Phase II Trial of Nivolumab and HPV-16 Vaccination in Patients with HPV-16-Positive Incurable Solid Tumors

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1.0 INTRODUCTION AND STUDY RATIONALE

Infection with Human Papillomavirus (HPV) causes substantial cancer burden including nearly all cervical, majority of oropharyngeal, and a significant percentage of anal, penile, vulvar, and vaginal cancers. The HPV vaccines currently available are only preventive, must be given before sexual debut/HPV exposure, and have little market penetration. Additionally, the latent period between exposure and diagnosis of cancer is decades-long; therefore, even if vaccine use is increased in the near term, high risk HPV-related cancers will continue to increase and persist for decades to come.

Recent work has identified signaling pathways between tumor and immune cells which act to suppress immune rejection. Inhibition of signaling with antibodies to key proteins, cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death receptor-1 (PD-1), has resulted in very promising tumor shrinkage and prolonged survival in patients with a variety of cancers, including lung cancer, a tumor long-considered non-immunogenic. However, only a subset of patients responds, emphasizing the importance of addressing other mechanisms of resistance to immune response. This strategy of checkpoint inhibition may have particular relevance for virally-driven cancers, which should be vulnerable based on expression of non-self, viral-specific antigens, and theoretically, could synergize with the effects of vaccination.

Melief et al. have developed and investigated ISA101 and its prototype, designated HPV-16-SLP, a series of overlapping, fully synthetic long peptides (SLP) based on the sequence of HPV-16 oncoproteins E6 and E7 (1, 2). This vaccine showed strong immunogenicity and clinical responses in patients with HPV-related vulvar intraepithelial neoplasia, the latter directly correlating with induced immune response (1). The vaccine is being evaluated in patients with advanced cervical cancer in combination with standard chemotherapy. Although a pilot trial of standard chemotherapy in combination with ISA101 treatment in cervical cancer treatment has shown synergy in the induction of potent immune responses (Melief et al, unpublished results), another highly promising treatment is combination of vaccination and checkpoint blocking. If successful, as proposed in the current protocol, at a later stage all three treatment modalities could be combined (i.e. chemo-immunotherapy with both vaccine and nivolumab). For now, it is obviously not prudent to do this without first exploring the added value of ISA101 vaccination in combination with nivolumab without chemotherapy.

ISA 101 is a therapeutic HPV-16 vaccine consisting of 9 overlapping long E6 peptides (25-mer to 32-mer E6 peptides) and 4 overlapping long E7 peptides (35-mer E7 peptides) (SLP-HPV-16 vaccine). These peptides overlap by 10 to 18 residues and cover the complete sequence of HPV16 E6 and E7 onco-proteins. These long peptides have the capacity to effectively deliver antigens to dendritic cells (DC). Proper DC activation by adjuvant then induces $CD4^+$ and $CD8^+$ T-cell responses by MHC class I and MHC class II presentation of the HPV16 E6/E7 processed epitope peptides. Properly activated CD4 T cells increase surface CD40Ligand (CD40L) expression, causing DC activation through CD40L-CD40 triggering. This in turn leads to CD8 T cell activation associated

with expansion of CD8+ cytotoxic T cells capable to reach and kill tumor cells that express E6 and E7 epitopes.

In a preclinical model of HPV16-induced cervical cancer, vaccination with a HPV-16-SLP vaccine, consisting of a single SLP, designed for the MHC type of the homozygous inbred mouse strain C57BL/6, elicited a strong HPV16 specific cytotoxic CD8+ T cell response in mice, which in an adjuvant setting resulted in increased survival and eradication of existing tumors (3). In a second preclinical disease model in rabbits, mimicking persistent HPV infections and related papilloma induced lesions, the long peptide vaccination approach resulted in similar effects: a strong induction of a papilloma-specific immune response, clearing of viral DNA and regression of established cottontail rabbit papilloma virus-induced wart growth (4).

In patients with high-grade premalignant vulvar lesions (VIN) the prototype HPV-16-SLP vaccine produced regression of lesions in 15/19 patients at 12 months of follow up and 9/19 had complete disappearance of disease that was durable in all 9 at 24 months (1). Importantly, complete clinical responses were correlated with stronger interferon-gamma-associated T cell responses and all complete responders developed HPV-16 specific immunity. Subsequently a pilot study in 12 patients with advanced cervical cancer indicated that T-cell responses are enhanced when combining appropriately timed chemotherapy (carboplatin/paclitaxel) and the HPV-16 vaccine (1, 2). The mechanisms of enhanced immune response upon delivery of a single vaccine dose 2 weeks after the last dose in the second cycle of carboplatin/taxol chemotherapy appeared to be depletion of myeloid derived suppressor cells without depletion of T cell function or numbers. An additional Phase 2 trial in patients with advanced cervical cancer, CervISA, is being conducted to evaluate the optimal dose of ISA101, with or without interferon alpha,that produces a robust immune response. Results from this are expected to soon (first half of 2015).

Nivolumab (BMS-936558, Bristol Myers Squibb) is a fully human, IgG4 (kappa) isotype mAb that binds PD-1 on activated immune cells and disrupts engagement of the receptor with its ligands PD-L1 (B7-H1/CD274) and PD-L2 (B7-DC/CD273), thereby reportedly abrogating inhibitory signals and augmenting the host antitumor response. In early clinical trials, nivolumab has demonstrated activity in several tumor types, including melanoma, renal cell cancer (RCC), and NSCLC (5). In particular, substantial activity has been noted in the squamous histology subgroup of NSCLC, for whom objective response rates averaged 33% across dose levels, and progression free survival approached ~6 months (6). In general, nivolumab has been well tolerated to date, with a favorable safety profile consistent with predicted toxicities based on an immunostimulatory mechanism of action (7).

