following the last dose of hu14.18-IL2.

9.2 Phase IB Schedule of Events

Table 9.2-1: Phase IB Schedule of Events Through Cycle 1									
(cycle = 21 days)			**Cycle 1**						
DAY	**Screening[B,C]**	**Post-Registration[D]**	**-8 to -4**	**1**	**2**	**3**	**4**	**5**	**8[N]**
Radiotherapy			**X[A]**						
Hu14.18-IL2[M]				**X**	**X**	**X**			
Complete history/PE	**X**								
Physical Exam	**X**			**X**					
Adverse event assessment[R] & conmed review				**X**	**X**	**X**	**X**		**X**
Weight	**X**			**X**	**X**	**X**	**X**		**X**
Performance Status	**X**			**X**	**X**	**X**	**X**		**X**
Pregnancy Test (serum or urine, subjects of childbearing potential only)	**X**			**X[C]**					
ECG[Q]	**X**								
Biopsy of Lesions (treated and not treated)[L]		**X**						**X**	
Tumor measurement[G]	**X**								
Skin Photography[G]	**X**								
Evaluation of injection site[G]	**X**			**X**	**X**	**X**	**X**		**X**
Vital signs (T, BP, P, RR)[H]	**X**			**X**	**X**	**X**	**X**		**X**
CBC, diff, plts	**X**			**X[C]**		**X**			**X**
CMP[E]	**X**			**X[C]**		**X**			**X**
Phosphate, LDH, Mg, CRP	**X**			**X[C]**		**X**			**X**
INR[L]		**X**						**X**	
Urinalysis[I]	**X**								
TSH				**X**					
HIV, HBsAg, Hep C Ab	**X**								
Research Blood Samples	**See table 9.5-1**								

Table 9.2-2: Phase IB Schedule of Events, Cycle 2 through Follow-Up								
(Cycles 2-4 = 21 days, Cycles 5+ = 28 days)	**Cycle 2 +**						**End of Treatment[J]**	**Follow-up[K]**
DAY	**1[F]**	**2[O]**	**3[O]**	**4[O]**	**5**	**8[N,O]**		
Radiotherapy								
Hu14.18-IL2[M]	**X**	**X**	**X**					
Physical Exam	**X[F]**						**X**	
Adverse event assessment[R] & conmed review	**X**	**X**	**X**	**X**		**X**	**X**	
Weight	**X**	**X**	**X**	**X**		**X**	**X**	
Performance Status	**X**	**X**	**X**	**X**		**X**	**X**	
Biopsy of Lesions (treated and not treated)[L]					**X[L]**			
Tumor measurement[G]	**X**							**X**
Skin Photography[G]	**X**							**X**
Evaluation of injection site[G]	**X**	**X**	**X**	**X**		**X**	**X**	
Vital signs (T, BP, P, RR)[H]	**X**	**X**	**X**	**X**		**X**	**X**	
CBC, diff, plts	**X[F]**		**X**			**X**	**X**	
CMP[E]	**X[F]**		**X**			**X**	**X**	
Phosphate, LDH, Mg, CRP	**X[F]**		**X**			**X**	**X**	
INR[L]					**X**			
Urinalysis[I]	**X[F]**						**X**	
Survival & subsequent anti-cancer therapy								**X**
Research Blood Samples	**See table 9.5-1**							

A. Subjects to have first study treatment (RT) within 14 days from registration. Final RT dose must be completed at least 4, but no more than 8 days prior to first dose of hu14.18-IL2. If additional RT treatments (per section 5.2.9) are to be given to other lesion(s) after the initial course of RT then this must occur at least 24 hours after and at least 24 hours prior to any treatments with hu14.18-IL2. One cycle of hu14.18-IL2 may be skipped to accommodate scheduling and administration of RT to such additional lesions.
B. All screening procedures, except pregnancy test, to be completed within 28 days prior to registration. Pregnancy test for women of childbearing potential to be completed within 14 days prior to registration.
C. Labs (CBC, diff, platelets, CMP, phosphate, LDH, Mg, CRP, pregnancy test), physical exam, vitals, weight, and performance status should be repeated prior to first cycle of IT-IC if not completed within previous 14 days.

D. Post-registration procedures to be completed after registration and prior to start of any study treatment.
E. CMP – AST, ALT, total bilirubin, alkaline phosphatase, albumin, total protein, sodium, potassium, calcium, creatinine, BUN, glucose.
F. Day 1 of cycles 2+ may be delayed up to one week to allow for scheduling around holidays, subject vacations, and clinic scheduling. Safety labs (all labs not located in the research immune assessments section of the calendar) and the physical exam do not need to be repeated on day 1 of each cycle if performed in the previous 7 days. Physical exam, AEs and conmed review can be completed up to 3 days prior to day 1 of cycle 2+.
G. Imaging: Head MRI or CT with contrast at screening. CT Chest with or without contrast and CT Abdomen/Pelvis with contrast to be completed at screening and every 12 weeks (+/- 14 days) from cycle 1 day 1. Imaging can be delayed up to an additional 14 days if treatment and/or cycle delays occurred since the prior imaging. If contrast allergy, may substitute CT chest w/out contrast + MRI abdomen/pelvis. Skin photography for measurement of superficial lesions recommended. Subjects who go off treatment for reasons other than progression should continue to have scans every 12 weeks (+/- 14 days) until progression, withdrawal of patient consent, start of new anti-cancer therapy, or until 2 years from end of study therapy.

   Evaluation of the injection site must include measurement of the injected lesions (caliper, tape measure, etc) on all cycles day 1, when clinically feasible per treating physician. Evaluation of the injection site on all indicated days to include evaluation of reactions at the injection site.
H. Vital signs (temperature, blood pressure, pulse, respiration rate): Vital signs must always be obtained pre-treatment for all study drugs. On hu14.18-IL2 administration days during cycle 1, vital signs will be obtained prior to treatment, and then hourly until six hours post each injection. This can be reduced to a minimum of 2 hours post injection for all subsequent hu14.18-IL2 administrations at the discretion of the treating physician.
I. Urinalysis with microscopy, culture if > 5 WBCs/hpf
J. End of treatment visit to occur 30 days (+/- 3 days) from last protocol therapy. New AEs to be collected through end of treatment visit. Existing AEs to be collected through resolution or until deemed permanent.
K. Follow-up: After completion of therapy, subjects will be followed every 12 weeks (+/- 14 days) from C1D1, until disease progression, or until 2 years from end of study treatment. After 2 years, or disease progression, subjects to be followed every 6 months for survival and subsequent anti-cancer therapy.
L. Biopsies to be completed at baseline, cycle 1 day 5, and day 5 of cycles 2 & 4. Day 5 biopsies may be completed on days 4-9 of the applicable cycle. The cycle 1 biopsy may be omitted at the discretion of the PI, the protocol chair or their clinical designee if the biopsy is felt to be unsafe. INR to be drawn up to four days prior to biopsies.
M. The time interval between day 1, 2, and 3 doses of hu14.18-IL2 is 24 hours +/- 6 hours
N. Day 8 visit window is +/- 3 days. If felt appropriate by the investigator, the day 8 visit can occur remotely. In the case of remote visits lab tests would be completed at a local lab. The day 8 c-reactive protein test may be skipped if the visit is completed remotely, but may be added to a subsequent visit if requested by the treating investigator. Day 8 research samples

that are missed due to a remote visit may also be added to the following visit if requested by the treating investigator. Collection of vital signs and weight may be skipped; AEs, conmeds, and performance status can be reviewed and evaluated by phone or telemed visit. A discussion of the status of the injection site with the subject will be an acceptable alternative to the standard injection site evaluation when remote visits are used.

O. Procedures, labs, and assessments scheduled for days 2, 3, 4, and 8 of each cycle need only occur if the subject will be receiving hu14.18-IL2 during that cycle. Exceptions: Subjects must still have biopsies performed, have research immune labs drawn at the scheduled time-points, and tumor measurements must still be done per the specified imaging schedule.
P. This footnote removed in protocol amendment 4
Q. ECG required at screening, then as clinically indicated.
R. In all cases, subjects in Phase IB will continue to be monitored for at least 90 days after completion of study treatment for late toxicities associated with RT or IT-hu14.18-IL2. Potential sub-acute and late local toxicities due to palliative radiation therapy will be monitored for 12 months following the radiation therapy.

9.3 Phase IC Schedule of Events

Table 9.3-1: Phase IC Schedule of Events Through Cycle 1											
(cycle = 21 days)			**Cycle 1**								
DAY	**Screening[B,C]**	**Post-Registration[D]**	**-8 to -4**	**-7 to -1**	**1**	**2**	**3**	**4**	**5**	**8[P]**	**8-14[G]**
Radiotherapy			**X[A]**								
Nivolumab[G]				**X** *(dose 1)*							**X** *(dose 2)*
Hu14.18-IL2[O]					**X**	**X**	**X**				
Complete history/PE	**X**										
Physical Exam	**X**			**X**							**X**
Adverse event assessment[T] & conmed review					**X**	**X**	**X**	**X**		**X**	**X**
Weight	**X**				**X**	**X**	**X**	**X**		**X**	**X**
Performance Status	**X**				**X**	**X**	**X**	**X**		**X**	**X**
Pregnancy Test (serum or urine, subjects of childbearing potential only)	**X**			**X[C]**							
ECG[S]	**X**										
Biopsy of Lesions (treated and not treated)[N]		**X**							**X**		
Tumor measurement[H]	**X**										
Skin Photography[H]	**X**										
Evaluation of injection site[H]	**X**				**X**	**X**	**X**	**X**		**X**	
Vital signs (T, BP, P, RR, pulse oximetry)[I,J]	**X**				**X**	**X**	**X**	**X**		**X**	**X**
CBC, diff, plts	**X**			**X[C]**	**X[F]**		**X**			**X**	**X**
CMP[E]	**X**			**X[C]**	**X[F]**		**X**			**X**	**X**
Phosphate, LDH, Mg, CRP[F]	**X**			**X[C]**	**X[F]**		**X**			**X**	**X**
Amylase, Lipase	**X**			**X[C]**							**X**
INR[N]		**X**							**X**		

Urinalysis[K]	**X**				**X**[F]						
TSH, T4 Free, Cortisol[Q]				**X**							
HIV, HBsAg, Hep C Ab	**X**										
Research Blood Samples	**See table 9.5-1**										

Table 9.3-2: Phase IC Schedule of Events, Cycle 2								
(Cycle = 21 days)	**Cycle 2**							
DAY	**1[F]**	**2[G]**	**3[G]**	**4[G]**	**5**	**1-7[G]**	**8[P,G]**	**15-21[G]**
Nivolumab[G]						**X** *(dose 3)*		**X** *(dose 4)*
Hu14.18-IL2[O]	**X**	**X**	**X**					
Physical Exam	**X**					**X**		**X**
Adverse event assessment[T] & conmed review	**X**	**X**	**X**	**X**		**X**	**X**	**X**
Weight	**X**	**X**	**X**	**X**		**X**	**X**	**X**
Performance Status	**X**	**X**	**X**	**X**		**X**	**X**	**X**
Biopsy of Lesions (treated and not treated)[N]					**X**			
Tumor measurement[H]						**X**		**X**
Skin Photography[H]						**X**		**X**
Evaluation of injection site[H]	**X**	**X**	**X**	**X**			**X**	
Vital signs (T, BP, P, RR, pulse oximetry)[I,J]	**X**	**X**	**X**	**X**		**X**	**X**	**X**
CBC, diff, plts	**X**[F]		**X**			**X**	**X**	**X**
CMP[E]	**X**[F]		**X**			**X**	**X**	**X**
Phosphate, LDH, Mg, CRP[F]	**X**[F]		**X**			**X**	**X**	**X**
Amylase, Lipase						**X**		**X**
INR[N]					**X**			
Urinalysis[K]	**X**[F]							
TSH, T4 Free, Cortisol[Q]						**X**		
Research Blood Samples	**See table 9.5-1**							

Table 9.3-3: Phase IC Schedule of Events, Cycle 3							
(Cycle = 21 days)	**Cycle 3**						
DAY	**1[F]**	**2[G]**	**3[G]**	**4[G]**	**5**	**8[P,G]**	**8-14[G]**
Nivolumab[G]							**X** *(dose 5)*
Hu14.18-IL2[O]	**X**	**X**	**X**				
Physical Exam	**X**						**X**
Adverse event assessment[T] & conmed review	**X**	**X**	**X**	**X**		**X**	**X**
Weight	**X**	**X**	**X**	**X**		**X**	**X**
Performance Status	**X**	**X**	**X**	**X**		**X**	**X**
Biopsy of Lesions (treated and not treated)[N]							
Tumor measurement[H]							**X**
Skin Photography[H]							**X**
Evaluation of injection site[H]	**X**	**X**	**X**	**X**		**X**	
Vital signs (T, BP, P, RR, pulse oximetry)[I,J]	**X**	**X**	**X**	**X**		**X**	**X**
CBC, diff, plts	**X[F]**		**X**			**X**	**X**
CMP[E]	**X[F]**		**X**			**X**	**X**
Phosphate, LDH, Mg, CRP[F]	**X[F]**		**X**			**X**	**X**
Amylase, Lipase							**X**
INR							
Urinalysis[K]	**X[F]**						
TSH, T4 Free, Cortisol[Q]							**X**
Research Blood Samples	**See table 9.5-1**						

Table 9.3-4: Phase IC Schedule of Events, Cycle 4								
(Cycle = 21 days)	**Cycle 4**							
DAY	**1[A]**	**2[G]**	**3[G]**	**4[G]**	**5**	**1-7[G]**	**8[P,G]**	**15-21[G]**
Nivolumab[G]						**X** *(dose 6)*		**X** *(dose 7)*
Hu14.18-IL2[O]	**X**	**X**	**X**					
Physical Exam	**X**					**X**		**X**
Adverse event assessment[T] & conmed review	**X**	**X**	**X**	**X**		**X**	**X**	**X**
Weight	**X**	**X**	**X**	**X**		**X**	**X**	**X**
Performance Status	**X**	**X**	**X**	**X**		**X**	**X**	**X**
Biopsy of Lesions (treated and not treated)[N]					**X**			
Tumor measurement[H]								**X**
Skin Photography[H]								**X**

Evaluation of injection site[H]	**X**	**X**	**X**	**X**			**X**	
Vital signs (T, BP, P, RR, pulse oximetry)[I,J]	**X**	**X**	**X**	**X**		**X**	**X**	**X**
CBC, diff, plts	**X**[F]		**X**			**X**	**X**	**X**
CMP[E]	**X**[F]		**X**			**X**	**X**	**X**
Phosphate, LDH, Mg, CRP[F]	**X**[F]		**X**			**X**	**X**	**X**
Amylase, Lipase						**X**		**X**
INR[N]					**X**			
Urinalysis[K]	**X**[F]							
TSH, T4 Free, Cortisol[Q]								
Research Blood Samples	**See table 9.5-1**							

Table 9.3-5: Phase IC Schedule of Events, Cycle 5 through Follow-Up

(Cycles = 28 days)	**Cycle 5 +**								**End of Treatment[L]**	**Follow-up[M]**
DAY	**1[F]**	**2[G]**	**3[G]**	**4[G]**	**5**	**8[P,G]**	**8-14[G]**	**22-28[G]**		
Radiotherapy										
Nivolumab[G]							**X (dose 8+)**	**X (dose 9+)**		
Hu14.18-IL2[O]	**X**	**X**	**X**							
Physical Exam							**X**	**X**	**X**	
Adverse event assessment[T] & conmed review	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	
Weight	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	
Performance Status	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	
Tumor measurement[H]								**X**		**X**
Skin Photography[H]								**X**		**X**
Evaluation of injection site[H]	**X**	**X**	**X**	**X**		**X**			**X**	
Vital signs (T, BP, P, RR, pulse oximetry)[I,J]	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	
CBC, diff, plts	**X**[F]		**X**			**X**	**X**	**X**	**X**	
CMP[E]	**X**[F]		**X**			**X**	**X**	**X**	**X**	
Phosphate, LDH, Mg, CRP[F]	**X**[F]		**X**			**X**	**X**	**X**	**X**	
Amylase, Lipase							**X**	**X**	**X**	

Urinalysis[K]	**X**[F]								**X**	
TSH, T4 Free, Cortisol[Q]								**X**	**X**	
Survival & subsequent anti-cancer therapy										**X**
Research Blood Samples	**See table 9.5-1**									

A. Subjects to have first study treatment (radiation) within 14 days from registration. Final RT dose must be completed at least 4, but no more than 8 days prior to first dose of hu14.18-IL2, and at least 24 hours prior to nivolumab. If additional RT treatments (per section 5.2.9) are to be given to other lesion(s) after the initial course of RT then this must occur at least 24 hours after and at least 24 hours prior to any treatments with hu14.18-IL2 or nivolumab. One cycle of hu14.18-IL2 may be skipped to accommodate scheduling and administration of radiation to the any such additional lesions.
B. All screening procedures, except pregnancy test, to be completed within 28 days prior to registration. Pregnancy test for women of child bearing potential to be completed within 14 days prior to registration.
C. Labs (CBC, diff, platelets, CMP, phosphate, LDH, Mg, CRP, Amylase, Lipase, pregnancy), physical exam, vitals, weight, and performance status should be repeated prior to first cycle of nivolumab if not completed within previous 14 days.
D. Post-registration procedures to be completed after registration and prior to start of any study treatment.
E. CMP – AST, ALT, total bilirubin, alkaline phosphatase, albumin, total protein, sodium, potassium, calcium, creatinine, BUN, glucose.
F. Day 1 of cycles 2+ may be delayed up to one week to allow for scheduling around holidays, subject vacations, and clinic scheduling. Safety labs (all labs not marked as research blood samples) do not need to be repeated on day 1 of each cycle if performed in the previous 7 days. On nivolumab treatment days safety labs do not need to be repeated if completed in previous 2 days. Physical exam, AEs and conmed review can be completed up to 3 days prior to day 1 of cycles 2+.
G. Nivolumab to be given every 2 weeks (+/- 3 days). Nivolumab doses must be a minimum of 10 days apart. Dose 7 of Nivolumab may be delayed up to 7 days in order to more closely aligned with Cycle 5 Day 1 hu14.18-IL2.
Nivolumab treatment visits, including planned assessments, need only occur if nivolumab treatment is planned. Procedures, labs, and assessments scheduled for days 1, 2, 3, 4, and 8 of each cycle need only occur if the subject will be receiving hu14.18-IL2 during that cycle. However, if a subject is still receiving any study drug, they must be evaluated at least once every 4 weeks, with evaluations to match cycle 5+ day 1 (including evaluation of injection site). Exceptions: Subjects must still have biopsies performed, have research immune labs drawn at the scheduled time-points, and tumor measurements must still be done per the specified imaging schedule.
H. Imaging: Head MRI or CT with contrast at screening. CT Chest with or without contrast and CT Abdomen/Pelvis with contrast to be completed at screening and every 12 weeks (+/- 14

days) from cycle 1 day 1. Imaging can be delayed up to an additional 14 days if treatment and/or cycle delays occurred since the prior imaging. If contrast allergy, may substitute CT chest w/out contrast + MRI abdomen/pelvis. Skin photography for measurement of superficial lesions recommended. Subjects who go off treatment for reasons other than progression should continue to have scans every 12 weeks (+/- 14 days) until progression, withdrawal of patient consent, start of new anti-cancer therapy, or until 2 years from end of study therapy.