1.1 STUDY RATIONALE

1.1.1 Rationale for the combination of nivolumab and ISA101

In HPV-related oropharyngeal cancer, recent data suggest a key role of the programmed death-1 (PD-1) pathway in creating a favorable microenvironment for HPV infection and

subsequent tumor progression (8). Melief et al. have shown that the combination of checkpoint inhibition with antibody to PD-ligand 1 (PD-L1) enhances the antitumoral immune response created by vaccination with the single HPV SLP mentioned above in an E7-expressing murine tumor model obtained by co-transformation of primary C57BL/6 mouse lung epithelial cells with HPV-16 E6 and E7 and activated ras oncogene as previously described (TC-1) (9) (*unpublished)*. In addition, in this particular HPV16 E6/E7 TC-1 tumor, HPV16-specific T cells did not enter the tumor microenvironment without HPV SLP vaccination. These findings suggest that the effect of checkpoint inhibition (i.e. PD-L1 blockade) is at least in part dependent on vaccine-induced infiltration of HPV-specific tumor infiltrating lymphocytes (TIL). Nasman et al have reported that the number of CD8-positive TILs in HPV-related oropharyngeal cancer is directly correlated with prognosis (10). Badoual et al. reported that vaccination with a non-replicative delivery vector targeting dendritic cells increased PD-1 expression on T cells and enhanced tumor regression with PD-1 blockade in a mouse model of HPV-positive oropharyngeal carcinoma (11). These lines of evidence strongly support the concept that the SLP anti-HPV vaccine will enhance the efficacy of checkpoint inhibition therapy. The rationale to combine vaccine with checkpoint inhibition is further strengthened by preliminary data from an ongoing trial with pembrolizumab (MK3475, Merck), another PD-1 antibody reported by Seiwert et al. (12). They reported an ORR of 20% (4/20), including one CR in patients with HPV-positive OPSCC. Although these data are proof of principle that checkpoint inhibition is effective for a subset of patients, they also underline the importance of studying combination immunotherapy approaches to increase response rate.

1.1.2 Rationale for ISA 101 dose and schedule

In completed and ongoing clinical studies with HPV-16-SLP, the precursor vaccine to ISA101,, over 180 patients with HPV-induced pre-malignancy of the cervix and vulva have been administered the vaccine at least once at doses ranging from 20 to 300 µg per peptide [ISA101 Investigators Brochure, June 2014 (13)]. Previous clinical trials utilizing HPV-16-SLP formulated with Montanide as monotherapy have demonstrated an acceptable safety profile for patients with malignancy, induction of robust T cell immune responses compared to pre-vaccination values, and clinical efficacy in patients with high grade VIN (1, 14). In patients with cervical dysplasia and advanced cervical cancer, it has been possible to show induction of specific T cell responses utilizing the HPV-16-SLP monotherapy approach (2, 15), but these remained below the level associated with clinical responses in the VIN trial, and therefore, as expected without convincing clinical impact. This indicates the need for improvement by co-treatment in patients with late stage HPV16-positive cervical cancer.

A phase I/II clinical trial, exploring the aforementioned chemo-immunotherapy co-treatment, called CervISA (NCT02128126), sponsored by ISA, is currently ongoing in Europe. This trial is investigating the safety and tolerability of different dose levels of ISA101 with or without pegylated interferon-alpha (IFNα) in combination with carboplatin and paclitaxel in patients with recurrent/metastatic incurable cervical cancer. Dose determination for the current trial has been chosen on bases of prior trials that have shown 100 mcg to be safe and to elicit appropriate anti HPV-16 immune response (1, 14).

1.1.3 Rationale for nivolumab dose and schedule

The dose and schedule of nivolumab in this study will be 3 mg/kg every 2 weeks, based upon a February 24, 2012 analysis of safety, efficacy, and exposure-response data from a Phase 1 study (16). Anti-tumor activity was observed in NSCLC subjects at dose levels of 1, 3 and 10 mg/kg every 2 weeks. Anti-tumor activity appeared to approach a plateau at dose levels of 3 mg/kg and above. Consistent with these observations, the results of exposure-response analyses showed that the probability of a tumor response tended to approach a plateau for trough concentrations produced by 3 and 10 mg/kg administered every 2 weeks. nivolumab was adequately tolerated up to 10 mg/kg, the highest dose level tested, and no maximum tolerated dose (MTD) was identified. While the spectrum, frequency, and severity of nivolumab-related AEs were generally similar across the dose levels tested, the 10 mg/kg dose level had numerically higher rates of Grade 3/4 drug-related SAEs and AEs leading to discontinuation. Based on these observations, a dose of 3 mg/kg every 2 weeks was chosen for further study.

1.1.4 Rationale for initial tumor assessment at 11 weeks

Accumulating clinical evidence indicates that patients treated with immune system stimulating agents may develop progression of disease (by conventional response criteria) before demonstrating clinical objective responses and/or stable disease. This phenomenon was observed in the Phase 1 study of nivolumab. Two hypotheses have been put forth to explain this phenomenon. First, enhanced inflammation within tumors could lead to an increase in tumor size appearing as enlarged index lesions and as newly visible small non-index lesions. Over time, both the malignant and inflammatory portions of the mass may then decrease leading to overt signs of clinical improvement. Another hypothesis is that the kinetics of tumor growth may initially outpace anti-tumor immune activity in some individuals. With sufficient time, the anti-tumor activity will dominate and become clinically apparent. For these reasons, the initial tumor assessment in the phase I trial was conducted at 8 weeks, and it is unknown if an earlier assessment would demonstrate similar activity due to premature termination of study treatment.

To mitigate the risk of detecting false-progression early in the course of treatment with nivolumab in combination with ISA101, the initial tumor assessment in this study will take place at Week 11 (±5 days). Thereafter, all subsequent tumor assessments will take place regularly every 6 weeks (±5 days) until documented disease progression or treatment discontinuation, whichever occurs later.

1.1.5 Rationale for collection of tumor tissue and evaluation of tumor immune related biomarkers

This study will prospectively examine tumor tissues and blood samples from patients in the clinical trial to identify potential pharmacodynamic markers reflecting therapy effect and biomarkers of response or resistance to therapy.

Tumor tissue will be obtained at baseline, at Week 11 (response evaluation), and at progression (as possible).

Among the tumor markers to be studied are the B7 family ligands, PD-L1 (B7-H1), PD-L2 (B7-DC), B7-H3 and B7-x, which have been reported to be up-regulated in tumors by mRNA profiling. While PD-L1 is the major ligand expressed by tumor cells in solid malignancies, immunohistochemistry analysis by the Pardoll/Topalian/Taube group has demonstrated PD-L2 expression on tumor infiltrating dendritic cells and macrophages (17). Allison and Sharma have published data demonstrating expression of B7-H3 and B7-x as predictors of poor clinical outcome in patients with prostate cancer (18), which we will now extend to study in patients with HPV-related cancers to be treated with anti-PD-1 and ISA101. Among the lymphocyte receptors to be analyzed, PD-1, LAG-3, 2B4, BTLA and Tim3 have all been associated with inhibition of lymphocyte activity and in some cases induction of lymphocyte anergy that can be reversed upon antibody blockade in animal models (19). Thus, they appear to play non-redundant roles in feedback inhibition of T cell responses when their cognate ligands are present. In addition to providing inhibitory signals to activated effector T cells, some of these receptors, particularly PD-1, LAG-3 and Tim3 (as well as CTLA-4), are highly expressed on regulatory T cells (Treg), where they amplify Treg inhibitory activity (17). Ligands for LAG-3, 2B4, BTLA and Tim3 are MHC II, CD48, HVEM and galectin 9 respectively, some of which have been previously reported as expressed in various human tumors on gene expression arrays. These studies will provide us with a comprehensive view of cellular subsets and checkpoint molecule expression in tumors from untreated patients and how cellular subsets and key immune regulatory molecules are impacted intratumorally after treatment with anti-PD-1 and ISA101. Determining which inhibitory molecules/pathways are present despite treatment with anti-PD-1 and the ISA101 vaccine should inform selection of rational combination therapy in the future. Importantly, all of these parameters of the immune microenvironment will be correlated with clinical outcome.

1.2 RESEARCH HYPOTHESIS

In patients with recurrent/metastatic incurable HPV-16 positive cancer the combination of ISA101 vaccination and checkpoint inhibition will have an acceptable safety profile and will produce HPV-specific immune responses and clinical efficacy.

1.3 OBJECTIVES

1.3.1 Primary Objective

To evaluate the objective response rate (ORR) by RECIST 1.1 criteria of the combination of ISA 101 vaccination and nivolumab in patients with recurrent/metastatic incurable HPV-16 positive solid malignancies.

1.3.2 Secondary Objectives

- To evaluate the toxicity of the ISA 101 vaccination and nivolumab individually and of the combination;

- To evaluate the HPV-specific immune responses of the combination of ISA 101 vaccination and nivolumab;
- To evaluate response rate by immune-related criteria of the combination of ISA 101 vaccination and nivolumab;
- To evaluate the progression free survival (PFS) of the combination of ISA 101 vaccination and nivolumab;
- To evaluate the immune-related PFS of the combination of ISA 101 vaccination and nivolumab;
- To evaluate the overall survival (OS) of the combination of ISA 101 vaccination and nivolumab;

1.3.3 Exploratory Objectives

- To explore potential predictive biomarkers of response to the combination of ISA 101 vaccination and nivolumab in tumor specimens and peripheral blood.

1.4 MECHANISM OF ACTION

1.4.1 Mechanism of action of ISA101 vaccine

The proposed mode of action in relation to therapy of HPV-associated malignancies, is based on the fact that SLP of 25-35 amino acids in length require an obligatory processing step by DC in order for the resulting short peptides to bind to MHC class I and II molecules and become transported to the cell surface. DC can very efficiently process and present long peptides following direct cytoplasmic uptake and only DC can do this efficiently. Short exact MHC class I-binding peptides, in contrast, bind exogenously to MHC class I molecules of all nucleated cells that express surface MHC class I. Since most of these cells are not professional antigen-presenting cells such as DC, this leads to suboptimal antigen processing and presentation in the absence of co-stimulatory molecules. In addition the use of exact MHC class I-binding peptides does not stimulate CD4+ T cell immunity, which is a prerequisite for optimal expansion of CD8+ killer cells and for CD8+ T cell memory.

Pharmacokinetic parameters for ISA101 were not determined in the pre-clinical studies. Studies with other SLP vaccines, however, indicate that the distribution of the peptides is mainly restricted to the draining lymph nodes (20). Furthermore, the investigational product is not metabolized in the conventional sense: the peptides are taken up by antigen presenting cells, which process the peptides into smaller entities, called T-cell epitopes, which are presented on the surface of the antigen presenting cell (APC). In the meantime, the APC traffics to a draining lymph node, where naive T cells recognize the T-cell epitopes and are activated by these epitopes. The peptides are degraded within APCs through normal degradation pathways.

1.4.2 Mechanism of action of nivolumab

Cancer immunotherapy rests on the premise that tumors can be recognized as foreign rather than as self and can be effectively attacked by an activated immune system. An effective immune response in this setting is thought to rely on immune surveillance of tumor antigens expressed on cancer cells that ultimately results in an adaptive immune

response and cancer cell death. Meanwhile, tumor progression may depend upon acquisition of traits that allow cancer cells to evade immunosurveillance and escape effective innate and adaptive immune responses (21-23). Support for the role of immunosurveillance in many tumor types, including NSCLC, is suggested in retrospective analyses demonstrating a correlation between tumor infiltrating lymphocytes in surgically resected specimens and recurrence free survival (24-26). Current immunotherapy efforts attempt to break the apparent tolerance of the immune system to tumor cells and antigens by either introducing cancer antigens by therapeutic vaccination or by modulating regulatory checkpoints of the immune system.

T-cell stimulation is a complex process involving the integration of numerous positive as well as negative co-stimulatory signals in addition to antigen recognition by the T-cell receptor (TCR). Collectively, these signals govern the balance between T-cell activation and tolerance. PD-1 is a member of the CD28 family of T-cell co-stimulatory receptors that also includes CD28, CTLA-4, ICOS, and BTLA (27). PD-1 signaling has been shown to inhibit CD-28-mediated upregulation of IL-2, IL-10, IL-13, interferon –γ (IFN-γ) and Bcl-xL. PD-1 expression also been noted to inhibit T cell activation, and expansion of previously activated cells. Evidence for a negative regulatory role of PD-1 comes from studies of PD-1 deficient mice, which develop a variety of autoimmune phenotypes (28). These results suggest that PD-1 blockade has the potential to activate anti-self T-cell responses, but these responses are variable and dependent upon various host genetic factors. Thus, PD-1 deficiency or inhibition is not accompanied by a universal loss of tolerance to self-antigens.