Evaluation of the injection site must include measurement of the injected lesions (caliper, tape measure, etc) on all cycles day 1, when clinically feasible per treating physician. Evaluation of the injection site on all indicated days to include evaluation of reactions at the injection site.

I. Vital signs (temperature, blood pressure, pulse, respiration rate): Vital signs must always be obtained pre-treatment for all study drugs. On hu14.18-IL2 administration days during cycle 1, vital signs will be obtained prior to treatment, and then hourly until six hours post each injection. This can be reduced to a minimum of 2 hours post injection for all subsequent hu14.18-IL2 administrations at the discretion of the treating physician. Vital signs during the administration of Nivolumab should be done per institutional guidelines.
J. Vital signs on days of treatment with Nivolumab should include pulse oximetry prior to dosing.
K. Urinalysis with microscopy, culture if > 5 WBCs/hpf
L. End of treatment visit to occur 30 days (+/- 3 days) from last protocol therapy. New AEs to be collected through end of treatment visit. Existing AEs to be collected through resolution or until deemed permanent.
M. Follow-up: After completion of therapy, subjects will be followed every 12 weeks (+/- 14 days) from C1D1, until disease progression, or until 2 years from end of study treatment. After 2 years, or disease progression, subjects to be followed every 6 months for survival and subsequent anti-cancer therapy.
N. Biopsies to be completed at baseline, cycle 1 day 5, and day 5 of cycles 2 & 4. Day 5 biopsies may be completed on days 4-9 of the applicable cycle. The cycle 1 biopsy may be omitted at the discretion of the PI, the protocol chair, or their clinical designee if the biopsy is felt to be unsafe. INR to be drawn up to four days prior to biopsies.
O. The time interval between Day 1, 2, and 3 doses of hu14.18-IL2 is 24 hours +/- 6 hours
P. Day 8 visit window is +/- 3 days. If subject is scheduled to be in clinic for another study related appointment during this window, those visits may be combined. If felt appropriate by the investigator, the day 8 visit can occur remotely. In the case of remote visits lab tests would be completed at a local lab. The day 8 c-reactive protein test may be skipped if the visit is compelted remotely, but may be added to a subsequent visit if requested by the treating investigator. Day 8 research samples that are missed due to a remote visit may also be added to the following visit if requested by the treating investigator. Colletion of vital signs and weight may be skipped; AEs, conmeds, and performance status can be reviewed and evaluated by phone or telemed visit. A discussion of the status of the injection site with the subject will be an acceptable alternative to the standard injection site evaluation when remote visits are used.

Q. TSH, T4 free, and cortisol to be completed prior to first dose of Nivoluab, prior to the third dose of Nivolumab, prior to the fifth dose (or start of maintenance) of Nivolumab, then every 8 weeks (+/- 1 week) or more often as clinically indicated, and at end of treatment. If a subject discontinues nivolumab (but remains on treatment with hu14.18-IL2) then TSH, T4 free, and cortisol should be completed as clinically indicated.
R. This footnote removed in protocol amendment 4
S. ECG required at screening, then as clinically indicated.
T. In all cases, subjects in Phase IC will continue to be monitored for at least 90 days after completion of study treatment for late toxicities associated with any component of therapy (RT, IT-hu14.18-IL2, nivolumab). Potential sub-acute and late local toxicities due to palliative radiation therapy will be monitored for 12 months following the radiation therapy.

9.4 Phase ID Schedule of Events

Table 9.4-1: Phase ID Schedule of Events Through Cycle 1											
(cycle = 21 days)			**Cycle 1**								
DAY	**Screening[B,C]**	**Post-Registration[D]**	**-8 to -4**	**-7 to -1**	**1**	**2**	**3**	**4**	**5**	**8[Q]**	**15-21[H]**
Radiotherapy			**X[A]**								
Nivolumab[H]				**X**							**X**
Ipilimumab[E,H]				**X**							**X**
Hu14.18-IL2[P]					**X**	**X**	**X**				
Complete history/PE	**X**										
Physical Exam	**X**			**X**							**X**
Adverse event assessment[U] & conmed review					**X**	**X**	**X**	**X**		**X**	**X**
Weight	**X**				**X**	**X**	**X**	**X**		**X**	**X**
Performance Status	**X**				**X**	**X**	**X**	**X**		**X**	**X**
Pregnancy Test (serum or urine, subjects of childbearing potential only)	**X**			**X[C]**							
ECG[T]	**X**										
Biopsy of Lesions (treated and not treated)[O]		**X**							**X**		
Tumor measurement[I]	**X**										
Skin Photography[I]	**X**										
Evaluation of injection site[I]					**X**	**X**	**X**	**X**		**X**	
Vital signs (T, BP, P, RR, pulse oximetry)[J,K]	**X**				**X**	**X**	**X**	**X**		**X**	**X**
CBC, diff, plts	**X**			**X[C]**	**X**		**X**			**X**	**X**
CMP[F]	**X**			**X[C]**	**X**		**X**			**X**	**X**
Phosphate, LDH, Mg, CRP[G]	**X**			**X[C]**	**X**		**X**			**X**	**X**
Amylase, Lipase	**X**			**X[C]**							**X**
Uric Acid				**X[C]**							**X**

INR[O]		**X**							**X**		
Urinalysis[L]	**X**				**X**						
TSH, T4 Free, Cortisol[R]				**X**							
HIV, HBsAg, Hep C Ab	**X**										
Research Blood Samples	**See table 9.5-1**										

Table 9.4-2: Phase ID Schedule of Events, Cycle 2 through Cycle 4							
(Cycles = 21 days)	**Cycle 2 - 4**						
DAY	**1[G]**	**2[H]**	**3[H]**	**4[H]**	**5**	**8[H,Q]**	**15-21[H]**
Radiotherapy							
Nivolumab[H]							**X**
Ipilimumab[E,H]							**X[E]**
Hu14.18-IL2[P]	**X**	**X**	**X**				
Physical Exam	**X[G]**						**X**
Adverse event assessment[U] & conmed review	**X**	**X**	**X**	**X**		**X**	**X**
Weight	**X**	**X**	**X**	**X**		**X**	**X**
Performance Status	**X**	**X**	**X**	**X**		**X**	**X**
Biopsy of Lesions (treated and not treated)[O]					**X[O]**		
Tumor measurement[I]							**X**
Skin Photography[I]							**X**
Evaluation of injection site[I]	**X**	**X**	**X**	**X**		**X**	
Vital signs (T, BP, P, RR, pulse oximetry)[J,K]	**X**	**X**	**X**	**X**		**X**	**X**
CBC, diff, plts	**X[G]**		**X**			**X**	**X**
CMP[F]	**X[G]**		**X**			**X**	**X**
Phosphate, LDH, Mg, CRP[G]	**X[G]**		**X**			**X**	**X**
Amylase, Lipase							**X**
Uric Acid							**X**
INR[O]					**X**		
Urinalysis[L]	**X[G]**						
TSH, T4 Free, Cortisol[R]							**X**
Research Blood Samples	**See table 9.5-1**						

Table 9.4-3: Phase ID Schedule of Events, Cycle 5 through Follow-Up									
(Cycles = 28 days)	**Cycle 5 +**							**End of Treatment[M]**	**Follow-up[N]**
DAY	**1[G]**	**2[H]**	**3[H]**	**4[H]**	**8[H,Q]**	**8-14[H]**	**22-28[H]**		
Radiotherapy									
Nivolumab[H]						**X**	**X**		
Ipilimumab[E,H]									
Hu14.18-IL2[P]	**X**	**X**	**X**						
Physical Exam	**X[G]**					**X**	**X**	**X**	
Adverse event assessment[U] & conmed review	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Weight	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Performance Status	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Tumor measurement[I]	**X**								**X**
Skin Photography[I]	**X**								**X**
Evaluation of injection site[I]	**X**	**X**	**X**	**X**	**X**			**X**	
Vital signs (T, BP, P, RR, pulse oximetry)[J,K]	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
CBC, diff, plts	**X[G]**		**X**		**X**	**X**	**X**	**X**	
CMP[F]	**X[G]**		**X**		**X**	**X**	**X**	**X**	
Phosphate, LDH, Mg, CRP[G]	**X[G]**		**X**		**X**	**X**	**X**	**X**	
Amylase, Lipase						**X**	**X**	**X**	
Uric Acid						**X**	**X**	**X**	
Urinalysis[L]	**X[G]**							**X**	
TSH, T4 Free, Cortisol[R]	**X**						**X**	**X**	
Survival & subsequent anti-cancer therapy									**X**
Research Blood Samples	**See table 9.5-1**								

A. Subjects to have first study treatment (RT) within 14 days from registration. Final RT dose must be completed at least 4, but no more than 8 days prior to first dose of hu14.18-IL2, and at least 24 hours prior to nivolumab + ipilimumab. If additional palliative RT treatments (per section 5.2.9) are to be given to other lesion(s) after the initial course of RT then this must occur at least 24 hours after and at least 24 hours prior to any treatments with hu14.18-IL2, nivolumab, or ipilimumab. One cycle of hu14.18-IL2 may be skipped to accommodate scheduling and administration of radiation to and such additional lesions.

B. All screening procedures, except pregnancy test, to be completed within 28 days prior to registration. Pregnancy test for women of child bearing potential to be completed within 14 days prior to registration.
C. Labs (CBC, diff, platelets, CMP, phosphate, LDH, Mg, CRP, Amylase, Lipase, Uric Acid, pregnancy test), physical exam, vitals, weight, and performance status should be repeated prior to first cycle of ipilimumab + nivolumab if not completed within previous 14 days.
D. Post-registration procedures to be completed after registration and prior to start of any study treatment.
E. Ipilimumab for up to 4 doses only.
F. CMP – AST, ALT, total bilirubin, alkaline phosphatase, albumin, total protein, sodium, potassium, calcium, creatinine, BUN, glucose.
G. Day 1 of cycles 2+ may be delayed up to one week to allow for scheduling around holidays, subject vacations, and clinic scheduling. Safety labs (all labs not marked as research blood samples) and the physical exam do not need to be repeated on day 1 of each cycle if performed in the previous 7 days. On ipilimumab and/or nivolumab treatment days safety labs do not need to be repeated if completed in previous 2 days. Physical exam, AEs and conmed review can be completed up to 3 days prior to day 1 of cycles 2+.
H. Cycles 1-4, combined treatment with Nivolumab + Ipilimumab must be given no less than 18 days from the previous combined dose and should be given no earlier than seven days prior to next scheduled dose of hu14.18-IL2.

   Doses of Nivolumab starting with dose 5 (maintenance) are every 2 weeks (+/- 3 days), and must be a minimum of 10 days apart.

   Nivolumab treatment visits, including planned assessments, need only occur if nivolumab treatment is planned. Procedures, labs, and assessments scheduled for days 1, 2, 3, 4, and 8 of each cycle need only occur if the subject will be receiving hu14.18-IL2 during that cycle. However, if a subject is still receiving any study drug, they must be evaluated at least once every 4 weeks, with evaluations to match cycle 5+ day 1 (including evaluation of injection site). Exceptions: Subjects must still have biopsies performed, have research immune labs drawn at the scheduled time-points, and tumor measurements must still be done per the specified imaging schedule.
I. Imaging: Head MRI or CT with contrast at screening. CT Chest with or without contrast and CT Abdomen/Pelvis with contrast to be completed at screening and every 12 weeks (+/- 14 days) from cycle 1 day 1. Imaging can be delayed up to an additional 14 days if treatment and/or cycle delays occurred since the prior imaging. If contrast allergy, may substitute CT chest w/out contrast + MRI abdomen/pelvis. Skin photography for measurement of superficial lesions recommended. Subjects who go off treatment for reasons other than progression should continue to have scans every 12 weeks (+/- 14 days) until progression, withdrawal of patient consent, start of new anti-cancer therapy, or until 2 years from end of study therapy.

   Evaluation of the injection site must include measurement of the injected lesions (caliper, tape measure, etc) on all cycles day 1, when clinically feasible per treating physician.

Evaluation of the injection site on all indicated days to include evaluation of reactions at the injection site.

J. Vital signs (temperature, blood pressure, pulse, respiration rate): Vital signs must always be obtained pre-treatment for all study drugs. On hu14.18-IL2 administration days during cycle 1, vital signs will be obtained prior to treatment, and then hourly until six hours post each injection. This can be reduced to a minimum of 2 hours post injection for all subsequent hu14.18-IL2 administrations at the discretion of the treating physician. Vital signs during the administration of Nivolumab +/- Ipilimumab should be done per institutional guidelines.
K. Vital signs on days of treatment with Nivolumab should include pulse oximetry prior to dosing.
L. Urinalysis with microscopy, culture if > 5 WBCs/hpf
M. End of treatment visit to occur 30 days (+/- 3 days) from last protocol therapy. New AEs to be collected through end of treatment visit. Existing AEs to be collected through resolution or until deemed permanent.
N. Follow-up: After completion of therapy, subjects will be followed every 12 weeks (+/- 14 days) from C1D1, until disease progression, or until 2 years from end of study treatment. After 2 years, or disease progression, subjects to be followed every 6 months for survival and subsequent anti-cancer therapy.
O. Biopsies to be completed at baseline, cycle 1 day 5, and day 5 of cycles 2 & 4. Day 5 biopsies may be completed on days 4-9 of the applicable cycle. The cycle 1 biopsy may be omitted at the discretion of the PI, the protocol chair, or their clinical designee if the biopsy is felt to be unsafe. INR to be drawn up to four days prior to biopsies.
P. The time interval between day 1, 2, and 3 doses of hu14.18-IL2 is 24 hours +/- 6 hours
Q. Day 8 visit window is +/- 3 days. If felt appropriate by the investigator, the day 8 visit can occur remotely. In the case of remote visits lab tests would be completed at a local lab. The day 8 c-reactive protein test may be skipped if the visit is compelted remotely, but may be added to a subsequent visit if requested by the treating investigator. Day 8 research samples that are missed due to a remote visit may also be added to the following visit if requested by the treating investigator. Collection of vital signs and weight may be skipped; AEs, conmeds, and performance status can be evaluated by phone or telemed visit. A discussion of the status of the injection site with the subject will be an acceptable alternative to the standard injection site evaluation when remote visits are used.
R. TSH, T4 free, and cortisol to be completed prior to first dose of Nivolumab + Ipilimumab, prior to the third dose of Nivolumab + Ipilimumab, prior to the fifth dose (or start of maintenance) of Nivolumab, then every 8 weeks (+/- 1 week) or more often as clinically indicated, and at end of treatment. If a subject discontinues both nivolumab and ipilimumab (but remains on treatment with hu14.18-IL2) then TSH, T4 free, and cortisol should be completed as clinically indicated.
S. This footnote removed in protocol amendment 4
T. ECG required at screening, then as clinically indicated.
U. In all cases, subjects in Phase ID will continue to be monitored for at least 90 days after the completion of study treatment for late toxicities associated with any component of therapy (RT, IT-hu14.18-IL2, nivolumab, ipilimumab). Potential late local toxicities due to palliative radiation therapy will be monitored for 12 months following the radiation therapy.

9.5 Schedule of Research Blood Assessments

9.5-1: Schedule of Research Blood Assessments for All Phases			
Time-point[A]	**Blood Volume**	**Tube Type**	**Assays**
Post-Registration	90 ml	Heparinized syringes[B] or green top tubes	Immune cell assays[C]
Cycle 1 Day 1			
Pre-treatment	20 ml	Red top	sIL-2R, PKs
2 hours post-treatment	10 ml	Red top	PKs
6 hours post-treatment	10 ml	Red top	PKs
Cycle 1 Day 2 pre-treatment	10 ml	Red top	PKs
Cycle 1 Day 3			
Pre-treatment	10 ml	Red top	PKs
2 hours post-treatment	10 ml	Red top	PKs
6 hours post-treatment	10 ml	Red top	PKs
Cycle 1 Day 4	10 ml	Red top	sIL-2R, PKs
Cycle 1 Day 8	10 ml	Red top	sIL-2R, anti-IC
Cycle 2 Day 1 pre-treatment	10 ml	Red top	Anti-IC
Cycle 3 Day 1 pre-treatment	90 ml	Heparinized syringes[B] or green top tubes	Immune cell assays
	10 ml	Red top	Anti-IC
Cycle 5 Day 1 pre-treatment	90 ml	Heparinized syringes[B] or green top tubes	Immune cell assays
	10 ml	Red top	Anti-IC
Cycles 4, 7, 10, then every 3rd cycle thereafter[D]			
Day 1 pre-treatment	10 ml	Red top	sIL-2R, PKs, anti-IC
Day 3 pre-treatment	10 ml	Red top	PKs
Day 3, 2 hours post-treatment	10 ml	Red top	PKs
Day 4	10 ml	Red top	sIL-2R, PKs
Day 8	10 ml	Red top	sIL-2R, anti-IC
All other cycles (not listed above), Day 1, pre-treatment	10 ml	Red top	Anti-IC
At time of protocol required disease assessment & imaging (i.e. every 12 weeks)[E]	90 ml	Heparinized syringes[B] or green top tubes	Immune cell assays
End of Treatment	90 ml	Heparinized syringes[B] or green top tubes	Immune cell assays
	10 ml	Red top	Anti-IC

A. Pre- and post- treatment designations refer to pre- and post-treatment with hu14.18-IL2. Pre-treatment samples should be drawn within 60 minutes prior to hu14.18.IL2. Post-treatment samples can be drawn +/- 15 minutes from target time. All other samples if not otherwise indicated should be drawn pre-treatment with any study drug (if applicable)
B. Heparinized syringes preferred, green top tubes acceptable
C. sIL-2R = soluble IL2 receptor α levels, PKs = hu14.18-IL2 pharmacokinetics, anti-IC = anti-hu14.18-IL2 antibodies. Immune cell assays = *in vitro* immune cell assays
D. Starting at cycle 7: all indicated samples will be drawn, however, sIL-2R and PK assays will only be performed on select subjects, based on anti-IC levels or other lab or clinical findings which may warrant performing these assays.
E. If subject is having imaging at an outside facility (i.e., not at the study site) this sample can be drawn at the next scheduled study visit

9.6 Clinical Assessment, Biopsy Schedule and *In Vivo* Testing

9.6.1 Clinical Assessment for each Treatment Cycle

The detailed schedules for treatment administration and clinical monitoring are included as tables in sections 9.1 - 9.4.