In vitro, nivolumab binds to PD-1 with high affinity (EC50 0.39-2.62 nM), and inhibits the binding of PD-1 to its ligands PD-L1 and PD-L2 (IC50 ~ 1 nM). Nivolumab binds specifically to PD-1 and not to related members of the CD28 family such as CD28, ICOS, CTLA-4 and BTLA. Blockade of the PD-1 pathway by nivolumab results in a reproducible enhancement of both proliferation and IFN-γ release in the mixed lymphocyte reaction (MLR). Using a CMV-re-stimulation assay with human PBMC, the effect of nivolumab on antigen specific recall response indicates that BMS-936558 augmented IFN-γ secretion from CMV specific memory T cells in a dose-dependent manner versus isotype-matched control. In vivo blockade of PD-1 by a murine analog of nivolumab enhances the anti-tumor immune response and result in tumor rejection in several immunocompetent mouse tumor models (MC38, SA1/N, and PAN02) (29).

1.4.3 HPV positive malignancies

The causal role of HPV infections in the development of preneoplastic lesions and subsequent carcinoma has been unambiguously established (30, 31). Genital infections with high-risk HPV are mainly acquired through sexual activity (32-34) and are highly prevalent in young sexually active individuals. In the majority of infected subjects the infection is cleared within one year (35, 36). However, infection with the high-risk HPV type 16 (HPV16) is associated with a greater risk for progression and is most common in patients with HPV-related cancer (30, 37). HPV-16 encodes the two tumor-specific oncoproteins E6 and E7 that can elicit a favorable immune response in which specific T-cells play a critical role in the control and elimination of the HPV infection. The virus-specific interferon-γ (IFNγ)-producing CD4+ cells (Th1 cells) and CD8+ cytotoxic T-

lymphocytes (CTL) are able to recognize peptides processed from the oncoproteins E6 and E7 and contribute to the virus elimination (38, 39).

However, in case of an uncontrolled persistent infection with a high-risk HPV type, the expression of the viral oncoproteins E6 and E7 contributes to the development of (pre)malignancies. Apparently, the spontaneous HPV-specific T-cell response fails in these patients and there is no or a negligible activation and expansion of the proper HPV16-specific CD4+ and CD8+ T cells (38, 39).

Persistent HPV infection causes a variety of solid cancers including cervical, vulvar, vaginal, oropharyngeal, anal and penile cancers. Usually HPV-related cancers are cured in the majority of cases when discovered at early stages with multimodality therapy including surgery, radiation therapy and chemotherapy. However, distant metastases may arise after longer intervals and in unusual sites (40, 41).

Appropriate treatment of incurable advanced disease, consisting mainly of platinum-based doublet systemic chemotherapy, has only limited efficacy with a response rate and median OS of approximately 30% and one year, respectively. Bevacizumab added to chemotherapy increases OS to a median of approximately 18 months in patients with cervical cancer. Therefore, novel treatments are needed to improve the survival of locoregionally recurrent and metastatic HPV-positive solid tumors.

1.4.4 HPV-16-SLP and ISA 101 clinical results

1.4.4.1 Clinical pharmacology summary

Early studies were performed with a similar prototype vaccine manufactured at the Leiden University Medical Center (LUMC), identified as HPV-16-SLP. ISA101 is manufactured by a contract manufacturing organization for current trials sponsored or supported by ISA Therapeutics. Data developed with HPV-16-SLP may provide important information to help guide the development of ISA101 with respect to mechanism of action, the safety profile and biologic activity. Data from studies performed with the prototype vaccine, HPV-16-SLP, as distinct from ISA101, are specifically identified within this document.

Clinical experience with HPV-16-SLP, the prototype of ISA101, has been obtained from several investigator-sponsored studies and available data have been provided to ISA. Initial clinical trials of HPV-16-SLP were mainly designed to assess the safety, tolerability and immunogenicity. ISA's access to the safety data in these trials data is limited, and the data may not be complete as these studies have not been conducted, monitored or audited by ISA. ISA engaged an independent reviewer, Dr M. van Poelgeest, in late 2011 to assess clinical safety data from studies conducted through November 18, 2011. This safety analysis focused on the two most common and important allergic reactions to ISA101: local injection site and systemic allergic reactions. This analysis provides the primary basis for understanding of the safety profile of HPV-16-SLP and hence, ISA101. More recent data on HPV-16-SLP have also been incorporated, as well as all available non-clinical and clinical data on ISA 101 through May 31, 2014.

Preclinical efficacy studies showed significant activity of the papilloma virus long peptide approach. In a preclinical model of HPV16-induced cervical cancer, vaccination with a single synthetic long peptide of HPV-16 E7 induced a strong HPV-16 specific immune response in inbred B6 mice, increased survival and mediated eradication of existing tumours. A second preclinical disease model in outbred rabbits used a set of long overlapping peptides of the entire sequence of cottontail rabbit papilloma virus E6 and E7, mimicking persistent HPV infections and related papilloma induced lesions. In this model, the long peptide vaccination approach resulted in similar effects: a strong induction of a papilloma-specific immune response, clearing of viral DNA and control of papilloma induced wart growth.

Pharmacokinetic parameters of ISA101 were not assessed in the preclinical studies. Studies with other synthetic long peptides, however, indicate that the distribution of the peptides is mainly restricted to DC in the draining lymph nodes (20).

1.4.4.2 Safety summary

Toxicology studies in rats (HPV-16-SLP) and rabbits (ISA101) showed that the vaccine induced high antibody levels against the immunogen and was well tolerated systemically after subcutaneous (s.c.) administration at high doses. In these studies, the vaccine was given together with an adjuvant, Montanide ISA 51 VG, hereafter referred to simply as Montanide, to mimic clinical studies. Doses in the rat were up to 120 µg per peptide per injection, while in the rabbit study the animal dose (300 µg per peptide per injection) was the same absolute dose as used in clinical trials, i.e. not adjusted for body weight or surface area.

Local inflammation at the injection site was volume dependent which suggests that the formulation was at least partially responsible for this effect. The irritation persisted throughout the studies; recovery started when treatment stopped but was not completely resolved within the 14-day recovery period. Systemic effects included transient, minor decreases in albumin content and albumin to globulin (A/G) ratios in the blood, increased spleen weight, and microscopic minor inflammatory changes in lungs, spleen and lymph nodes. These effects are considered indicative of an immune and/or inflammatory response. The immune responses are primarily local in nature and occur in microenvironments of the draining lymph nodes.