9.6.2 Biopsy Schedule and Purpose

All subjects will have their index lesion (lesion A) and lesion B biopsied pretreatment and at day 5 of cycle 1 (with a possible range of day 4-9 to facilitate subject scheduling). Subjects who have provided consent will have these same lesions biopsied or resected following cycles 2 and 4 on day 5 (with a possible range between day 4-9 to facilitate subject scheduling). Any response at the non-injected lesion B (measurable tumor shrinkage, or histological evidence of immune infiltrate) would be consistent with (but not prove) immune mediated antitumor activity at distant sites, as this lesion would not have been directly injected with hu14.18-IL2. The cycle 1 biopsy may be omitted at the discretion of the PI, the protocol chair, or their clinical designee if the biopsy is felt to be unsafe. Image guidance (ultra sound or CT) is commonly used to assist in most biopsies. The use of image guidance is determined on a case by case basis and is determined by the physician at the time of biopsy. Factors such as tumor size, type of biopsy, location of tumor are considered by the physician to ensure adequate tissue acquisition in the safest way possible.

The biopsies will be approximately 0.3 cm tissue samples (using a 2 or 4 mm punch or core) which contains a cutaneous or subcutaneous tumor nodule. If needed, a surgical consult will be obtained for biopsy/resection of the nodule(s) using local anesthesia. These biopsies, 1-4 samples per location per time point, will be collected by sterile technique. One half will be placed in in fixative and used to evaluate morphology, tumor necrosis, inflammatory infiltrate and molecular profiles. The other half will be flash frozen and stained for GD2, and used for other analyses.

9.7 Immunologic Monitoring

Assessments of hu14.18-IL2 pharmacokinetics, anti-hu14.18-IL2 antibodies, and soluble IL2 receptor α levels will be completed per table 9.5-1. The laboratory manual should be consulted for details regarding processing.

9.7.1 ***In vitro*** **immune cell assays** will be performed on selected subjects. All pre-treatment immune cells will be obtained per table 9.5-1, with additional guidance provided in the laboratory manual. Additional exploratory immune monitoring assays may be performed on selected subjects using existing blood and tissue samples.

9.7.1.1 ADCC/NK function. In selected subjects, antibody-dependent cell-mediated cytotoxicity and Natural Killer cell functions will be monitored.

9.7.1.2 Monitoring of T cell response. Flow cytometry and functional analyses will be performed on selected subjects. Aliquots of the baseline blood may be used for providing genetic information about polymorphisms that may be relavant to the proposed mechanisms of action of hu14.18-IL2, such as FcR genotype and KIR/KIR-ligand genotype[55, 56], confirming HLA-A*0201 status, and monitoring the T cell receptor repertoire.

9.7.1.3 Determination of HLA-A*0201 status. Select subjects will be screened for HLA-A*0201 expression by flow cytometry of PBMCs stained with anti HLA-A*02-specific mAb BB7.2 (BD Bioscience, Pharmingen). Samples positive by flow cytomtery will have the presence of the HLA-A*0201 allele confirmed by PCR.

9.7.1.4 Analyses for HLA-A*0201+ subjects. For the ~35% of selected subjects who are HLA-A0201+, we will stain PBMC with HLA-A*0201 pentamer reagents, each presenting an immunodominant peptide from shared melanoma associated antigens (MAA). Positive and negative control HLA-A0201 pentamer reagents will be used. Data are acquired on a LSR II cytometer and analyzed with FlowJo software. Following exclusion of doublets, $CD4^+$, $CD14^+$, $CD19^+$, dye aggregates and dead cells, the frequency of antigen-specific $CD8^+$ T cells is determined. The lower limit of detection will be defined as 2 SD above the mean of non-specific pentamer binding in a cohort of HLA-A2-healthy donors.

9.7.1.5 Intracellular cytokine staining (ICS) after stimulation with immunodominant peptides and/or overlapping TAA peptide libraries, to be performed selectively for HLA-A*0201+ and HLA-A*0201- subjects. PBMC from selected subjects are stimulated with each of the single HLA-A*0201 peptide (matched to the pentamer reagent for HLA-A*0201+ subjects) or overlapping peptide libraries (all subjects) for 6 hrs in the presence of Brefeldin A and monensin. PBMC are stimulated with CMV pp65 and/or EBV BZLF1 overlapping peptide pools and PMA/Ionomycin as positive controls, or with HLA Class I Ig-like C1 type domain overlapping peptide pool as negative controls, respectively. Following stimulation, cells are stained with phenotypic and functional markers. Data will be acquired with a BD LSR II and analyzed using FlowJo software.

Following exclusion of doublets, $CD14^+$, $CD19^+$, dye aggregates and dead cells, the frequencies of cytokine-producing $CD4^+$ and $CD8^+$ T cells are determined. The lower limit of detection will be defined as 2 SD above the mean of non-specific pentamer binding in a cohort of HLA-A2- healthy donors. Co-staining with pentamers and ICS enables the detection of antigen-specific T cells and their effector function, and this approach may be investigated. Cytokine released into media, following peptide stimulation may be assayed via standard ELISA or via flow cytometry with BD Bioscience Enhanced Sensitivity Cytokine Bead Array kits and analyzed with FCAP Array.

9.8 Blood drawing totals and requirements

All subjects entered into this study are planned to participate in all specified treatment, evaluations, specimen collection, and blood testing with their written consent. A subject may choose to withdraw consent and leave the study at any time. The blood drawing requirements for screening through the first week of cycle 3 of treatment for all studies included in tables in sections 9.1 - 9.5 adds up to approximately 486 mL of blood, less than the 500 mL obtained in a standard blood donation with the Red Cross. This estimate is based on an 8 week schedule where a subject has a relatively short screening period. Volume estimates decrease with a longer screening period.

As the removal of 500 mL of blood (1 unit) on an every 8 week schedule is considered acceptable for individuals > 55 kg, this corresponds to 8.8 mL/kg over this initial time period. Thus, the required volume of 486 mL needed for full participation in the first 3 cycles of this study is acceptable. We will make certain that no participant in this study donates more than 8 mL/kg through cycle 3 day 1. Throughout the study subjects' blood counts will be monitored to ensure only safe amounts of blood are obtained.

If the full blood volume for these research-only labs is not able to be drawn from a subject due to clinical reasons this will not be considered a protocol deviation.

9.9 Biopsy tissue handling guidelines

9.9.1 Review of outside diagnostic material

Prior to therapy at the University of Wisconsin, slides of subjects' prior diagnostic material will be reviewed by a pathologist to ensure accuracy of diagnoses and presence of melanoma at the resection site, as appropriate.

9.9.2 Biopsy handling and pathologic analysis

All biopsies will be dissected fresh with a representative portion frozen in embedding compound (OCT) and preserved at -80º C for subsequent immunostaining for GD2 (which requires frozen sections). If adequate specimen is available a portion of any biopsy from the index lesion may also be flash frozen and preserved at -80º C without OCT embedding for future mRNA extraction and real time PCR quantification of the expression level of genes involved in radiation and immune response. The remainder of the specimen will be fixed in 10% formalin and submitted for permanent, paraffin-embedded sections for high-quality

hematoxylin and eosin stained sections for morphologic analysis of the tumor and for evaluation of necrosis, apoptosis, and any inflammatory infiltrate. Lymphoid infiltrates will be quantified and characterized as peritumoral versus intratrumoral. These sections also will be assessed by immunohistochemical analysis for the presence of CD3, and CD4 or CD8 positive T cells, NK cells (CD56 and CD16), and macrophages (CD68) (antibodies for which all function well on formalin-fixed tissues), as well as PD-L1. Tissue blocks and OCT embedded samples will be stored in the Ranheim laboratory during the course of the study.

9.9.3 Handling of fresh tissue samples

The standard protocol for frozen section analysis will include assessment of activation markers on infiltrating T cells (CD69) and for evidence of Tregs (CD25 and FoxP3) by double immunolabeling with CD3, CD4, or CD8, as appropriate. In addition, we will stain for HLA class I and II to assess changes in HLA level on tumor cells, as enhanced class I expression was noted in the mouse studies. Finally, we will assess the expression of GD2 on tumor cells (the target antigen of hu14.18-IL2), as well as PD-L1 before and after study treatment, as well as the presence of hu14.18-IL2 bound to tumor cells after study treatment.

9.9.4. Quantification of the histologic analyses

For the primary parameters to be assessed on the resected melanoma (i.e., necrosis, apoptosis, and cellular infiltrate), an objective scoring system will be established by a pathologist, grading each specimen with a score of 0, +, ++, +++. The specific criteria for each category will be determined based on the appearance of specimens from the initially enrolled subjects in order to provide a spectrum from which meaningful comparisons can be derived. In addition, purely quantitative assessment of necrosis of tumor cells also will be measured and scored with a value ranging from 0% - 100% of tumor area. These and all pathology analyses will be performed by a pathologist in a non-blinded fashion, but will be repeated, using the scoring system thus established, by a second blinded pathologist. For quantitation of expression levels of HLA class I and II and the GD2 antigen on a per cell basis, it may be useful to use flow cytometric analyses in addition to attempting to qualitatively compare “brightness” of staining between histologic frozen sections in different tumors. As such, if adequate fresh tissue is available, it will be disaggregated into a single-cell suspension, and these parameters will be evaluated by flow cytometry where possible.

9.9.5 Additional preliminary analyses

For select samples that do show a cellular infiltrate, *in situ* hybridization will be performed on frozen sections to determine the expression of intracellular gamma interferon in T cells and NK cells infiltrating into the tumor, and FoxP3 on T cells. Depending upon what is observed, other cytokines may be evaluated selectively by *in situ* hybridization.

9.10 Creation of cell lines

Epstein-Barr virus transformed B cells (lymphoblastoid B cell lines (LCLs), i.e., EBV-B cells) may be generated by *in vitro* culture of lymphocytes with supernatant from B95-8 cells (Human herpesvirus 4 (HHV-4) ATCC® VR-1492™).

Melanoma tumor cells from tumor biopsies may be expanded *in vitro* to establish melanoma cell lines or utilized as fresh tumor cell lysates.

The creation of cell lines is optional for all subjects.

9.11 Blood and tissue banking

After study related testing is completed the blood and tissue samples from subjects who consent to future banking will be stored in a laboratory at the University of Wisconsin - Madison for future IRB approved cancer research. Samples may be shipped to collaborating laboratories outside of UW for the completion of testing related to IRB approved cancer rearch being conducted at UW. If samples are shipped to outside collaborating labs all remaining samples after completion of testing will be sent back to UW.

If a subject consents to both the creation of cell lines and to the banking of samples the cell lines would also be banked for future research. These samples would be kept until they are exhausted. Both coded and de-identified samples may also be shared with other researchers within the University of Wisconsin – Madison for use in IRB approved research. The study PI will review each request for samples to ensure IRB approval and scientific feasibility of the project. Subjects would be permitted to withdraw their samples by informing the study team (orally or in writing), as long as their samples remain identifiable. See section 13.1 for a list of data elements to be maintained for banked samples.

10.0 MEASUREMENT OF EFFECT

Serial clinical measurements of tumor size will be done by standard radiologic and physical exam measurements, appropriate for each subject's clinical status. Tumor measurement will take place after cycle 4, and again every 12 weeks. The clinical response to treatment will be determined at each of the disease status evaluation time points. The updated Immune-Related Response Criteria (irRC)[12] will be utilized for measurement of treatment effect. If tumor lesions are surgically resected as part of this study, the overall response will be determined both with and without the surgically resected lesion(s).

10.1 Measurable Tumor Lesions

Palpable lesions will be considered measurable if they can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$ 10 mm (1.0 cm). Lesions noted on radiographic studies will be considered measurable if they can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$ 20 mm (2.0 cm) with conventional techniques, or as $\geq$ 10 mm (1.0 cm) with **spiral** CT scan.

For the irRC, only index and measurable new lesions are taken into account (in contrast to conventional WHO criteria, which do not require the measurement of new lesions, nor do they include new lesion measurements in the characterization of evolving tumor burden).

At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (up to five lesions per organ, up to a total of 10 visceral lesions and five cutaneous index lesions) is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions (≥5 × 5 mm; up to 5 new lesions per organ: 5 new cutaneous lesions and 10 visceral lesions) are added together to provide the total tumor burden.

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out immune-related progressive disease, irPD).

10.2. Definitions of Overall Response

Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening). The irRC were derived from WHO criteria and, therefore, the thresholds of response remain the same.

10.2.1 Target Lesions

All measurable lesions, up to a maximum of 5 lesions per organ and 10 lesions in total, representative of all involved organs. Target lesions should be selected on the basis of their size (those with the longest diameters), and their suitability for accurate repeated measurements.

The sum of the longest diameters of all target lesions will be calculated at baseline and reported as the ***baseline sum of longest diameters***. This ***baseline sum of longest diameters*** will be used as the reference by which to characterize the objective tumor response. For lesions measurable in 2 or 3 dimensions, always report the measurement in 1 dimension, and this should always include the longest diameter at the time of each assessment.

10.2.1.1 Complete Response (CR)

The disappearance of all target lesions. To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than 4 weeks** after the criteria for response are first met.

10.2.1.2 Partial Response (PR)

A decrease of ≥30% in tumor burden compared with baseline in the sum of the longest diameters of target lesions, taking as reference the ***baseline sum of longest diameters***, in two observations. To be assigned a status of partial response, changes in tumor measurements must be confirmed by two consecutive observations performed **at least 4 weeks** after the criteria for response are first met.

10.2.1.3 Progressive Disease (PD)

At least 20% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart.

10.2.1.4 Stable Disease (SD)

Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease. A 30% or greater decrease in tumor burden compared with baseline cannot be established nor can 20% or greater increase compared with nadir. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of four weeks.

10.2.2 Nontarget Lesions

Measurements of these lesions (all other lesions or sites of disease) are not required, but the presence or absence of each should be noted throughout follow-up.

10.2.2.1 Complete Response (CR)

The disappearance of all nontarget lesions and normalization of tumor marker levels, if applicable. To be assigned a status of complete response, changes in tumor measurements must be confirmed by two consecutive observations performed **no less than 4 weeks** after the criteria for response are first met.

10.2.2.2 Partial Response (PR)

A ≥30% decrease in tumor burden compared with baseline..

10.2.2.2 Stable Disease (SD)

A 30% decrease in tumor burden compared with baseline cannot be established nor 20% increase compared with nadir.

10.2.2.3 Progressive Disease (PD)

The appearance of one or more new lesion(s) and/or unequivocal progression of existing nontarget lesions.

10.2.2.4 Symptomatic Deterioration

Subjects with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having ***symptomatic deterioration***.

10.2.3 Evaluation of Subject's Best Overall Response

The best overall response is the best response recorded from baseline until disease progression/ recurrence, taking as reference for progressive disease the smallest measurements recorded since registration. The table below provides overall responses for all possible combinations of tumor responses in target and nontarget lesions, with or without new lesions.

To be assigned a status of overall CR, PR, or SD, changes in tumor measurements must be confirmed by two consecutive observations performed **no less than 4 weeks** after the criteria for response are first met.

Table 10.2.3-1 Overall Response for all Possible Combinations of Tumor Response

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

CR = complete response; PR = partial response; SD = stable disease;
PD = progressive disease; Non-PD = CR, PR, or SD

10.2.4 First Documentation of Response
The time between initiation of treatment and first documentation of PR or CR.

10.2.5 Confirmation of Response
To be assigned a status of CR or PR, changes in tumor measurements must be confirmed by repeat assessments performed **no less than 4 weeks** after the criteria for response are first met.

10.2.6 Duration of Response
Duration of overall response: The period measured from the time that measurement criteria are met for complete or partial response (whichever status is recorded first) until the first date that recurrent or progressive disease is objectively documented, taking as reference the smallest measurements recorded since treatment started.

10.2.6.1 Duration of Overall Complete Response
The period measured from the time measurement criteria are met for complete response until the first date that recurrent disease is objectively documented.

10.2.6.2 Duration of Stable Disease
A measurement from baseline until the criteria for disease progression are met, taking as reference the smallest measurements recorded since registration. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 4 weeks.

10.2.7 Time to Progression

This interval will be measured from the date of entry on the study to the appearance of new metastatic lesions or objective tumor progression.

10.2.8 Treatment After Progression

Subjects who are determined to have progressive disease, per irRECIST, prior to or at the time of their week 12 disease assessment, will be permitted to continue treatment at the discretion of the treating physician. This is permitted in the absence of a decline in performance status, and as long as there is less than a 50% increase in tumor burden from beginning of treatment. Those subjects may continue to be treated through the week 24 disease assessment timepoint per investigator discretion unless there is a decline in performance status, a need for additional treatment, including new CNS lesions that require immediate treatment.

10.2.9 Methods of Measurement

Imaging based evaluation is preferred to evaluation by clinical examination. The same imaging modality must be used throughout the study to measure disease.

10.2.9.1 CT and MRI

CT and magnetic resonance imaging (MRI) are the best currently available and most reproducible methods for measuring target lesions. Conventional CT and MRI should be performed with contiguous cuts of 10 mm or less in slice thickness. Spiral CT should be performed by use of a 5 mm contiguous reconstruction algorithm. This specification applies to tumors of the chest, abdomen, and pelvis, while head and neck tumors and those of the extremities require specific procedures.

10.2.9.2 Chest X-Ray

Lesions on chest X-ray (CXR) are acceptable as measurable lesions when they are clearly defined and surrounded by an aerated lung. However, CT is preferable.

10.2.9.3 Clinical Examination

Clinically-detected lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For skin lesions, documentation by color photography, including a ruler to estimate size of the lesion, is recommended.

10.3 Definition of "Local Histological Response"

10.3.1 Reason to also evaluate local response

While the overall goal of this hu14.18-IL2 protocol is to evaluate the toxicity of IT administration as well as systemic antitumor responses, our preclinical data document responses of measurable lesions receiving hu14.18-IL2 treatment. Thus this is also an objective of the present study, and has been shown in a separate European study (Weide et al) of IT administration of a separate IC [45]. We will therefore provide assessments of local responses for the lesion(s) receiving direct IT-hu14.18-IL2 treatment. Standard immune RECIST criteria apply to size measurements of all measurable disease identified prior to

treatment (target lesions). In contrast, these local histological assessments of the single lesion receiving IT-hu14.18-IL2 administration are based, primarily, on histologic criteria. Details regarding histological methods are included below in section 10.3.2. Thus, the following definitions apply only to the site receiving hu14.18-IL2 treatment, that is subsequently resected or biopsied, for the purposes of evaluating the local responses in this protocol. All decisions about duration of protocol therapy will be determined by the subject's overall response.