Clinical experience with ISA101, per se, is limited as clinical studies were initiated in June 2013. Two trials with ISA101 are enrolling patients as of the date of writing this protocol,

- CervISA: An open label phase I/II study in patients with advanced or metastatic (stage IVb) or recurrent HPV-16 positive cervical cancer for whom no curative treatment options exist.
- VACCAIN1: A study of ISA101 administered by intradermal injection without Montanide in patients with Anal Intraepithelial Neoplasia (AIN).

In supporting clinical studies with HPV-16-SLP-Montanide approximately 180 patients with HPV-induced pre-malignant lesions and malignancies of the cervix and vulva have been administered the vaccine at least once at doses ranging from 50 to 300 μg per peptide. Of these patients, approximately 18 (~10%) have terminated vaccination due to local and/or systemic adverse events.

The most important adverse events associated with HPV-16-SLP systemic effects include fever, chills, nausea, malaise and fatigue. Local reactions at the vaccination site include pain, redness swelling and itching. Adverse effects of HPV-16-SLP appear to be reported at a greater frequency and severity in patients with premalignant disease compared to patients with advanced cervix carcinoma. Generally the adverse events after HPV-16-SLP vaccination do not exceed grade 2 according to the CTC criteria. However, a number of more serious adverse events (CTC criteria grade 3), have been reported, including systemic allergic reactions and more serious local reactions such as ulcerations or fistulas at the vaccination sites that have led to withdrawal from study treatment in approximately 7% and 5.5% of subjects, respectively. Visible, grade 2 ulceration, abscess formation and fistulas with granulomatous inflammation have been observed for approximately 24 months after vaccination, particularly in patients with pre-malignant disease. In addition, a significant number (~10-20%) of patients with pre-malignant disease have reported fatigue, fever and chills of up to CTC grade 3. For details see the ISA101 Investigator Brochure.

Local swelling and discoloration of different size and intensity has persisted for a prolonged period (one year or more) in the majority of patients who have received HPV-16-SLP in Montanide. In some cases these complaints require specific treatment of the vaccination site by a dermatologist. In some patients, local excision led to alleviation of the symptoms. Long term follow up for up 12 months of the first VIN study (P88/89) revealed visible or palpable lesions in about 75% of the patients; about half of the biopsies showed a granulomatous inflammatory reaction, sometimes resulting in the formation of scar tissue.

In addition to local reactions, systemic allergic reactions leading to study withdrawal have been reported in approximately 7% of patients who have received HPV-16-SLP. One patient also reported difficulty in breathing. Patients have responded to an anti-histamine, in those instances when it was given. Vaccinations should therefore only be administered in a clinic where immediate treatment of severe allergic reactions is possible.

An odorous breath lasting up to approximately > 24 hours, most likely due to the DMSO component in the vaccine formulation, was also a frequently reported side effect.

Table 1. HPV-16-SLP safety overview in malignant diseases; local injection site reactions

Malignant diseases (N=69)				
Range (x-y%) - severity grade		**Grade 1 (%)**	**Grade 2 (%)**	**Grade 3 (%)**
Grade1: 29-31%	Erythema	25-27	29-31	0

Grade 2: 69-70% Grade 3: 0%	Pain	96-98	2-4	0
	Induration or swelling	48-50	35-37	0
	Nodules	NA		
	Ulceration	NA		
	Itching	25-27	7-8	0
	Pigmentation	NA		

Table 2. HPV-16-SLP safety overview in malignant diseases; systemic reactions, withdrawals and SAEs

Malignant diseases (N=69)					
Systemic reactions (%)* Max severity				**Withdrawals (number, reasons)**	**SAE** (number, type)**
	Grade 1 (%)	**Grade 2 (%)**	**Grade 3(%)**		
Fever	10-12	10-12	0	N=3 • 1 death duo to local recurrence • 1 inconvience • 1 systemic side effects	N=0
Chills	17-19	0	0		
Malaise	9-11	2-3	0		
Nausea	7-9	0	0		
Vomiting	2-3	0	0		
Dizziness	1-2	0	0		
Rash	9-10	1-2	0		
Headache	0	2-3	0		
Fatigue	10-12	3-4	0		
Flu like symptoms	9-11	3-4	0		
Tingling extremities	2-3	0	0		
Swelling extremities	6-8	0	0		
Burning eyes	1-2	0	0		
Itching other skin	0	2-3	0		

* 34 patients died in the Phase I End-stage CxCA study in the follow-up phase between 2-17 months, due to PD.
** In study CHDR0919, the pilot chemo-immunotherapy study (N=18 of which 6 patient did not receive vaccination), 7 SAEs in 7 patients were reported: 2 deaths due to metastatic disease (one received HPV-16-SLP vaccination); 4 complications as result of advanced oncological disease (3 received HPV-16-SLP vaccination); 1 complication with standard treatment of oncological disease (no HPV-16-SLP vaccination). Other adverse event data from this study are not yet available.

For a more detailed description of the local and systemic side effects in the various studies with HPV-16-SLP, see ISA101 Investigator's Brochure (42).

The vegetable sourced adjuvant Montanide is based on mineral oil and is a more pure form of mineral oil than Freund's incomplete adjuvant (IFA). It is an adjuvant that non-specifically induces an immune response and functions as a depot for the peptides, to assure a slow and consistent release. In preclinical mouse experiments the adjuvants IFA or Montanide were shown to be safe and to support robust anti-T cell immunity induced by long peptides.

In this study Montanide will be used for emulsification of the peptides before injection, resulting in a formulation of DMSO/WFI/Montanide of 20/30/50%. The dose level of ISA101 peptides and therefore the amount of Montanide will be based on available data from the CervISA trial. In clinical trials conducted previously, HPV-16-SLP was also

formulated in DMSO/WFI (or PBS)/Montanide 20/30/50%, and administered at the same highest dose and same volume of Montanide.

The subcutaneous route of administration has been used in the preclinical experiments in rats and rabbits conducted by ISA Pharmaceuticals B.V. (ISA). These repeated dose toxicology studies involved dosing the vaccine reconstituted and emulsified in DMSO and Montanide in the same manner as proposed for the clinic. In both species the vaccine was well tolerated systemically after repeated s.c. administration although local inflammation at the site of injection could be attributed to the adjuvant Montanide [Investigator's Brochure, Montanide ISA 51 VG, August 2013].