10.3.2 Localized histologic clearance of tumor

Localized histologic clearance of tumor means that the lesion directly injected with hu14.18-IL2 has completely resolved without any remaining palpable or measurable tumor nodule, that is subsequently resected after cycles 1, 2 or 4 and found to have no remaining viable tumor cells, by microscopic examination.

10.3.3 Localized histologic partial clearance of tumor

The injected lesion will be biopsied on day 5 of cycle 1, 2 and 4 in all eligible subjects, and evaluated histologically and compared for these parameters to biopsies of the same lesions obtained prior to treatment. A semi-quantitative assessment of viable tumor cells, tumor cell necrosis and inflammatory infiltrate will be performed by Dr. Ranheim or the responsible pathologist, and each will be graded on a **0** to +++ scale. If a palpable or imageable lesion remains present after cycle 3, the resection/biopsy specimen will be histologically evaluated and determined to show "Localized histologic **partial clearance** of tumor" if it contains some viable tumor cells (based on Melan A or S100 staining of morphologically malignant and viable cells) but these are decreased in comparison to the pretreatment biopsy, being replaced by some increased degree of tumor necrosis and or inflammation. In these, the level of inflammatory infiltrate will also be graded on a + to ++++ scale, to enable semi-quantitative comparison of the level of inflammatory infiltrate in this same tumor when biopsied prior to treatment and at the 5-day timepoint of cycle 1.

10.3.4 Non-detectable local histological tumor clearance

For the purpose of this protocol, tumors evaluated microscopically following cycle 1,2 or 4 that are judged to not meet the above criteria for local histologic partial or full tumor clearance will be scored as "not showing a local histological antitumor response". In these, even if there is no evidence for tumor cell clearance, the level of inflammatory infiltrate will also be graded on a + to ++++ scale, to enable semi-quantitative comparison the level of inflammatory infiltrate in this same tumor when biopsied prior to treatment and at the 5-day timepoint of cycle 1.

10.3.5 Progressive local disease

In the event that the locally injected site appears clinically to be showing tumor progression (rather than transient induration/inflammation from the locally injected hu14.18-IL2), and meets criteria for tumor progression, based on size, then a biopsy (or resection) can be performed earlier than the scheduled biopsy after cycle 4 of treatment. If the histologic review shows a lesion that contains primarily viable melanoma and has no substantial increase in necrosis of tumor cells compared to the pretreatment biopsy, this lesion will be

judged as showing progressive local disease and this lesion will be indentified as having progressive disease in the overall disease assessment. Tumor measurements of all sites of known disease will then be obtained by standard radiologic and physical exam measurements, and decisions about continuation of protocol therapy will be based on the overall disease assessment. Alternatively, if that early biopsy shows that the enlarged lesion, is enlarged due to inflammatory infiltrate, and necrotic tumor cells, with a clear decrease in the number of viable tumor cells (compared to the pretreatment biopsy), this lesion (despite its enlargement) will be considered "Localized histologic **partial clearance** of tumor", and this lesion will not be identified as having progressive disease in the overall disease assessment. In this case, the subject can continue on study and the overall disease assessment will take place as described in section 10.

10.4 Additional Outcome Measures of Treatment Effect

10.4.1 Progression-free survival (PFS)

The length of time from the start of treatment until disease progression or death.

10.4.2 Overall survival (OS)

The length of time from the start of treatment until death from any cause.

10.4.3 Clinical Benefit (CB)

The status of achieving complete response, partial response or stable disease in response to treatment.

10.4.4 Duration of Response

The length of time from documentation of tumor response until disease progression.

11.0 INVESTIGATIONAL AGENTS

11.1 Hu14.18-IL2 FORMULATION

11.1.1 Treatments Administered

Subjects will receive hu14.18-IL2 at the indicated dose, as daily IT injections on 3 consecutive days each 21-day or 28-day cycle. These will be injected directly into and around the "index" tumor (Lesion A). **Details for the clinical administration of these IT-injections are provided in section 5.1.1.** Treatment may be stopped for severe or dose limiting toxicity. Hu14.18-IL2 may be discontinued or dose reduced following resolution of the toxicity prompting the interruption in dosing. See section 6.1 for definition of DLT and list of DLT exceptions. The hu14.18-IL2 is formulated as lyophilized product in 6 mL rubber stopper sealed glass vials containing 4mg hu14.18-IL2. Each 4mg vial of hu14.18-IL2 will be reconstituted in Sterile water for injection (SWFI) 0.5 mL, yielding a final concentration of 8mg/ml*. Subject IT-injections will all be in a volume of at least 1 mL, and no more than 2 ml. Doses less than 8 mg (1 mL) will be diluted with additional SWFI to a final total volume of 1 mL. **[Doses greater than 16 mg, that would require an IT volume greater than 2 mL at 8 mg/mL will be diluted separately (more concentrated) to enable an IT injection of no more than 2.0 mL, as follows: each 4mg vial of hu14.18-IL2 will be*

reconstituted in SWFI 0.25 mL, yielding a final concentration of 16 mg/ml]. The maximum single dose to be given to any subject will be capped at 2.0 mL of 16 mg/mL (32 mg/dose), and would only potentially apply to a subject of very unlikely size. Injections will be performed daily for 3 days. There will be no intra-subject dose escalation. Systemic non-steroidal analgesics (i.e. ibuprofen, acetaminophen) and topical anesthetics (i.e. EMLA cream or lidocaine 4% cream) can be used to treat local pain at the injection site.

11.1.2 Investigational Product

11.1.2.1 Identity of the Investigational Product

The investigational product is an immunocytokine (IC) referred to as "hu14.18-IL2". This agent has been shown, by ^{51}Cr release assays, to be equivalent to a prior investigational product identified as Merck KGA product number hu14.18-IL2 (BB IND-9798). The product is a sterile lyophilized white powder in alum crimped 6mL glass vials sealed with a halobutyl rubber stopper. Each vial contains 4 mg hu14.18-IL2, ready for reconstitution with SWFI.

11.1.2.2 Description of the Investigational Product

Please note, the material that is being used for this study of IT administration of hu14.18-IL2 is from a large batch produced in Europe by Merck Serono, and which is now held by Apeiron Biologics AG, Vienna. Unlike the product produced at the NCI and used previously in our trials of IV administration of this agent (which was vialed as a liquid containing 1 mg/ml), this new batch is prepared and vialed in a 6 mL glass vial as a lyophilized white pellet of 4 mg of hu14.18-IL2 per vial. It was initially designed to be recontituted and diluted with sterile WFI to yield a product of 1 mg/mL for use as IV infusion. Please note that higher concentrations will be used in this study. Dilution studies performed in Spring 2014 by Apeiron tested this material when diluted in lower volumes in order to yield solutions containing hu14.18-IL2 at 1, 8, 16 and 32 mg/mL, and assessed for aggregates. By visual inspection, no undissolved solids were seen at any of these concentrations. By SEC Analysis of DS aggregates, the material at 32 mg/mL was noted to have 2.71% aggregates; in contrast the material at 1, 8 and 16 mg/mL was found to be acceptable, with minimal aggregates noted (0.8%, 0.51% and 0.01% aggregates, respectively). To provide a margin of safety, we will use only the 16 mg/mL as the highest concentration for hu14.18-IL2 to be used for IT injection for this study, with the majority of injections using 8 mg/mL, in order to administer the required dose in the allowed 1.0 – 2.0 mL/dose.

11.1.2.3 Packaging and Labeling of the Investigational Drug Product

Hu14.18-IL2 will be provided to the trial site in open-labeled supplies for admixture and administration at the site. No drug will be dispensed directly to subjects. Hu14.18-IL2 was manufactured according to current Good Manufacturing Practices (GMP).

The information on the vial label will be in accordance with all applicable regulatory requirements. The vial label will include the drug name and quantity of drug contained in the vial, a retest date, lot number, and storage conditions, as well as a notice that federal law restricts the use of the product to investigational use.

The Investigator/Designee (i.e. experimental agent pharmacist) is advised to place a similar label on the syringe after the material is reconstituted and drawn up into the administration syringe from the vials containing the material. In accordance with applicable regulatory requirements, it is recommended that the label on the syringe identify the investigational drug, lot number, the date and time of reconstitution, the expiration date and time (i.e. maximum of 12 hours after reconstitution), amount of drug and volume in the syringe, and storage conditions prior to administration. The infusion syringe label should include the UW protocol study number (to be determined), the subject's initials, and the subject's study identification number.

11.1.2.4 Shipping, Storage, Issue, and Return of Investigational Drug Product
Vials containing lyophilized hu14.18-IL2 are shipped from the storage facility to the Investigator in temperature monitored isolated boxes at 2-8°C.

Upon receipt of the medication, the Investigator, or the responsible pharmacist, will inspect the medication and send back the acknowledgement of receipt form that is enclosed with the parcel, duly completed and signed, confirming that the material is in appropirate condition and quantity. A copy of the signed acknowledgement of receipt must be kept in the study files.

All study medication will remain cooled at 2-8°C and will be stored safely and separately from other drugs in a limited access room under the responsibility of the Investigator/Designee. The hu14.18-IL2 will be stored by the Investigator/Designee in a continuously monitored refrigerator at 2-8°C (reserved for medication storage only). Hu14.18-IL2 **MUST** NOT BE FROZEN. Please note that this differs from previous hu14.18-IL2 liquid formulations. The Investigator/Designee will be responsible for the storage, dispensing, inventory, and accountability of all clinical supplies, and will exercise accepted medical and pharmaceutical practices. An accurate, timely record of the disposition of all clinical supplies will be maintained. The supplies and inventory must be available for inspection by the designated representatives of the UWCCC and NCI upon request. Under no circumstances will the Investigator allow the investigational drug to be used other than directed by this protocol.

The Investigator/Designee must retain and maintain complete records of the disposition of all study medication during the life of the trial. A copy of the inventory record and a record of clinicaly supplies transferred to the investigator's research lab must be maintained and be available for review upon request by the UWCCC or NCI or Apeiron. This form shall include information on:

- Receipt, date, quantity, lot number
- All adminstered units, date, subject information and dose
- All unused units
- All units transferred to the investigators research lab, strictly for non-clinical use
- The date of transfer to the non-clinical research lab and location.

Records shall be maintained by the Investigator/Designee of any alternate disposition of the study medication. These records must show the identification and quantity of each unit transfered, and the person who transferred the test substance, as well as the person in the research laboratory that signed for acceptance of and responsibility for this material as a research reagent, clarifying where it will be stored, that it will only be used for in vitro and non-clinical use in mouse models, and that it will only be used within the laboratory of the principal investigator and the laboratories of collaborators named on this study, and only for research purposes. Such records shall be submitted to the study monitor for forwarding to the Sponsor.

After drug accountability has been verified by the monitor, the Investigator/Designee will initiate removal from the experimental pharmacy of all used investigational materials (medication and packaging) and will initiate submission of all unused hu14.18-IL2 to the investigator's research laboratory, strictly for preclinical (in vitro or experimental animal preclinical research in mice). Instructions for that use will be provided separately.

11.1.2.5 Stability of Investigational Drug Product

From the time of manufacture, lyophilied hu14.18-IL2 is stable for at least 96 months if appropriately stored. It is continuously monitored as part of an on-going stability program. If stability testing allows, the release date of hu14.18-IL2 may be extended. Apeiron will provide immediate notification to the Investigator should the product not meet stability specifications as it should then no longer be used for the clinical study.

The greatest concern to product stability is the potential for aggregation of the protein once it is resuspended. Aggregation may occur when the resuspended solution in a vial is handled roughly or shaken. Do not use a filter in dose preparation.

11.1.2.6 Preparation of the Reconstituted Solution

The following information summarizes the details regarding the storage, formulation, dilution, and handling of this lyophilized material:

Lyophilized hu14.18-IL2 4mg vials are to be stored at 2-8°C until the retest date indicated on the vial. Vials must not be frozen.

All manipulations shall be performed aseptically using sterile material. Vials can only be used until the retest date indicated on their label. Reconstituion shall be performed at ambient temperature. For reconstitution, aspirate 0.5 mL SWFI with a syringe and appropriate needle and add this volume to the 4 mg hu14.18-IL2 vial to achieve a final antibody concentration of 8 mg/mL (or add 0.25 mL SWFI to the 4 mg hu14.18-IL2 vial to achieve a final antibody concentration of 16 mg/mL, if required). After the addition of water, gently rotate the vial 5-10 times and make sure that the liquid has been evenly distributed and has covered the entire inside of the vial. The white pellet will completely dissolve within 10-30 seconds. Do not vortex or agitate to vial to avoid building of foam. If visible particles or parts of the pellet still remain in suspension, gently agitate the vial an additional 5-10 times, until the pellet completely dissolves. Inspect again for the presence

of particles before application or further handling. If solution is clear and free of particles, it might be processed further. Do not use any clowdy solution or suspension still containing visible particles. Hu14.18-IL2 reconstituted solution must be prepared on the day of administration. **No other drugs or diluent can be added to the reconstituted solution containing hu14.18-IL2.** In-line filters must not be used during preparation or administration. Any stock solution remaining after the subject's dose is taken from the vial may not be used to prepare another dose or used for any other purpose.

The 8 mg/mL (or 16 mg/ml) immunocytokine solution now contains:
4% sucrose, 160mM L-Arginine, 20mM citric acid, 0.4% polysorbate 20,, pH 5.4(or 8% sucrose, 320 mM L-arginine, 40mM citric acid, 0.8% polysorbate 20, pH 5.2).
The solution is now ready to be diluted to the correct application volume.

After reconstitution, the product should be injected as soon as possible and not later than 12 hours after reconstitution.

Calculate the appropriate dose volume based upon the subject dose as follows:

Dose (mg) = Dose level (mg/M^2) X actual BSA (in M^2)

Dose volume (ml) = Dose (mg) ÷ 8mg/mL (or ÷ 16 mg/mL, for doses > 16 mg/dose)

Carefully draw up the dose volume into a syringe, label with an expiration of 12 hours.

Minimum dose volume is 1 mL; for doses <8mg/1mL, QS to a final total volume of 1.0 mL with SWFI. Maximum dose volume is 2.0 mL.

11.1.2.7 Stability of the Reconstituted Solution
Hu14.18-IL2 in the syringe to be used for injection should be maintained at room temperature after resuspension. **It must be handled gently without shaking.** It must not be exposed to direct sunlight or heat. Hu14.18-IL2 solutions should not be used if the product has aggregated or precipitated.

All reconstituted hu14.18-IL2 should be used as soon as possible after mixture. Hu14.18-IL2 infusions must only be prepared on the day of infusion and should be administered to subjects within 12 hours after reconstitution.

11.1.2.8 Administration of Investigational Product
The subject's baseline height and weight will be used to calculate the body surface area (BSA). The BSA determination will be multiplied by the dose to determine the subject's daily dose of hu14.18-IL2. BSA calculation will be repeated on Day 1 of each cycle (or within the previous 7 days). However, the dose should only be adjusted if a subject has a ≥ 10% change of body weight relative to the baseline prestudy evaluation.

The actual dosage of hu14.18-IL2 administered will be documented in the dispensing document in the subject's file by the Investigator/Designee.

The dose of hu14.18-IL2 will be injected intratumorally (see sections 5.1 for injection details). Subjects will be treated and observed in the hospital for 6 hours following each injection in cycle 1. Monitoring for subsequent cycles will be as detailed above in section 5.1. Subjects will be under close observation by the Investigator/physician or a designated skilled staff member during the injection of hu14.18-IL2. Vital signs and other laboratory testing will be evaluated per good clinical practice (See Clinical Assessment, Tables in sections 9.1 - 9.4 for details). Subjects will return for biopsy on days 5 of cycles 1, 2 and 4, and will return for clinical and lab evaluation on day 8 of each treatment cycle.

The investigator will use clinical judgment, depending on the subject's status, in releasing the subject from close monitoring.

11.1.2.9 Activity

The activity assays have shown that 1 mg of the hu14.18-IL2 fusion protein is approximately equivalent to 1-3 x 10^6 Units of IL2), depending on the assay used (unpublished data, J. Hank and S. Gillies).

11.1.3 Material Transfer Agreement for Supply of hu14.18-IL2 to the UWCCC from Apeiron

The investigational agent used in this protocol, hu14.18-IL2 and hereinafter referred to as Agent, is supplied by Apeiron. The Agent is provided to the UWCCC for this trial under an agreement between Apeiron and the UWCCC. Therefore, the following obligations/guidelines, in addition to the provisions in the related Investigator-Sponsored Research Agreement between Apeiron and UWCCC contained within the terms of award, apply to the use of the Agent in this study:

1. Agent may not be used for any purpose outside the scope of this protocol, nor can Agent be transferred or licensed to BMS nor to any party not participating in the clinical study. Apeiron data for Agent are confidential and proprietary to Apeiron and shall be maintained as such by the investigators. The protocol documents for the study utilizing Agent contain confidential information and must not be shared or distributed without the permission of Apeiron.
2. Clinical trial data, results, and raw data developed under Collaborative Agreement will be made available as specified in the related Investigator-Sponsored Research Agreement between Apeiron and UWCCC.
3. Publication of clinical trial data is regulated in the related Investigator-Sponsored Research Agreement between Apeiron and UWCCC.

11.2 NIVOLUMAB FORMULATION

11.2.1 NAME of MEDICINAL PRODUCT
Nivolumab (Opdivo) 10mg/mL concentrate for infusion

11.2.2 QUALITATIVE AND QUANTITATIVE COMPOSITION
Each mL of concentrate contains 10 mg of nivolumab.
One 10 mL vial contains 100 mg of nivolumab.

Nivolumab is produced in Chinese hamster ovary cells by recombinant DNA technology.
Excipients with known effect:
Each mL of concentrate contains 0.1 mmol sodium (2.50 mg sodium).

11.2.3 PHARMACEUTICAL FORM
Concentrate for solution for infusion (sterile concentrate).
Clear to opalescent, colorless to pale yellow liquid, that may contain light (few) particulates.
The solution has a pH of 5.5 to 6.5 and an osmolality of approximately 340 mOsm/kg.

11.2.4 CLINICAL PARTICULARS

11.2.4.1 Therapeutic indications
Melanoma
OPDIVO as monotherapy is indicated for the treatment of advanced (unresectable or metastatic) melanoma in adults.

11.2.5.2 Dosing and administration
Treatment must be initiated and supervised by physicians experienced in the treatment of cancer.
The recommended dose of nivolumab in 3 mg/kg administered intravenously over 30 minutes every 2 weeks. Dose escalation or reductin is not recommended. Dosing delay or discontinuation may be required based on individual safety and tolerability.