Although the use of the adjuvant Montanide has been associated with a number of side effects, most notably of a local nature, the evidence that this adjuvant is needed for robust T cell response induction by ISA101 is strong and the side effects are considered acceptable for a therapeutic vaccine modality that is capable of the induction of vigorous T cell immune responses against HPV-16 E6/E7 in patients with HPV induced malignancies.

1.4.4.3 Antitumor activity summary

Clinical experience with ISA101, per se, is limited as clinical studies were initiated in June 2013.

CervISA: An open label phase I/II study in patients with advanced or metastatic (stage IVb) or recurrent HPV-16 positive cervical cancer for whom no curative treatment options exist. This is a sequential group study of patients who have advanced (stage IIIb-IVa with involvement of lymph nodes beyond the renal vein) or metastatic (stage IVb) or recurrent HPV-16 positive cervical cancer for whom no curative treatment options exist. All subjects will receive ISA101 in Montanide by subcutaneous injection at the dose levels described in Table 1. The study was designed to enroll cohorts of six patients each to evaluate the safety and HPV-specific immune responses following different vaccination regimens. Patients will receive chemotherapy considered standard of care for this disease: starting with carboplatin at an AUC of 6 plus paclitaxel at a dose of 175 mg/m^2; dose reductions in chemotherapy are allowed, consistent with the standard of care, with continuation of vaccination (with or without pegylated IFNα, depending on the assigned cohort). The maximum total treatment duration for a patient is six cycles (1 cycle is 21 days) of carboplatin and paclitaxel for a total of 18 weeks, if there are no dose interruptions or delays. On Day 15 (±3 days) of cycles 2, 3 and 4, the vaccination scheme of ISA101 with or without pegylated IFNα (depending on cohort assignment in Table 1) will start. The patients will be vaccinated with a fixed dose of ISA101 every three weeks for a total of three ISA101 vaccinations. Four dose levels of ISA101 may be assessed. The primary endpoints of the study are safety and HPV-specific immune responses. The secondary endpoints are antitumor efficacy according to RECIST 1.1 (overall response rate, disease control rate, progression free survival). Exploratory endpoints include general responsiveness of the immune system. As of May, 2014, only the first cohort was evaluated, and patient recruitment is ongoing. The AEs and SAEs observed to date appear to be expected complications of advanced cervical cancer and/or standard chemotherapy. Virtually all of the AEs and SAEs that are reported as "related" to the protocol-specified therapy (e.g. thrombocytopenia, neutropenia, anemia), are

expected complications of chemotherapy. No new or unexpected safety concerns potentially related to ISA101 have been identified in this ongoing study to date.

VACCAIN1: A study of ISA101 administered by intradermal injection without Montanide in patients with Anal Intraepithelial Neoplasia (AIN). Study ISA101-AIN is an investigator initiated study, which is designed to assess therapeutic vaccination against HPV-16 with ISA101 for the treatment of anal intraepithelial neoplasia in HIV-positive (HIV^+) men. It should be noted that this study is being conducted using ISA101 *without Montanide* administered by intradermal (i.d.) injection. The Academic Medical Center in Amsterdam has started a sequential group dose-response study with ISA101 in HIV-positive MSM, with 3 different dosage schedules, based on intra-patient dose escalation: schedule I: 1,5,10 μg, schedule II: 5,10,20 μg and schedule III: 10,20,40 μg of ISA101 administered i.d. with a three-week interval. Each dosage schedule will be evaluated with or without the co-administration of pegylated interferon-α (Pegintron 1 μg/kg s.c.) on the day of vaccine administration. Each vaccination schedule is to be tested in 5 patients. The vaccination regimen that induces the best HPV-16-specific response with an acceptable safety profile will be considered the optimal schedule in this clinical setting. The size of this dose group will be increased to a total of 20 patients by vaccinating an additional 15 patients.

Data developed with HPV-16-SLP may inform the development of ISA101 with respect to mechanism of action, the safety profile and biologic activity. However, due to changes in manufacturing and analytical methods, HPV-16-SLP and ISA101 are considered different products from a regulatory point of view. The clinical trials described below are all investigator-sponsored studies of the prototype vaccine HPV-16-SLP. As summarized in the following tables, multiple studies evaluating HPV-16-SLP vaccination have been conducted in the past. These studies using HPV-16-SPL have included patients with different types of high-risk pre-malignant and malignant HPV-induced diseases (Table 3).

Table 3. HPV-16-SLP vaccination clinical trials.

Study ID	Indication	Study design	Key (immunological) results
Pre-malignant diseases			
P88/89 Phase II	High-grade VIN	22 patients: 4 vaccinations at 3 week interval	Clinical responses in women with HPV-16 positive, grade VIN3, correlated with induction of HPV-16 specific immunity (1).
P06.227 Phase II	High-grade CIN	9 patients (planned 50): Two arms, placebo controlled, at 3 week interval	Vaccination of HSIL patients results in increased HPV-16- specific T-cell immunity. (43). Study was terminated after 9 of planned 50 patients were recruited, due to lack of recruitment.
P06.226	Low-grade CIN or persistent PAPII	50 patients: 4 vaccinations at 3 week interval, three arms, placebo controlled	Robust HPV-16-specific T-cell responses detected after vaccination (44).

Study ID	Indication	Study design	Key (immunological) results
P08.062 Phase II	High-grade VIN or VaIN	39 patients: Two arms, 4 vaccinations at 3 week interval	Patients with high-grade VIN (or VaIN) Analysis is pending.
Malignant diseases			
P88/89 Phase I	End-stage cervical cancer	43 patients: 4 vaccinations at 3 week interval	The vaccinations resulted in a strong and broad T-cell response (2).
P88/89 Phase II	FIGO stage 1B1 cervical cancer	6 patients: 4 vaccinations at 3 week interval	The HPV-16 E6 and E7 SLP increases the number and activity of HPV-16-specific CD4(+) and CD8(+) T-cells to a broad array of epitopes in all patients (45).
P05.086 Phase II	End-stage gynecological cancer	20 patients: 4 vaccinations at 3 week interval	The HPV-16-SLP vaccine induced a broad IFNγ-associated T-cell response in patients with advanced or recurrent HPV-16-induced gynecological carcinoma but neither induced tumor regression nor prevented progressive disease (15).
CHDR0912 Pilot chemo-immuno therapy	Advanced or recurrent cervical cancer where carboplatin/paclitaxel is appropriate	18 patients: 6 patients carboplatin/paclitaxel only 12 patients vaccinated on D15 of cycle 2 of carboplatin/paclitaxel	Interim analysis a single vaccine dose of HPV-16-SLP +Montanide could induce strong T cell responses if the timing in relation to chemotherapy delivery was optimized (46) and *unpublished observations*).

1.4.5 Nivolumab clinical results

Nivolumab has demonstrated clinical activity in subjects with a variety of malignancies in the following indications and studies with available data:

Non-small cell lung cancer (NSCLC)

MDX1106-03: completed Phase 1 multidose escalation study with nivolumab monotherapy (total number of subjects [N] = 129). The ORR was 17%; the most active doses were 3 mg/kg and 10 mg/kg. Only a single response was reported at 1 mg/kg. Responses were observed in subjects with NSCLC (both squamous and nonsquamous subtypes) with a median duration of response of 17 months.

CA209012: ongoing Phase 1 study with nivolumab monotherapy or in combination with platinum-based chemotherapy or erlotinib (N = 52 with monotherapy, N = 137 with combination therapy). Preliminary analysis at 24 weeks for subjects treated with nivolumab monotherapy (N = 20, median follow-up 66.1 weeks), nivolumab + ipilimumab (N = 49, median follow-up 38.1 weeks), nivolumab + chemotherapy (N = 56, median follow-up 75.2 weeks), and nivolumab + erlotinib (N = 21, median follow-up 71.9 weeks) in CA209012 showed that among responders, the median durations of responses ranged from 25.4 weeks to 45 weeks for nivolumab + chemotherapy, while the

median durations of responses for nivolumab monotherapy, nivolumab + ipilimumab, and nivolumab + erlotinib were not reached at the time of analysis.

Melanoma
MDX1106-03: completed Phase 1 multidose escalation study with nivolumab monotherapy (N = 107). The ORR was 31% for melanoma subjects (N = 107) in MDX1106-03 who were administered nivolumab monotherapy Q2W at doses ranging from 0.1 mg/kg to 10 mg/kg. The majority of responses were durable and exceeded 6 months.

CA209038: ongoing exploratory, open-label, pharmacodynamic study of nivolumab monotherapy and nivolumab in combination with ipilimumab (N = 85). A total of 85 subjects with advanced melanoma were treated with nivolumab 3 mg/kg monotherapy in CA209038 (preliminary data). The ORR was 32% for ipilimumab-naive subjects and 18% in ipilimumab-progressed subjects.

CA209004: ongoing Phase 1b study with nivolumab in combination with ipilimumab (N = 126). Of the 125 response-evaluable subjects in CA209004, 53 subjects received concurrent therapy with nivolumab + ipilimumab, and 40 subjects (in an expansion treatment group) received concurrent therapy at the selected registrational dose and schedule The ORR (modified World Health Organization criteria) for all subjects in the concurrent-regimen group and concurrent expansion group was 42% and 43%, respectively. The 12-month OS rate was 85% (95% CI: 75 - 95) for subjects in the concurrent treatment group.

Renal cell carcinoma (RCC)
MDX1106-03: completed Phase 1 multidose escalation study with nivolumab monotherapy (N = 34). The ORR was 21% for RCC subjects (N = 34) in MDX1106-03, who were administered nivolumab monotherapy Q2W at doses of 1 mg/kg or 10 mg/kg. The majority of responses were durable and exceeded 6 months.

CA209010: completed Phase 2 dose-ranging monotherapy study in subjects with advanced/metastatic clear-cell RCC who received prior anti-angiogenic therapy (N = 167). Of the 168 randomized subjects with advanced/metastatic clear-cell RCC in CA209010, the ORR in the 0.3-, 2-, and 10-mg/kg treatment groups were similar (20%, 22%, and 20%, respectively). Median PFS (mPFS) was reached for each treatment group (2.7 months, 4.0 months, and 4.2 months in the 0.3-, 2-, and 10-mg/kg treatment groups, respectively). No dose-response relationship was noted for ORR or mPFS. The median OS was reached for the 0.3-mg/kg treatment group (18.2 months), but was not reached for the 2-mg/kg or 10-mg/kg treatment groups.

CA209016: ongoing Phase 1 dose-escalation study of nivolumab in combination with VEGFR-TKIs or ipilimumab in subjects with metastatic RCC (N = 53 in combination with TKIs, N = 44 in combination with ipilimumab). A total of 44 subjects with metastatic RCC were treated with nivolumab (1 or 3 mg/kg) and ipilimumab (1 or 3 mg/kg). In addition, a total of 53 subjects with metastatic RCC were treated with

nivolumab (2 or 5 mg/kg) and sunitinib (n = 33) or nivolumab (2 mg/kg) and pazopanib (n = 20) in CA209016. The confirmed ORR was 43% and 48% in subjects treated in 3-mg/kg nivolumab/1-mg/kg ipilimumab and 1-mg/kg nivolumab/3-mg/kg ipilimumab cohorts, respectively, and 52% and 45% in subjects treated in sunitinib and pazopanib cohorts, respectively.

1.4.5.1 Clinical pharmacology summary

Single-dose PK of nivolumab was studied in 39 subjects with cancer. The single-dose PK of nivolumab was linear and dose-proportional in the range of 0.3 mg/kg to 10 mg/kg. The mean terminal T-HALF of nivolumab ranged between 17 and 25 days across the dose range of 0.3 mg/kg to 10 mg/kg. Geometric mean total clearance varied from 0.13 mL/h/kg to 0.19 mL/h/kg, while mean volume of distribution varied between 83 mL/kg and 113 mL/kg across doses. The clearance and half-life of nivolumab are consistent with that of IgG4.