Nivolumab may be given prior to hu14.18-IL2 injections if given on the same day, but may not be given after hu14.18-IL2 on the same day.

Special warnings and precautions for use
Nivolumab is associated with immune-related adverse reactions. Patients should be monitored continuously (at least up to 5 months after the last dose) as an adverse reaction with nivolumab may occur at any time during or after discontinuation of nivolumab therapy.

11.2.5 PHARMACOLOGICAL PROPERTIES

11.2.5.1 Pharmacodynamic properties

Pharmacotherapeutic group: Antineoplastic agents, monoclonal antibodies. ATC code: L01XC17.

Mechanism of action
Nivolumab is a human immunoglobulin G4 (IgG4) monoclonal antibody (HuMAb), which binds to the programmed death-1 (PD-1) receptor and blocks its interaction with PD-L1 and PD-L2. The PD-1 receptor is a negative regulator of T-cell activity that has been shown to be involved in the control of T-cell immune responses. Engagement of PD-1 with the ligands PD-L1 and PD-L2, which are expressed in antigen presenting cells and may be expressed by tumors or other cells in the tumor microenvironment, results in inhibition of T-cell proliferation and cytokine secretion. Nivolumab potentiates T-cell responses, including anti-tumor responses, through blockade of PD-1 binding to PD-L1 and PD-L2.

11.2.6 PHARMACEUTICAL PARTICULARS

11.2.6.1 List of excipients

Sodium citrate dihydrate
Sodium chloride
Mannitol (E421)
Pentetic acid (diethylenetriaminepentaacetic acid)
Polysorbate 80
Sodium hydroxide (for pH adjustment)
Hydrochloric acid (for pH adjustment)
Water for injections

11.2.6.2 Incompatibilities

In the absence of compatibility studies, this medicinal product must not be mixed with other medicinal products.

11.2.6.3 Shelf life

Unopened vial: 2 years
After opening
From a microbiological point of view, once opened, the medicinal product should be infused or diluted and infused immediately.

After preparation of infusion
From a microbiological point of view, the product should be used immediately.

If not used immediately, chemical and physical in-use stability of OPDIVO has been demonstrated for 24 hours at 2ºC to 8ºC protected from light and a maximum of 4 hours at

20°C-25°C and room light (this 4-hour period of the total 24 hours should be inclusive of the product administration period).

11.2.6.4 Special precautions for storage
Store in a refrigerator (2°C-8°C).
Do not freeze.
Store in the original package in order to protect from light.
For storage conditions after first opening or dilution of the medicinal product (await updated information from BMS)

11.2.6.5 Nature and contents of container
10 mL of concentrate in a vial (Type I flint glass) with a stopper (coated butyl rubber) and a grey flip-off seal (aluminium). Pack size of 1 vial.

11.2.6.6 Special precautions for disposal and other handling
Preparation should be performed by trained personnel in accordance with good practices rules, especially with respect to asepsis.

11.2.6.7 Calculating the dose:
The prescribed dose for the subject is given in mg/kg. Based on this prescribed dose, calculate the total dose to be given. More than one vial of nivolumab concentrate may be needed to give the total dose for the subject.

- Each 10 mL vial of nivolumab concentrate provides 100 mg of nivolumab
- The total nivolumab dose in mg = the subject's weight in kg × the prescribed dose in mg/kg.
- The volume of nivolumab concentrate to prepare the dose (ml) = the total dose in mg, divided by 10 (the nivolumab concentrate strength is 10 mg/ml).
- Dose may be rounded per institutional standard.

11.2.6.8 Preparation and administration:
Take care to ensure aseptic handling when you prepare the infusion. The infusion should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents.

Do not shake the product. Inspect parenteral drug products visually for particulate matter and discoloration prior to administration. Discard vial if solution is cloudy, is discolored, or contains particulate matter other than a few translucent-to-white amorphous particles.

Preparation
- Allow the vials to stand at room temperature for approximately 5 minutes prior to preparation of infusion.
- Withdraw the required volume of nivolumab and transfer into an intravenous bag.

- Dilute with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to perpare a diluted solution with a final concentration ranging from 0.35 mg/mL to 10mg/mL (undiluted). Mix diluted solution by gentle inversion.
- Store the diluted solution for no more than 24 hours under refrigeration (2ºC to 8ºC, 36ºF to 46ºF) or at room temperature (20ºC to 25ºC, 68ºF to 77ºF) for no more than 4 hours. The maximum 4-hour period under room temperature includes administration period.
- Discard partially used vials or empty vials of nivolumab.
- Preparation and dosing rounding per insitutional standard.

Administration

- Do not mix nivolumab with, or administer as an infusion with other medicinal products.
- Flush the intravenous line with 0.9% Sodium Chloride Injeciton, USP or 5% Detroxe Injection, USP after each dose.
- Administer diluted solution over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein-binding in-line filter (0.2-micron to 1.2-micron size).

11.2.6.9 MARKETING AUTHORISATION HOLDER

Bristol-Myers Squibb Company
Princeton, NJ 08543 USA

11.3 IPILIMUMAB FORMULATION

11.3.1 NAME OF THE MEDICINAL PRODUCT

Ipilimumab (Yervoy®) 5 mg/mL concentrate for solution for infusion.

11.3.2 QUALITATIVE AND QUANTITATIVE COMPOSITION

Each mL of concentrate contains 5 mg ipilimumab.
One 10 mL vial contains 50 mg of ipilimumab.
One 40 mL vial contains 200 mg of ipilimumab.

Ipilimumab is a fully human anti-CTLA-4 monoclonal antibody (IgG1κ) produced in Chinese hamster ovary cells by recombinant DNA technology.

Excipients with known effect:
Each mL of concentrate contains 0.1 mmol sodium, which is 2.30 mg sodium.

11.3.3 PHARMACEUTICAL FORM

Concentrate for solution for infusion (sterile concentrate).

Clear to slightly opalescent, colourless to pale yellow liquid that may contain light (few) particulates and has a pH of 7.0 and an osmolarity of 260-300 mOsm/kg.

11.3.4 CLINICAL PARTICULARS

11.3.4.1 Therapeutic indications

Ipilimumab is indicated for the treatment of advanced (unresectable or metastatic) melanoma in adults.

11.3.4.2 Dosage and administration

Treatment must be initiated and supervised by specialist physicians experienced in the treatment of cancer.

Unresectable Metastatic Melanoma

The recommended induction regimen of Ipilimumab is 3 mg/kg administered intravenously over a 30 minute period every 3 weeks for a total of 4 doses. Subjects should receive the entire induction regimen (4 doses) as tolerated, regardless of the appearance of new lesions or growth of existing lesions. Assessments of tumour response should be conducted only after completion of induction therapy.

Liver function tests (LFTs) and thyroid function tests should be evaluated at baseline and before each dose of ipilimumab. In addition, any signs or symptoms of immune-related adverse reactions, including diarrhea and colitis, must be assessed during treatment with ipilimumab with modifications to doses per recommendations.

11.3.5 PHARMACOLOGICAL PROPERTIES

11.3.5.1 Pharmacodynamic properties

Pharmacotherapeutic group: Antineoplastic agents, monoclonal antibodies, ATC code: L01XC11.

11.3.5.2 Mechanism of action

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a key regulator of T-cell activity. Ipilimumab is a CTLA-4 immune checkpoint inhibitor that blocks T-cell inhibitory signals induced by the CTLA-4pathway, increasing the number of reactive T-effector cells which mobilize to mount a direct T-cel immune attack against tumour cells. CTLA-4 blockade can also reduce T-regulatory cell function, which may contribute to an anti-tumour immune response. Ipilimumab may selectively deplete Tregulatory cells at the tumour site, leading to an increase in the IT T-effector/T-regulatory cell ratio which drives tumour cell death.

11.3.5.3 Pharmacodynamic effects

In subjects with melanoma who received ipilimumab, the mean peripheral blood absolute lymphocyte counts (ALC) increased throughout the induction dosing period. In Phase 2 studies, this increase was dose-dependent. In MDX010-20, ipilimumab at 3 mg/kg with or without gp100 increased ALC throughout the induction dosing period, but no meaningful change in ALC was observed in the control group of subjects who received an investigational gp100 peptide vaccine alone.

In peripheral blood of subjects with melanoma, a mean increase in the percent of activated HLA-DR+ CD4+ and CD8+ T cells was observed after treatment with ipilimumab, consistent with its mechanism of action. A mean increase in the percent of central memory (CCR7+ CD45RA-) CD4+ and CD8+ T cells and a smaller, but significant, mean increase in the percent of effector memory (CCR7- CD45RA-) CD8+ T cells also was observed after treatment with ipilimumab.

11.3.5.4 Immunogenicity

Less than 2% of subjects with advanced melanoma who received ipilimumab in Phase 2 and 3 clinical trials developed antibodies against ipilimumab. None had any infusion-related or peri-infusional hypersensitivity or anaphylactic reactions. Neutralizing antibodies against ipilimumab were not detected. Overall, no apparent association was observed between antibody development and adverse reactions.

11.3.5.5 Clinical trials

Overall survival (OS) advantage of ipilimumab at the recommended dose of 3 mg/kg in subjects with previously-treated advanced (unresectable or metastatic) melanoma was demonstrated in a Phase 3 study (MDX010-20). Patients with ocular melanoma, primary CNS melanoma, active brain metastases, human immunodeficiency virus (HIV), hepatitis B, and hepatitis C were not included in the pivotal clinical trial. Clinical trials excluded patients with ECOG performance status > 1 and mucosal melanoma. Patients without liver metastasis who had a baseline AST > 2.5 x ULN, patients with liver metastasis who had a baseline AST > 5 x ULN, and patients with a baseline total bilirubin ≥ 3 x ULN were also excluded.

11.3.6 PHARMACEUTICAL PARTICULARS

11.3.6.1 List of excipients

Tris hydrochloride (2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride)
Sodium chloride
Mannitol (E421)
Pentetic acid (diethylenetriaminepentaacetic acid)
Polysorbate 80
Sodium hydroxide (for pH-adjustment)
Hydrochloric acid (for pH-adjustment)
Water for injections

11.3.6.2 Incompatibilities

In the absence of compatibility studies, this medicinal product must not be mixed with other medicinal products.

11.3.6.3 Shelf life

Unopened vial: 3 years

After opening
From a microbiological point of view, once opened, the medicinal product should be infused or diluted and infused immediately. The chemical and physical in-use stability of the undiluted or diluted concentrate (between 1 and 4 mg/ml) has been demonstrated for 24 hrs at 25°C and 2°C to 8°C. If not used immediately, the infusion solution (undiluted or diluted) may be stored for up to 24 hours in a refrigerator (2°C to 8°C) or at room temperature (20°C to 25°C).

11.3.6.4 Special precautions for storage
Store in a refrigerator (2°C-8°C).
Do not freeze.
Store in the original package in order to protect from light.
For storage conditions after first opening or dilution of the medicinal product, see section 11.3.6.3.

11.3.6.5 Nature and contents of container
10 mL of concentrate in a vial (Type I glass) with a stopper (coated butyl rubber) and a flip-off seal (aluminium). Pack size of 1.
40 mL of concentrate in a vial (Type I glass) with a stopper (coated butyl rubber) and a flip-off seal (aluminium). Pack size of 1.
Not all pack sizes may be marketed.

11.3.6.6 Special precautions for disposal and other handling
Preparation should be performed by trained personnel in accordance with good practices rules, especially with respect to asepsis.

11.3.6.7 Calculating the dose:
The prescribed dose for the subject is given in mg/kg. Based on this prescribed dose, calculate the total dose to be given. More than one vial of ipilimumab concentrate may be needed to give the total dose for the patient.

- Each 10 mL vial of ipilimumab concentrate provides 50 mg of ipilimumab; each 40 mL vial provides 200 mg of ipilimumab.
- The total ipilimumab dose in mg = the subject's weight in kg × the prescribed dose in mg/kg.
- The volume of ipilimumab concentrate to prepare the dose (ml) = the total dose in mg, divided by 5 (the ipilimumab concentrate strength is 5 mg/ml).
- Dose may be rounded per institutional standard.

11.3.6.8 Preparation and administration:
Take care to ensure aseptic handling when you prepare the infusion. The infusion should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents.

Do not shake the product. Inspect parenteral drug products visually for particulate matter and discoloration prior to administration. Discard vial if solution is cloudy, there is pronounced discoloration (solution may have a pale-yellow color), or there is foreign particulate matter other than translucent-to-white amorphous particles.

Preparation

- Allow the vials to stand at room temperature for approximately 5 minutes prior to preparation of infusion.
- Withdraw the required volume of ipilimumab and transfer into an intravenous bag.
- Dilute with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to perpare a diluted solution with a final concentration ranging from 1 mg/mL to 4 mg/mL. Mix diluted solution by gentle inversion.
- Store the diluted solution for no more than 24 hours under refrigeration (2ºC to 8ºC, 36ºF to 46ºF) or at room temperature (20ºC to 25ºC, 68ºF to 77ºF).
- Discard partially used vials or empty vials of ipilimumab.
- Preparation and dosing rounding per insitutional standard.

Administration

- Do not mix ipilimumab with, or administer as an infusion with other medicinal products.
- Flush the intravenous line with 0.9% Sodium Chloride Injeciton, USP or 5% Detroxe Injection, USP after each dose.
- Administer diluted solution over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein-binding in-line filter.

11.3.6.9 MARKETING AUTHORISATION HOLDER

Bristol-Myers Squibb Pharma EEIG
Uxbridge Business Park
Sanderson Road
Uxbridge UD8 1DH
United Kingdom

12.0 STATISTICAL CONSIDERATIONS

Rationale for Moving Through Phases IA, IB, IC and ID

The first goal of this protocol is 1) to establish the MTD/MAD of IT-hu14.18-IL2 in combination with RT, nivolumab and ipilimumab. This will be determined sequentially by first establishing the MTD/MAD of IT-hu14.18-IL2 when administered: 1) alone, 2) in combination with RT, and 3) in combination with RT and nivolumab (see Schema table). After the MTD/MAD of IT-hu14.18-IL2 in combination with RT, nivolumab and ipilimumab is established, the second goal of this study is to evaluate antitumor activity of this treatment, both at the local site treated with RT and hu14.18-IL2 and at distant sites.

Our preclinical published data show the potent efficacy of the combination of RT + anti-CTLA-4 mAb + hu14.18-IL2 in mice that have GD2+ tumors. Furthermore, substantial clinical data have documented the superior clinical anti-tumor activity of the combination of ipilimumab and nivolumab vs. either alone as single agent treatment. Thus, our greatest interest is to evaluate antitumor effects (local and distant) when using this regimen (combining RT + nivolumab + ipilimumab + hu14.18-IL2) for patients with advanced GD2+ melanomas that have already received (or declined) at least one FDA approved treatment demonstrating an impact on survival (anti-CTLA-4, anti PD-1/PD-L1, IL2, etc.).

In addition, published clinical data[51, 52] have shown local antitumor activity for direct IT injection of IL2, as single agent therapy in melanoma. Preliminary preclinical data from our lab indicate some benefit is obtained when RT is combined with IT-IL2. For patients that have GD2- tumors, the IT injection of immunocytokine (at the same site that received RT), is a means for providing IL2 to the tumor site. Even though the anti-GD2 component of the IC will not bind to the tumor, the size of the IC molecule (180kD) should have it retained within the tumor microenvironment longer than an IT injection of soluble IL2 (15kD). Thus, we also plan to evaluate the local and distant clinical activity of RT + IT IL2 (in the form of hu14.18-IL2) + systemic nivolumab and ipilimumab in GD2- subjects.

From our most recent study, we find that ~50% of patients with advanced melanoma have GD2+ tumors[41]. Thus, we plan to treat a total of 28 subjects with the combination of RT + nivolumab + ipilimumab + hu14.18-IL2 , and expect approximately 14 subjects will have GD+ tumors and 14 subjects will have GD- tumors.

12.1 Primary Objectives and Endpoints

The first primary objective of the **Phase IA** component of this trial is to determine the MTD/MAD of IT-hu14.18-IL2 when given to subjects with advanced melanoma using a standard 3+3 dose escalation design where dose-limiting toxicities (DLT) are defined as any grade 3 or 4 toxicity using the CTCAE (version 5.0) with certain pre-defined exceptions (Section 6.1.1.3). The second primary objective of Phase 1A is to evaluate the safety and tolerability of IT-hu14.18-IL2 when given to subjects with advanced melanoma based on the endpoint of the incidence of adverse events (types and severity) graded using CTCAE (version 5.0); see Section 12.4.4.

The first primary objective of the **Phase IB** component of this trial is to determine the MTD/MAD of IT-hu14.18-IL2 after palliative RT in subjects with advanced melanoma using a standard 3+3 dose escalation design where dose-limiting toxicities (DLT) are defined as any grade 3 or 4 toxicity using the CTCAE (version 5.0) with certain pre-defined exceptions (Section 6.1.1.3). The second primary objective of Phase 1B is to evaluate the safety and tolerability of IT-hu14.18-IL2 + RT when given to subjects with advanced melanoma based on the endpoint of the incidence of adverse events (types and severity) graded using CTCAE (version 5.0); see Section 12.4.4.

The first primary objective of the **Phase IC** component of this trial is to determine the MTD/MAD of IT-hu14.18-IL2 in combination with RT + nivolumab in subjects with advanced melanoma using a standard 3+3 dose escalation design where dose-limiting toxicities (DLT) are defined as any grade 3 or 4 toxicity using the CTCAE (version 5.0) with certain pre-defined exceptions (Section 6.1.1.3). The second primary objective of Phase 1C is to evaluate the safety and tolerability of IT-hu14.18-IL2 + RT + nivolumab when given to subjects with advanced melanoma based on the endpoint if the incidence of adverse events (types and severity) graded using CTCAE (version 5.0); see Section 12.4.4.

The first primary objectives of the dose escalation component of **Phase ID** is to determine the MTD/MAD of IT-hu14.18-IL2 in combination with RT + nivolumab + ipilimumab in subjects with advanced melanoma using a standard 3+3 dose escalation design where dose-limiting toxicities (DLT) are defined as any grade 3 or 4 toxicity using the CTCAE (version 5.0) with certain pre-defined exceptions (Section 6.1.1.3). The Phase 1D cohort, in which the MTD or MAD for hu14.18-IL2 in combination with RT + nivolumab + ipilimumab is determined, will be expanded to 28 subjects at the Phase 1D-determined MTD/MAD. The second primary objective of Phase 1D is to determine the safety and tolerability of IT-hu14.18-IL2 + RT + nivolumab + ipilimumab when given to subjects with advanced melanoma based on the endpoint of the incidence of adverse events (types and severity) graded using CTCAE (version 5.0) on all subjects in Phase 1D (dose escalation component and expanded cohort); see Section 12.4.4. The third primary objective of Phase 1D is to evaluate objective tumor responses (endpoint), both locally and systemically as determined by updated Immune-Related Response Criteria (irRC), based on serial measurements (see tables 9.4-3,2,3) of tumor size using standard radiologic and physical examination (see Section 10.0). Thus, it will be determined whether the treatment regimen in Phase ID has sufficient clinical efficacy and is safe and tolerable to warrant further evaluation.

12.2 Secondary Objectives and Endpoints

Secondary objectives and endpoints (described in Table 12.2.1 below) pertain to a variety of clinical, immunologic, histologic, and pharmacokinetic parameters related to administration of IT-hu14.18-IL2 alone and in combination with RT, RT+nivolumab and RT+nivolumab+ipilimumab (See section 2.0).

For all clinical parameters and lab correlates we will first evaluate all eligible subjects as a group. Then we will separately perform a subgroup analysis for the GD2+ subjects separate from the GD2- subjects.

Table 12.2-1

Objective	Endpoint/Outcome Measure	Assessment Time point(s)	Methodology or Definition	Analysis
To assess PFS, OS, CB and duration of response in subjects who receive hu14.18-IL2+ RT+nivolumab+ipilimumab	Quantify the following for Phase 1D subjects: • PFS • OS • CB (CR+PR+SD)	9.4-2, 9.4-3	10.4.1, 10.4.2, 10.4.3, 10.4.4	12.4.5

	• Duration of response			
To evaluate pathologic (tissue) evidence of immune response at the injection site and untreated sites	Quantify the following in biopsy samples: • CD3+ T cells • CD3+CD4+ T cell subset • CD3+CD8+ T cell subset • CD56+ NK cells • CD56+CD16+ NK cell subset • CD68+ macrophages • T regulatory cells (CD25+ & FoxP3+) • Expression level of genes involved in radiation and imune response	9.1-1, 9.1-2, 9.2-1, 9.2-2, 9.3-1, 9.3-2, 9.3-3, 9.3-4, 9.4-1, 9.4-2	9.9.2	12GD2 .4.6
To assess PFS, CB and duration of response to hu14.18-IL2 + RT + nivolumab + ipilimumab based on resistance to prior treatment with anti-CTLA-4 and/or anti-PD1/PDL-1 antibody	For each subgroup of Phase 1D subjects (those with prior resistance to anti-CTLA-4 and/or anti-PD1/PDL-1 and those without resistance), measure and compare: • PFS • CB • Duration of response	9.4-2, 9.4-3	10.4.1, 10.4.2, 10.4.3, 10.4.4	12.4.8
To evaluate serial serum samples to determine the pharmacokinetics of hu14.18-IL2 administered intratumorally	In serial blood samples from all subjects, determine pK by quantification of: • Serum hu14.18-IL2	9.5-1	Laboratory Manual per 9.7	12.4.7
To evaluate each subjects' tumor cells for expression of GD2 and PD-L1, and determine if either antitumor activity or selected treatment-associated biologic effects are more likely for tumors that are GD2+ than GD2- and PD-L1+ then PD-L1-. Also, evaluate whether PD-L1 expression is induced or augmented from baseline following initiation of treatment	In biopsies from all subjects, quantify GD2 expression on tumor cells, and then: • Compare objective tumor response in GD2+ versus GD2-subjects. • Compare biologic effects (e.g., ADCC, presence of T regulator cells, etc.) in GD2+ versus GD2-subjects. In biopsies from all subjects, quantify PD-L1 expression on tumor cells, and then: • Compare objective tumor response in PD-L1+ versus PD-L1- subjects. • Compare biologic effects (e.g., ADCC, presence of T regulator cells, etc.) in PD-L1+ versus PD-L1- subjects. • Compare PD-L1 expression before and after treatment	9.1-1, 9.1-2, 9.2-1, 9.2-2, 9.3-1, 9.3-2, 9.3-3, 9.3-4, 9.4-1, 9.4-2	9.9.2, 9.9.3	12.4.9 12.4.10
To evaluate the immunologic activation induced in vivo by IT-hu14.18-IL2 using in vitro cellular, serologic and flow cytometry immune assays	In selected subjects, measure: • Antibody dependent cell-mediated toxicity function • Antibody independent cell-mediated toxicity (i.e., "Natural Killer") function • Soluble IL2α levels	9.5-1	9.7.1.1 9.9.2 Laboratory Manual per 9.7	12.4.6

To evaluate for histological evidence of antitumor activity	Quantify the following parameters in biopsy samples: • necrotic tumor cells • apoptosis • inflammatory infiltrate • cellular phenotype of infiltrate • presence of hu14.18-IL2 • expression of activation marker CD69 on T cells • expression levels of HLA class I and II	9.1-1, 9.1-2, 9.2-1, 9.2-2,9.3-1,9.3-2, 9.3-3,9.3-4, 9.4-1, 9.4-2	9.9.3 9.9.4	12.4.6
To evaluate circulating tumor cells, exosomes, endogenous antibodies, and/or DNA as explaratory biomarkers associated with clinical response to IT-hu14.18-IL2 + RT + nivolumab + ipilimumab	In selected subjects, blood samples will be evaluated for: • presence of circulating tumor cells • presence of tumor cell exosomes • presence of endogenous anti-hu14.18-IL2 antibodies • T cell repertoire • FcR genotype • KIR/KIR-ligand genotype	9.5-1	9.7.1 Laboratory Manual per 9.7	12.4.6
To evaluate induction of T cell responses to melanoma-associated antigens	In selected subjects who are HLA-A201+, PBMCs isolated from serial blood samples will be evaluated for: • frequency of antigen (MAA)-specific CD8+ T cells In selected HLA-A201+ and HLA-A201- subjects, PBMCs isolated from serial blood samples will be evaluated for: • frequency of cytokine-producing CD4+ and CD8+ T cells	9.5-1	9.7.1.3, 9.7.1.4, 9.7.1.5	12.4.6
To evaluate objective tumor responses, both locally and systemically, in response to treatment with: IT-hu14.18-IL2 alone (Phase IA), IT-hu14.18-IL2+RT (Phase IB), and IT-hu14.18-IL2+RT+nivolumab (Phase IC)	For subjects in phase IA, IB, and IC of the trial: • Using tumor measurements obtained via standard radiologic and physical examination, determine the clinical response to treatment using updated irRC	9.1-1, 9.1-2 9.2-1, 9.2-2 9.3-1, 9.3-2, 9.3-3, 9.3-4, 9.3-5	10.0	12.4.5
To assess PFS, OS, CB and duration of response in subjects who receive hu14.18-IL2+ RT+nivolumab	Quantify the following for Phase 1D subjects: • PFS • OS • CB (CR+PR+SD) Duration of response	9.4-2, 9.4-3	10.4.1, 10.4.2, 10.4.3, 10.4.4	12.4.5

12.3 Study Design/Sample Size Justification

As the primary objectives of Phases 1A, 1B, 1C and the dose escalation component of Phase 1D of this trial are to determine the MTD or MAD of hu14.18-IL2 when administered intratumorally, either alone or in combination with RT, RT+nivolumab, or RT+nivolumab+ipilimumab respectively, and to evaluate toxicities, no formal power

calculations are relevant. Rather, the sample size chosen was based on typical dose escalation designs (with 3-6 subjects per dose level). The total number of subjects treated in the study will depend on the number of dose levels tested and the number of subjects treated in each cohort before the MTD/MAD has been determined.

Table 12.3.1 below shows the probabilities of escalating to the next dose level, based on the true DLT rate at the current dose.

Table 12.3.1-2: Probabilities of dose escalations							
	True Toxicity Probability Rate at a Given Dose						
	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Probability of Escalation	0.91	0.71	0.49	0.31	0.17	0.08	0.03

Thus, if the true DLT probability is 0.30 at the current dose, there is a 0.49 probability of escalating to the next dose.

12.3.1 Phase IA

A traditional "3+3" dose escalation design will be used to determine the MTD/MAD of IT-hu14.18-IL2. The MTD will be defined as the highest safely tolerated dose where 0/3 or 1/6 (less than 33%) subjects experience a DLT and 2/3 or ≥ 2/6 subjects have experienced a DLT at the next higher dose level. The maximal administered dose (MAD) will be defined as the highest safely tolerated dose where 0/3 or 1/6 (less than 33%) subjects experience a DLT but no higher dose level has been assessed. Subjects will be accrued in cohorts of three. If no DLT is observed in these 3 subjects, a new cohort of 3 subjects will be treated at the next higher dose level. If one of the initial 3 subjects develops a DLT, then 3 additional subjects (total of 6) will be added to that same dose level. If DLT is observed in only one of 6 subjects at a given dose level, the next cohort of subjects will be allowed to start at the next higher dose level. If two or more subjects experience DLT at a particular dose level, then the dose escalation scheme will cease and the previous dose will be declared the MTD for Phase IA.

The primary objectives of Phase IA of this study are to evaluate safety and tolerability by evaluating toxicities and to determine the MTD or MAD of hu14.18-IL2 when administered intratumorally. A total of 9-18 subjects will be required to complete the 3 dose levels.

12.3.2 Phase 1B

Once Phase IA is completed, enrollment of subjects into Phase IB will begin. A traditional "3 + 3" dose escalation design will be used to determine the MTD/MAD of IT-hu14.18-IL2 after palliative RT. Initially 3 subjects will be treated at one dose level below MTD or MAD determined in Phase IA. If 0/3 or 1/6 DLTs, 3 subjects will be treated at Phase IA-determined MTD or MAD. If 0/3 or 1/6 DLTs occur, then this is the Phase IB-determined MTD or MAD. A total of 6-12 subjects will be required to complete the 2 dose levels. In the event that excessive toxicity is observed in the cohort employing the initial dose level in this

phase (i.e., one dose level below the Phase 1A determined MTD/MAD), then accrual of 3 subjects into a cohort employing IT-hu14.18-IL2 at two dose levels below the Phase 1A determined MTD/MAD will be performed.

The primary objectives of Phase IB of this study are to evaluate safety and tolerability by evaluating toxicities and to determine the MTD or MAD of hu14.18-IL2 when administered entratumorally in combination with RT. A total of 6-12 subjects will be required to complete the 2 dose levels.

12.3.3 Phase IC

Once Phase IB is completed, enrollment of subjects into Phase IC will begin. A traditional "3+3" dose escalation design will be used to determine the MTD or MAD of IT-hu14.18-IL2 combined with RT and nivolumab. Initially 3 subjects will be treated at one dose level below MTD or MAD determined in Phase IB. If 0/3 or 1/6 DLTs, 3 subjects will be treated at Phase IB-determined MTD or MAD. If 0/3 or 1/6 DLTs occur, then this is the Phase IC-determined MTD or MAD. A total of 6-12 subjects will be required to complete the 2 dose levels. In the event that excessive toxicity is observed in the cohort employing the initial dose level in this phase (i.e., one dose level below the Phase 1B determined MTD/MAD), then accrual of 3 subjects into a cohort employing IT-hu14.18-IL2 at two dose levels below the Phase 1B determined MTD/MAD will be performed.

The primary objectives of Phase IC of this study are to evaluate safety and tolerability by evaluating toxicities and to determine the MTD or MAD of hu14.18-IL2 when administered intratumorally in combination with RT and nivolumab. A total of 6-12 subjects will be required to complete the 2 dose levels.

12.3.4 Phase ID

Once Phase IC is completed, enrollment will begin of subjects into the dose-escalation component of Phase ID. A traditional "3+3" dose escalation design will be used to determine the MTD/MAD of IT-hu14.18-IL2 combined with RT, nivolumab and ipilimumab. Initially 3 subjects will be treated at one dose level below MTD or MAD determined in Phase IC. If 0/3 or 1/6 DLTs, 3 subjects will be treated at Phase IC-determined MTD or MAD. If 0/3 or 1/6 DLTs occur, then this is the Phase ID-determined MTD or MAD. In the event that excessive toxicity is observed in the cohort employing the initial dose level in this phase (i.e., one dose level below the Phase 1C determined MTD/MAD), then accrual of 3 subjects into a cohort employing IT-hu14.18-IL2 at two dose levels below the Phase 1C determined MTD/MAD will be performed.

The first primary objective of Phase ID of this studyis to determine the MTD or MAD of intratumoral hu14.18-IL2 in combination with palliative RT, nivolumab and ipilimumab. A total of 6-12 subjects will be required to complete the 2 dose levels.

When the MTD or MAD for Phase ID is determined, a total of 28 subjects will be enrolled at that dose (Phase ID expanded cohort) . The second primary objective of Phase ID of this study is to evaluate safety and tolerability by evaluating toxicities of intratumoral hu14.18-IL2 in combination with palliative RT, nivolumab and ipilimumab. The third primary objective of Phase ID is to evaluate the anti-tumor response (CR or PR or local histological tumor clearance) as defined in section 10 in the Phase ID expanded cohort. Based on the results of two previous phase II trials and a pilot clinical trial with hu14.18-IL2, and on clinical experience with the combination of ipilimumab and nivolumab in subjects with metastatic melanoma, we will not consider treatment with RT + nivolumab/ipilimumab + IT- hu14.18-IL2 promising in this subject population if the response probability is 0.10 or less. On the other hand, a response probability of at least 0.25 would be considered sufficient evidence to warrant further clinical investigation. In order to ensure sufficient accuracy in the estimation of the response rate, we would like to treat a sufficient number of subjects with treatment regimen under Phase ID. The following table provides power calculations for a range of underlying response probabilities using a one-sided binomial test at a significance level α=0.1.

Table 12.3.4-1: Power 1-β for testing H_0

	p_1						
	0.10	0.15	0.20	0.25	0.30	0.35	0.40
n=28	0.05	0.32	0.59	0.80	0.93	0.98	0.99
n=14	0.05	0.24	0.41	0.59	0.74	0.85	0.92

Assuming that the number of subjects with a response is binomially distributed, this Phase ID expansion component with 28 subjects has 0.80-0.93 power for detecting a true response probability of 0.25-0.30 using one-tailed test at a significance of 0.05. For GD2+ and GD2- subgroups each with a sample size of 14, the study would have 0.59-0.74 power without adjustment for multiplicity of testing.

DLT-Level Toxicities Occuring Outside the DLT Monitoring Period

To ensure that subjects are not exposed to an unacceptably high incidence of toxicity occurring outside of the DLT window, adverse events (grade 3-4, other than those defined in protocol section 6.1.1.3 as hu14.18-IL2 DLT exceptions) in subjects treated with the combination therapy of hu14.18-IL2 with local radiation, nivolumab and ipilimumab will be monitored continuously based on sequential probability ratio tests (SPRT).

The following table gives the SPRT boundaries for suspension of the study for the toxicity criteria specified above (adverse events of grade 3-4, excluding the hu14.18-IL2 DLT exceptions found in section 6.1.1.3, that are probably or definitely related to hu14.18-IL2). The SPRT boundaries test the null hypothesis that the probability of toxicity is no more than 0.59, the probability of the standard treatment with nivolumab and ipilimumab, against the alternative hypothesis that it is at least 0.85 at a one-tailed 0.05 (α) level test (the exact level is 0.0603) with power 0.951 (1-β). If the number of subjects with toxicity is equal to the boundary, the study will be suspended. Blank entries imply no decision can be made due to insufficient data.

Table 12.3.4-2: SPRT boundaries for H_0:p≤0.59 vs H_1:p≥0.85 at α=0.0603 with power 1-β=0.951														
# of Subjects	1	2	3	4	5	6	7	8	9	10	11	12	13	14
# of Events								8	9	9	10	11	12	12
# of Subjects	15	16	17	18	19	20	21	22	23	24	25	26	27	28
# of Events	13	14	14	15	16	17	17	18	19	20	20	21	22	23

For action taken in the event of excessive toxicity and decisions regarding protocol suspension or continuation, see Section 6.4.

12.4 Statistical Analysis Plan

12.4.1 Definitions:

Maximum Tolerated Dose (MTD):
The MTD is defined as the highest dose level at which less than 33% of the subjects experience a DLT. It will be estimated according to methods described in section 5.5.1.

Maximum Administered Dose (MAD):
The MAD is defined as the highest safely tolerated dose where less than 33% subjects experience a DLT but no higher dose level has been assessed. It will be estimated according to methods described in section 5.5.1.

Dose Limiting Toxicity (DLT):
DLT is defined in section 6.1.1.1.

Evaluable for safety:
All eligible subjects who receive any administration of hu14.18-IL2 will be considered evaluable for safety.

Evaluable for efficacy:
All eligible subjects who receive any administration of hu14.18-IL2 will be considered evaluable for efficacy.

Safety analysis population:
The safety analysis population consists of all subjects who are evaluable for safety.

Efficacy analysis population:
The efficacy analysis population consists of all subjects who are evaluable for efficacy.

Pharmacokinetic analysis population:
The pharmacokinetic analysis population will include all subjects for whom sufficient data is available to calculate the derived pharmacokinetic parameters on an as-treated basis.

12.4.2 General

Descriptive statistics will primarily be generated to summarize the data. For continuous variables, descriptive statistics may include the number of subjects reflected in the calculation (n), mean (standard deviation) and median (range of minimum to maximum); frequencies and percentages may be displayed for categorical data (e.g., toxicities, responses). Data analysis will primarily be performed using R version 3.1 or later, a publicly available statistical language for statistical computing and graphics (http://www.r-project.org) and SAS® (SAS Institute Inc., Cary, North Carolina) version 9.2 or greater. Analysis of pharmacokinetic parameters will be performed using the R package PK.

12.4.3 Demographics

All demographic variables (e.g., gender, age, weight, etc.) will be summarized by standard descriptive statistics, i.e., in terms of mean (standard deviations) and median (ranges) for variables on a continuous scale, and in terms of frequency/percentage tables for variables on a categorical scale.

12.4.4 Analysis of Safety Endpoints

Toxicities observed will be summarized in terms of types and severities by the NCI Common Terminology Criteria for Adverse Events (CTCAE) v5.0. This analysis will be performed on the safety analysis population. The number and severity of toxicity incidents will be summarized with frequency and proportion. The 95% confidence interval for the proportion of subjects with severe complications (grade 3 or higher toxicities) will be constructed.

12.4.5 Analysis of Efficacy Endpoints

Objective tumor responses (OR) and clinical benefit (CB) will be summarized using descriptive statistics. Furthermore, a point estimate along with the 95% confidence interval for the proportion of subjects with OR and CB will be provided. Kaplan-Meier method will be used to estimate the survival distribution of duration of response, progression-free survival and overall survival for the Phase ID expansion cohort. Clinical outcome of OR and CB will be summarized by dose level for Phase IC.

12.4.6 Analysis of Immunologic and Histological Parameters

Immunologic activation induced *in vivo* by IT- hu14.18-IL2 will be evaluated using both *in vivo* and *in vitro* analyses. Immunologic and histological assessments will be performed at various times as previously outlined in tables in sections 9.1 - 9.4 during the cycle of treatment and thus will give repeated measures over time from each subject. A descriptive profile of all immunologic and histological parameters will be given in tabular and graphical formats. Missing values will not be imputed; they will be treated as missing. Changes between assessment time points will be evaluated using a paired t-test or non-parametric Wilcoxon signed rank test, depending on the scale and distribution of the endpoint. Mixed effects models with subject specific random effects will be used to evaluate changes in immunologic parameters over time (cycles). The shape of the distribution of each immunologic parameter will be assessed before performing the analysis to validate the normality assumption. The peak levels of anti-idiotypic antibodies (i.e., anti-hu14.18-IL2)

will be computed for each cycle and summarized by standard descriptive statistics. The dose-response relationship between serum levels of fusion protein and hu14.18-IL2 dose will be examined by fitting a linear or nonlinear regression model. F-test from the analysis of variance and two-sample t-test will be used to evaluate differences and changes of levels of immunologic parameters among and between dose levels. Presence of necrotic tumor cells, inflammatory infiltrate, cellular phenotype of infiltrate, and presence of IC within the tumor will be summarized by descriptive statistics. Changes from baseline will be evaluated using a paired McNemar's test for binary outcomes. Differences in levels of tumor cell necrosis, immune cell density, and density of infiltrating lymphocytes between the locally injected lesion (Lesion A) and the untreated lesion (Lesion B) will be assessed using a paired t-test or non-parametric Wilcoxon signed rank test. The association between clinical response and the presence of necrotic tumor cells, inflammatory infiltrate, cellular phenotype of infiltrate, and presence of IC within the tumor will be assessed by calculating polychoric correlation coefficients.

12.4.7 Analysis of Pharmacokinetic Parameters

Pharmacokinetic assessments will be performed on multiple serum specimens for each subject, as no PK data yet exist for IT delivery of hu14.18-IL2. As patients will only be available for 6 hours following each of their IT-IC doses during cycle 1, insufficient data points for truly accurate PK determinations will be obtained. Even so, with the planned collections of serum 1, 2, 6 and approximately 24 hours after the IT-IC doses, PK parameters can still be calculated (24 hour sample taken at the next day's clinic visit). Each specimen will be evaluated for intact hu14.18-IL2, for hu14.18, and for IL2, because our murine study showed that the IL2 was cleaved off of the hu14.18-IL2 *in vivo* after ~4 hr. However, this cleavage was not seen in any of our human trials of hu14.18-IL2 given IV, nor in our *in vitro* studies incubating hu14.18-IL2 in human serum. Thus we do not anticipate cleavage of IL2 from the IC molecule in this study of IT administration, but we will evaluate for it using standard descriptive statistics. For each subject the $t_{1/2}$ α and $t_{1/2}$ β of each of these 3 determinants, as well as area under the curve (AUC) and clearance (CL) will be calculated. All PK parameters listed above ($t_{1/2}$ α, $t_{1/2}$ β, AUC, and CL) will be summarized by dose level with simple summary statistics: mean (standard deviations if numbers and distribution permit) and medians (ranges). The analysis of all PK parameters will be performed using the PK analysis population. Scatterplots will be used to explore possible associations between the dose and AUC. The Jonckheere-Terpstra trend test will be performed to determine the significance of the association between increasing dose level and AUC. Spearman's rank correlation analysis will be performed to determine the relationship between actual dose administered and the pharmacokinetic parameters. Additionally, logistic regression analyses will be performed to correlate PK parameters with toxicity (grade ≥ 3 vs. grade 0-2) and response (PR or CR or local histological tumor clearance vs. no PR or CR or local histological tumor clearance). AUC will be tested for dose-proportionality using the power model. The power model assumes a linear relationship between the natural log (ln)-transformed parameter and ln(dose), by including the ln(dose) term in the analysis of variance (ANOVA) model: $\ln(AUC) = \alpha + \beta * \ln(dose) + \text{random error}$, where α is the intercept, β is the slope, and ln(dose) is based on the dose level for each subject. The ln(dose) term, a continuous variable, is used to estimate the linear relationship

with the ln(AUC). A treatment term will be added to the above model as a classification variable to assess the goodness of fit of the statistical model. If the 95% confidence interval for the slope contains 1, then dose proportionality will be concluded. Possible relationships between anti-hu14.18-IL2 antibody, and PK parameters, will be evaluated.

12.4.8 Subgroup Analysis by Resistance to Prior Treatment with anti-CTLA-4 and/or anti-PD1/PD-L1 antibodies

In subjects who receive hu14.18-IL2 + RT + nivolumab + ipilimumab, clinical outcomes such as OR, duration of response, CB, PFS, and OS will be analyzed separately among subjects who were resistant to prior treatment with anti-CTLA-4 and/or anti-PD1/PD-L1 antibodies and those who lacked resistance in a subgroup analysis. This comparison is by necessity exploratory in nature and hypothesis generating.

12.4.9 Subgroup Analysis by GD2+ and GD2-

Clinical outcomes such as OR, duration of response, CB, PFS and OS and treatment-associated selected biologic effects will be analyzed separately among patients who are GD2+ and those who are GD2- in a subgroup analysis. In addition, these outcome measures will be compared using two-sample t-test, log rank test and chi-square/Fisher's exact test between the two groups of patients who are GD2+ and who are GD2-. This comparison is by necessity exploratory in nature and hypothesis generating. Immunohistochemical analyses in our most recent study of patients with Stage III and IV melanoma patients showed that half the patients were positive for GD2 on their melanoma. Therefore, assuming 14 patients with GD2+ and 14 patients with GD2- melanoma, this comparison will have power of 0.75-0.91 for detecting the effect size of 1-1.25 between the two groups, i.e. the difference in the mean between the two groups that is 1-1.25 times the standard deviation, according to a two-tailed two-sample t-test at a significance level of 0.05.

12.4.10 Analysis of PD-L1 Expression and Subgroup Analysis by PD-L1+ and PD-L1-

PD-L1 expression level will be compared between baseline and after initiation of treatment using linear mixed effects model after suitable transformation of PD-L1 expression level. Clinical outcomes such as OR, duration of response, CB, PFS and OS and treatment-associated selected biologic effects will be analyzed separately among patients who are PD-L1+ and those who are PD-L1- in a subgroup analysis. In addition, these outcome measures will be compared using two-sample t-test, log rank test and chi-square/Fisher's exact test between the two groups of patients who are PD-L1+ and who are PD-L1-. This comparison is by necessity exploratory in nature and hypothesis generating.

12.5 Estimate Accrual Time

Based on accrual to a separatae melanoma study performed at the UWCCC, also requiring patients with injectible/biopsiable melanoma lesions, we estimate ~12 patients to be enrolled yearly, and thus total accrual should be complete for this trial in ~4 years.

13.0 CONFIDENTIALITY

To protect the confidentiality of subjects in this study the blood and tissue samples will be coded. The coded samples may be labeled with any subset of the following: sample number, subject ID number, date of biopsy/blood draw, biopsy location (if applicable), and study number. The key will be saved in computer files that are protected via passwords and access rights. The research data will also be saved in secure computer files or in the OnCore database which is only accessible via username, password, and access rights. When not in use paper files will be stored in the research offices which are locked when not occupied, or in locked closets or cabinets associated with the Albertini, Sondel, Morris, or Ranheim lab. Research samples will be stored in the Albertini, Sondel, Morris, Ranheim lab, or a collaborating laboratory at the University of Wisconsin - Madison.

Table 13.0-1: Identifiers and information to be collected for this study:
Name
Medical record number
Date of birth
Gender, Race and Ethnicity
Address, telephone & fax numbers
E-mail address
Health Insurance Information
Tumor characteristics & genetic information
Medical history including dates of treatments and procedures
Information generated from participation in the study

13.1 Management of Banked Samples

Table 13.1-1 below outlines the data to be maintained for samples banked for future research. Information listed will be available to lab staff for use in selecting samples for potential future research projects. All future research projects using coded samples would be IRB approved before use of the samples.

Table 13.1-1: Data to be maintained with samples banked for future research
Study number & study subject ID number
Lab sample ID number
Diagnosis
Stage of disease
Therapeutic response
Date and types of treatments received
Dates and time points of sample collection
Biopsy locations
Results of lab analysis completed for the study

14.0 REFERENCES:

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APPENDIX 1: Eastern Cooperative Oncology Group (ECOG) Performance Status

Eastern Cooperative Oncology Group (ECOG) Performance Status[1]

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

[1] Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-55.

APPENDIX 2: Ipilimumab and Nivolumab Toxicity Management Algorithms

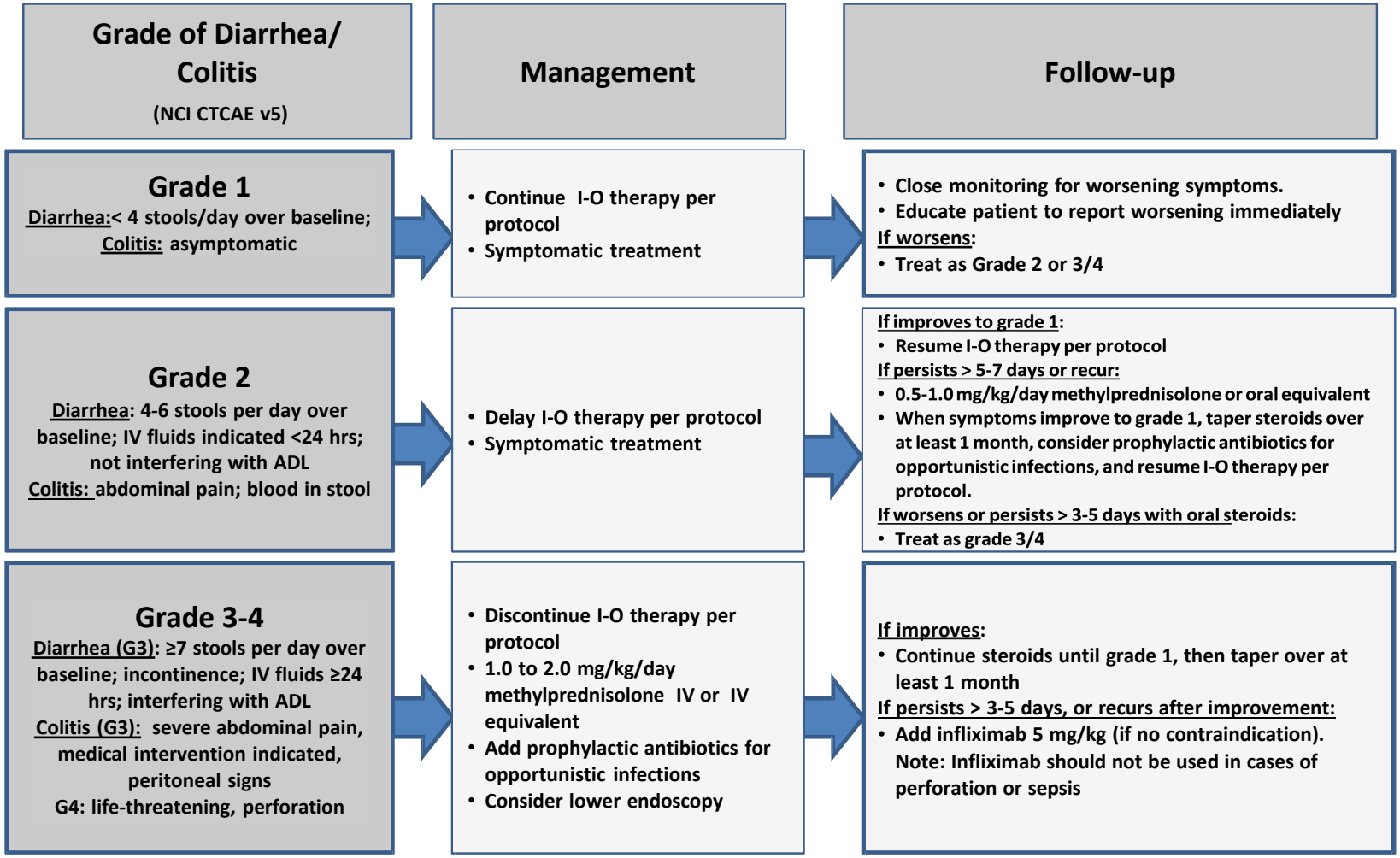
These guidelines may be supplemented by discussions with the Study Chair and Study PI. The guidance applies to all immuno-oncology agents and regimens. A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated.

These algorithms are recommendations only, and the dose modification sections of the protocol should always be followed. Not following the management algorithms, as long as the main dose modification sections of the protocol are adhered to, will not be considered a protocol deviation.

Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids. Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is recommended. The frequency and severity of the related adverse events covered by these algorithms will depend on the immuno-oncology agent or regimen being used.

GI Adverse Event Management Algorithm

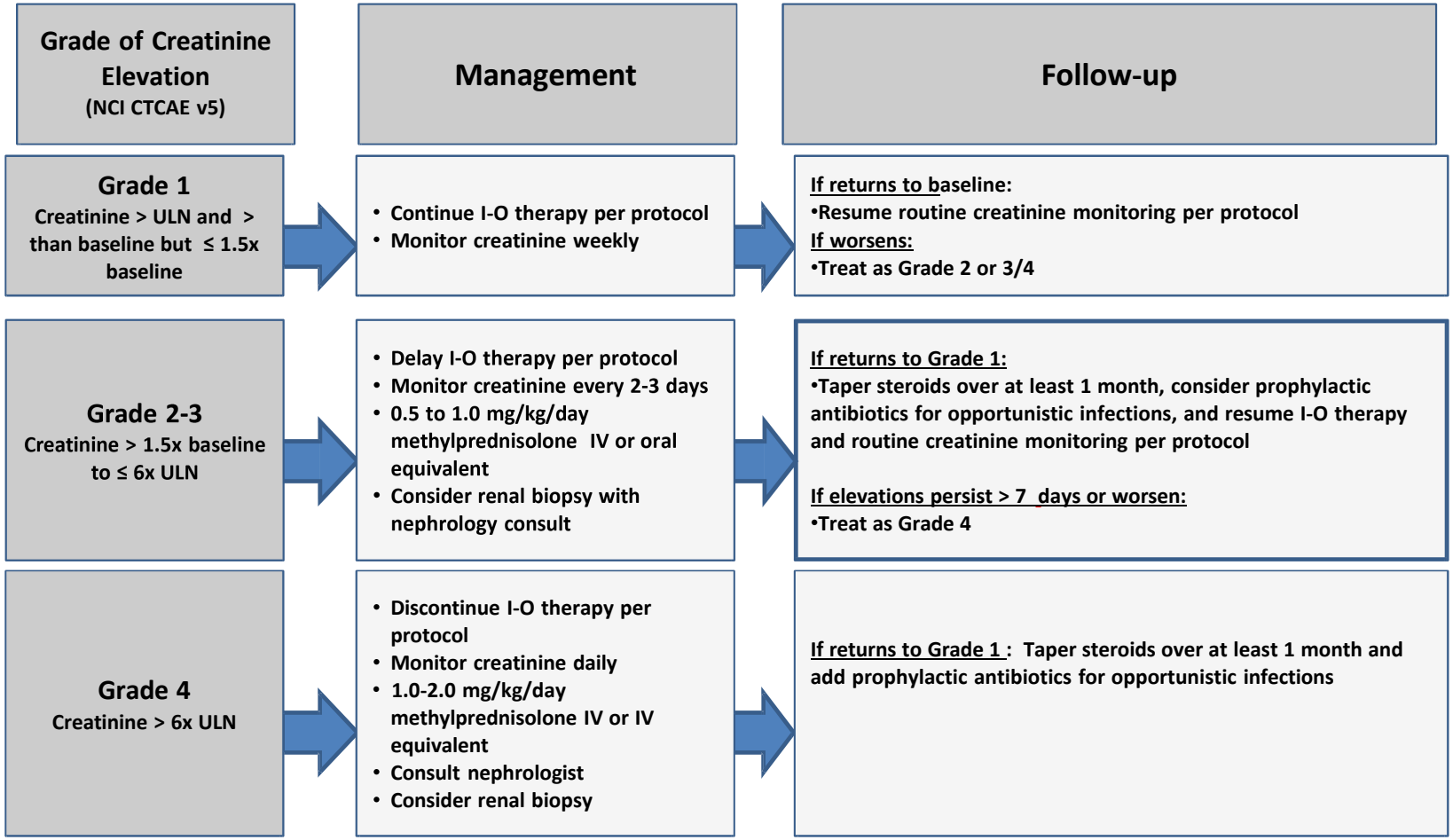
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Renal Adverse Event Management Algorithm

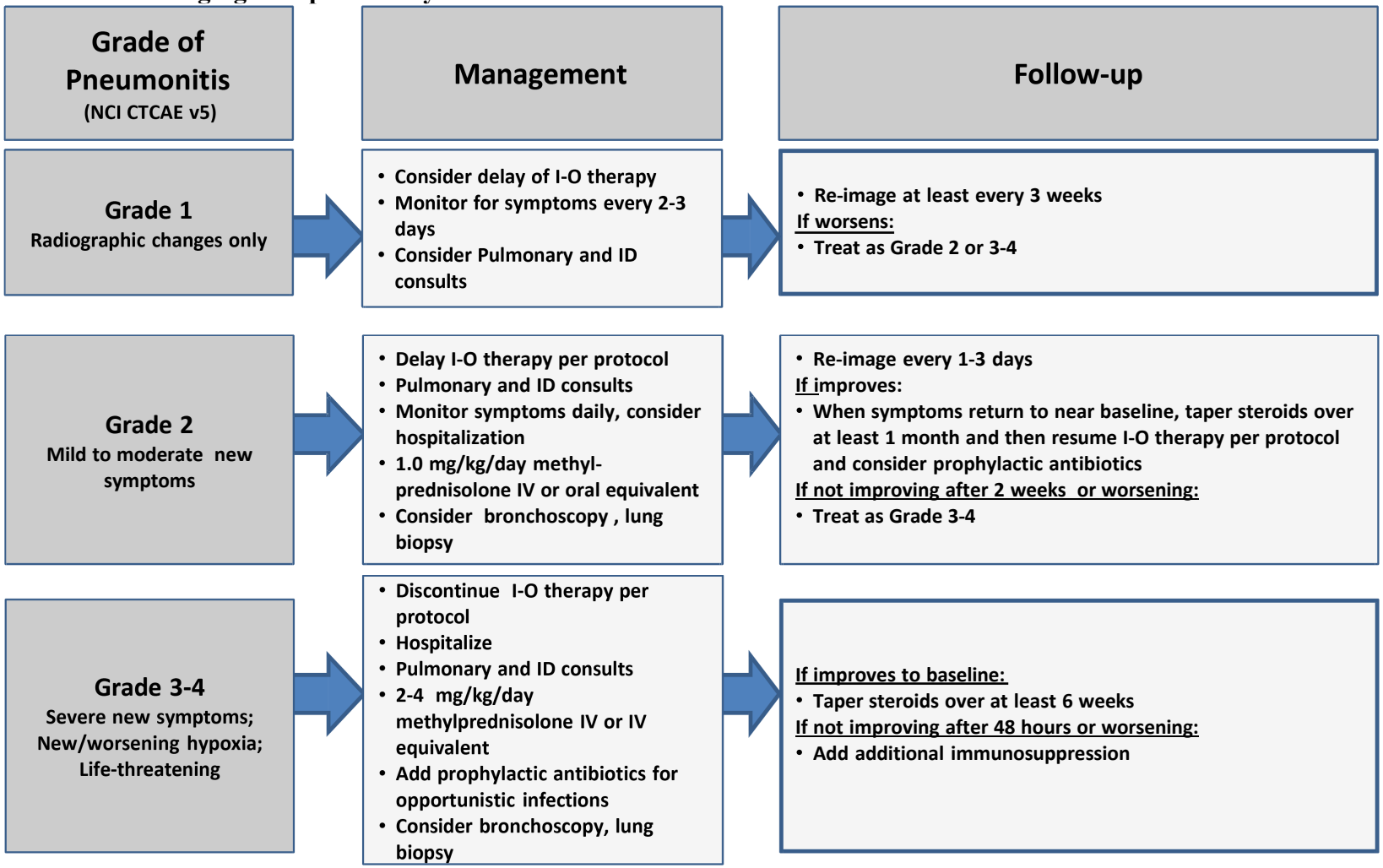
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm

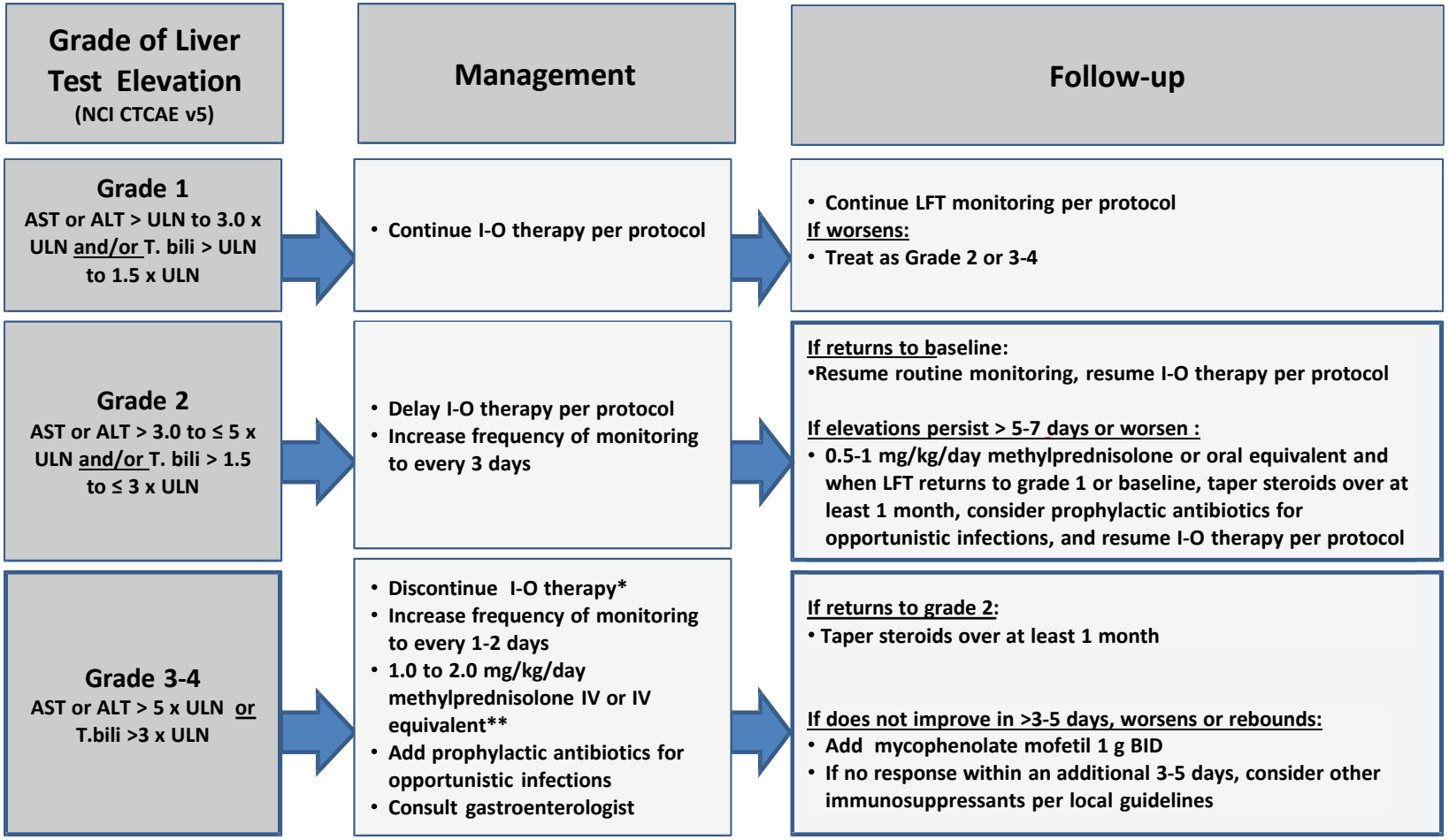
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids. Updated 05-Jul-2016

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.



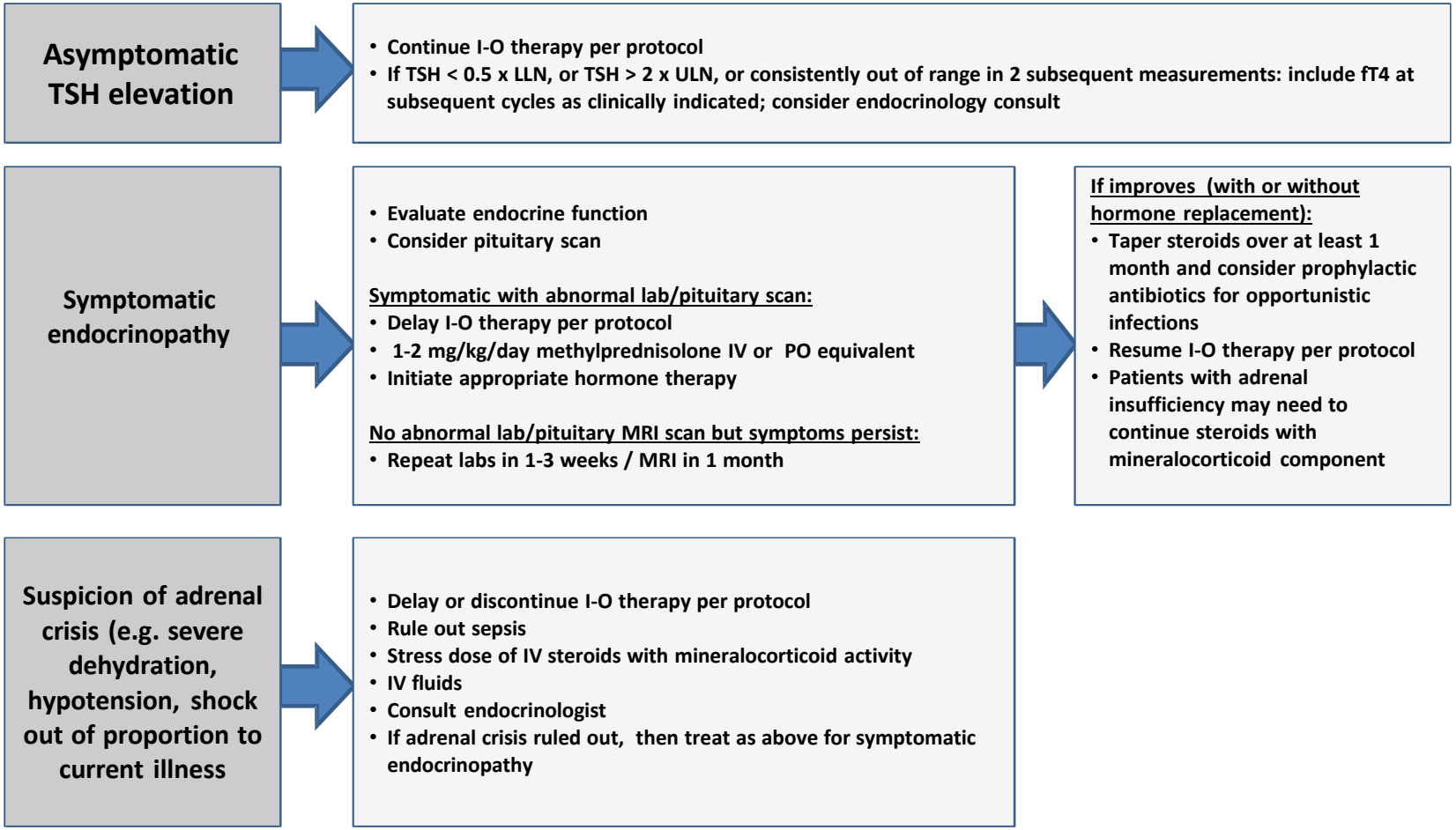
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

***I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN or T.bili ≤ 5 x ULN.**

**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Endocrinopathy Management Algorithm

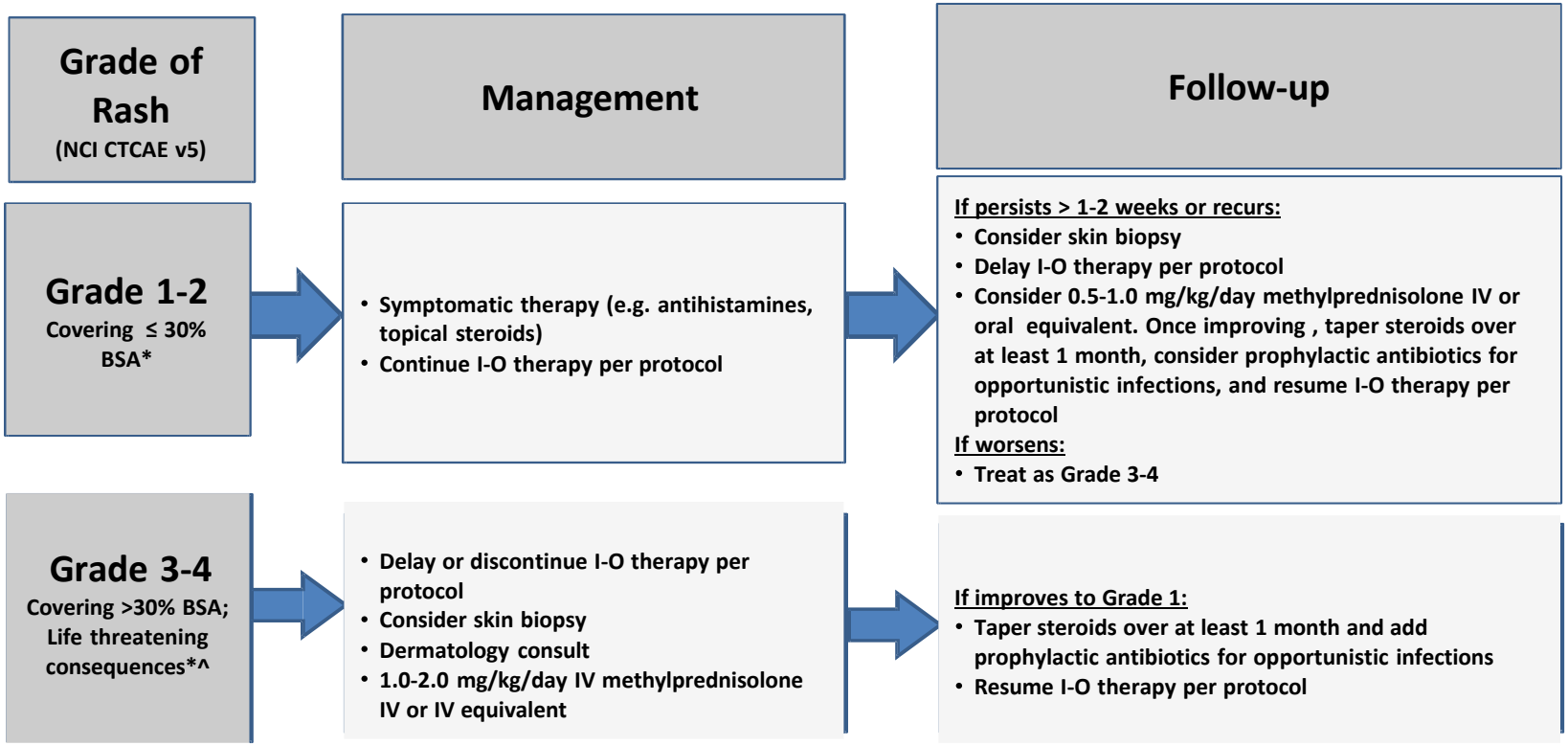
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

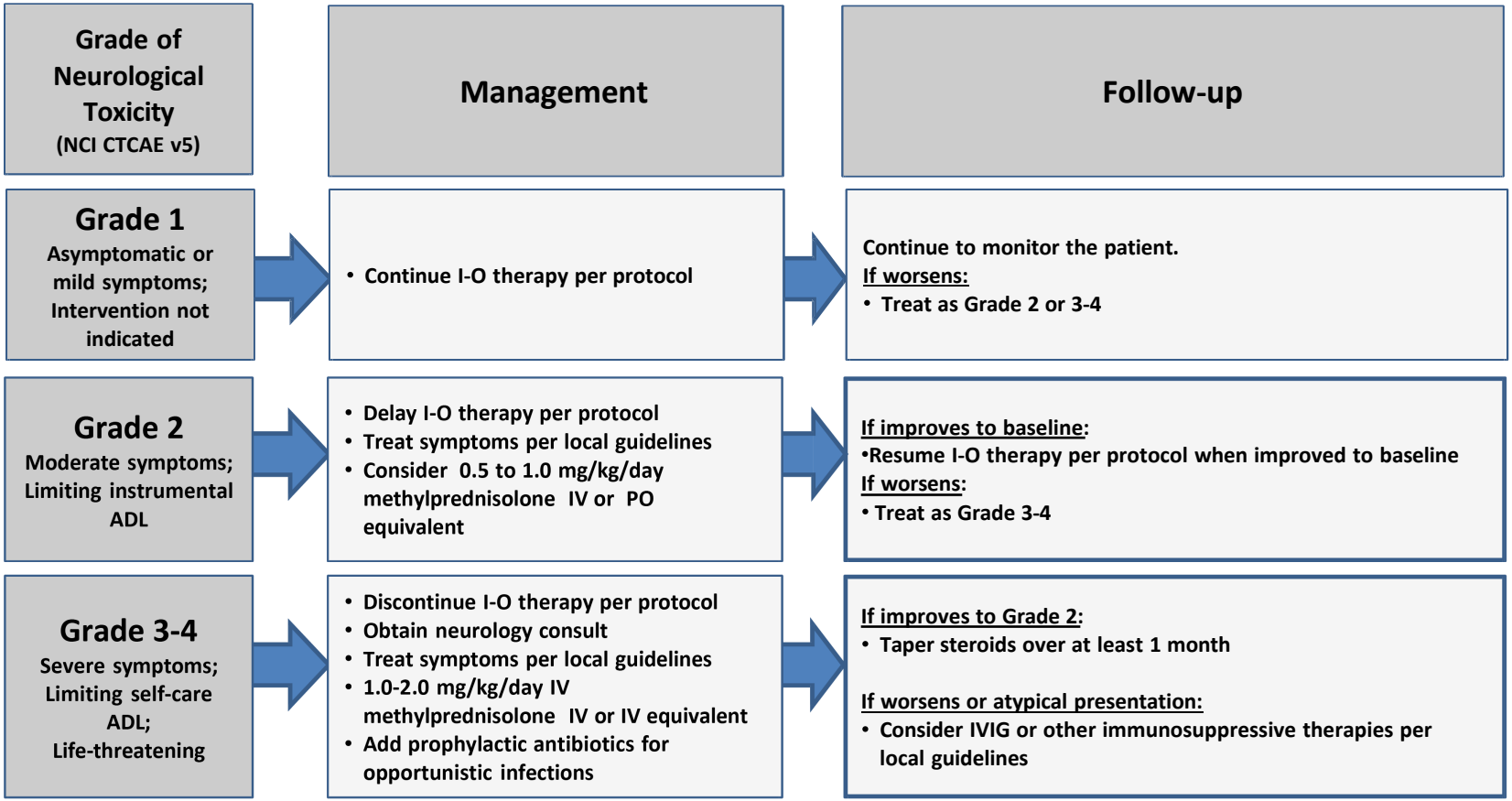


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*Refer to NCI CTCAE v5 for term-specific grading criteria.
^If SJS/TEN is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue I-O therapy.

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids

APPENDIX 3: Evaluation of Reactions at Hu14.18-IL2 Injection Sites[57]

Local Reaction to Injectable Product	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Pain	Does not interfere with activity	Repeated use of non- narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness*	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

APPENDIX 4: Phase IC & Phase ID Toxicity Decision Guide

<table>
<tr><td colspan="2" rowspan="2"></td><td colspan="3">Toxicity Attribution to Nivolumab and/or Ipilimumab</td></tr>
<tr><td>None</td><td>Unrelated or Unlikely</td><td>Possibly, Probably, or Definitely</td></tr>
<tr><td rowspan="4">Toxicity Attribution to hu14.18-IL2</td><td>None</td><td>Continue per protocol</td><td></td><td></td></tr>
<tr><td>Unrelated or Unlikely</td><td rowspan="2"></td><td rowspan="2">Continue per protocol</td><td>Stop nivolumab/ipilimumab, may resume per standard clinical practice (Section 6.2.2)</td></tr>
<tr><td rowspan="2">Possibly, Probably, or Definitely</td><td>Stop hu14.18-IL2, may later resume per protocol (Section 6.1)</td></tr>
<tr><td rowspan="2"></td><td>Continue nivolumab/ipilimumab</td><td>Stop nivolumab/ipilimumab, may resume per standard clinical practice (Section 6.2.2)</td></tr>
<tr><td>Stop hu14.18-IL2, may later resume per protocol (Section 6.1)</td><td>Stop hu14.18-IL2, may later resume per protocol (Section 6.1)</td></tr>
</table>