The multiple-dose PK of nivolumab given Q2W was determined from MDX1106-03 study as well as by population PK using data from 669 subjects across nivolumab studies. Multiple-dose PK of nivolumab following Q2W dosing was linear with dose-proportional increase in Cmax and AUC(TAU) in the studied range of 0.1 mg/kg to 10 mg/kg. nivolumab accumulation with Q2W dosing frequency was in the range of 2.9 to 3.3 based on AUC(TAU), 2.0 to 2.4 based on Cmax, and 3.1 to 4.8 based on Cmin.
A PPK model was developed by nonlinear mixed effect modeling using data from 669 subjects. nivolumab concentration-time data were well described by a linear, 2-compartment, 0-order IV infusion model with first-order elimination. nivolumab PK was found to be linear, dose independent, and time invariant. The geometric mean of terminal T-HALF was 25.6 days and the typical clearance was 8.8 mL/h, which are consistent with those of full human immunoglobulin antibodies. Clearance of nivolumab is independent of dose in the dose range (0.1 mg/kg to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights. Additional details are provided in IB (47).

1.4.5.2 Safety summary

A total of 39 and 306 subjects with selected recurrent or treatment-refractory malignancies have been treated in a completed Phase 1 single-dose study (MDX1106-01) and a completed Phase 1 multidose study (MDX1106-03), respectively. As the safety profile from MDX1106-03 to date is consistent with that observed for MDX1106-01, only data from the larger and more recent study (MDX1106-03) is presented in this IB. The baseline disease diagnosis by treatment for MDX1106-03 is provided in the final CSR (47). A review of the safety data by tumor type (RCC, NSCLC, mCRPC, CRC, and melanoma) did not show any clinically meaningful differences in the proportion of subjects with AEs noted across tumor types.

Overall, the safety profile of nivolumab monotherapy was generally manageable and was consistent with the mechanism of action of nivolumab. No MTD was reached at doses tested up to 10 mg/kg Q2W. The nature, frequency, and severity of any causality and treatment-related safety events were similar across tumor types.

The following were the key safety findings for the subjects in MDX1106-03 (47):

- Drug-related AEs (any grade) were reported in 75.2%, and drug-related Grade 3-4 AEs were reported in 17.0% of subjects. The most frequently reported drug-related AE was fatigue (28.1%). Other drug-related AEs Grade 3-4 reported in more than 2 subjects were pneumonitis (1.3%), lymphopenia (1.3%), diarrhea (1.0%), abdominal pain (1.0%), CD4 lymphocytes decreased (1.0%), and hypophosphatemia (1.0%).
- The most frequently reported drug-related SAE was pneumonitis (7 subjects, 2.3%). Drug-related Grade 3-4 pneumonitis was reported in 4 (1.3%).
- The most frequently reported drug-related select AE categories (any grade) were skin (24.5%), GI (14.1%), and endocrine (9.5%). AEs belonging to the pulmonary and renal select AE categories were unexpected, drug-related toxicities associated with the use of nivolumab. AEs belonging to select AE categories were generally manageable and reversible with the use of immunosuppressants.

The majority of the deaths were due to disease progression. Three subjects (1.0%) died due to study drug toxicity at the time of database lock; 2 out of 3 subjects died within 100 days of last dose of nivolumab, and 1 died > 100 days after the last dose of nivolumab. The reported causes of death for these 3 subjects were:

- Non-drug-related cardiopulmonary arrest due to complications from Grade 5 sepsis (drug-related)
- Drug-related sepsis
- Drug-related respiratory failure secondary to pneumonitis and progressive disease

After database lock for the final CSR, 2 subjects were reported to have died; both were subjects with NSCLC treated with 3-mg/kg nivolumab. The reported causes of death for these 2 subjects were:

- Drug-related pneumonitis
- Non-drug-related infection after experiencing drug-related Grade 3 pneumonitis

Although not considered to be the primary cause of death in all 5 subjects described above, pneumonitis was considered a contributory factor in each case (47).

1.4.5.3 Antitumor activity summary

Efficacy data from CA209003 was evaluated as of July 1, 2011, which provided a minimum follow-up of 8 months. Clinical antitumor activity was observed in melanoma, RCC, and NSCLC at all nivolumab doses tested. NSCLC subjects were treated at doses of 1, 3, and 10 mg/kg. Antitumor activity was mainly observed in the 3 and 10 mg/kg dose groups, and exposure-response appeared to be relatively flat at doses ≥ 3 mg/kg. At the 3 and 10 mg/kg dose levels, the RECIST-defined objective response rates for all histologies were 32% and 18%, respectively. The corresponding disease control rates (which included any subject who achieved a best overall response of CR, PR or SD) were 53% and 46%, respectively. PFS rates at 24 weeks were 41%, and 24%, respectively, indicating durable disease control. Differential activity was observed between squamous versus non-squamous histologies. Substantial activity was noted in the squamous histology subgroup (n=18), where the ORR and DCR in the 3 mg/kg dose

group were 50% and 67%, respectively (the ORR for squamous histology subjects across all dose groups was 33%). Responses were durable; as of the cutoff date, the median PFS in the 3mg/kg squamous group had not been reached, and 3 of 6 subjects had experienced PFS > 6 months. One subject who achieved PR (67% tumor reduction) experienced a response duration of 134 weeks. Activity in NSCLC is especially notable in that the majority of subjects had received 2 or more prior therapies. These preliminary data suggest that nivolumab induces substantial durable disease control in heavily pretreated subjects with NSCLC, and in particular in subjects with squamous histology.

1.5 OVERALL RISK/BENEFIT ASSESSMENT

Subjects with recurrent/metastatic incurable HPV-positive oropharyngeal and anogenital tract cancers represent a population for whom immunotherapy holds potential that has yet to be realized. HPV-16-SLP, the precursor vaccine to ISA 101, induced HPV-16 proliferative T cell responses in approximately 50% of patients with end-stage gynecological cancers, but did not cause any objective tumor regression. These data provide rationale to pursue combination strategies to amplify immune response. Similarly, the 20% ORR with anti-PD-1 therapy in HPV-positive OPSCC demonstrates the promise of this approach, and at the same time, the need to pursue relevant combinations. Combining ISA-101 with nivolumab is clearly rational and should be studied; however, it is only the first of several immunotherapy combinations of interest for these virally driven cancers.

ISA 101 has been very well-tolerated with injection site reactions the most common AE. Systemic reactions, typically readily manageable with anti-histamines or steroids, have also been observed. nivolumab has the potential for clinically relevant AEs including liver toxicities, thyroiditis, pneumonitis, and diarrhea. However, the activity and manageable AEs profile observed with nivolumab supports the combination with ISA 101.

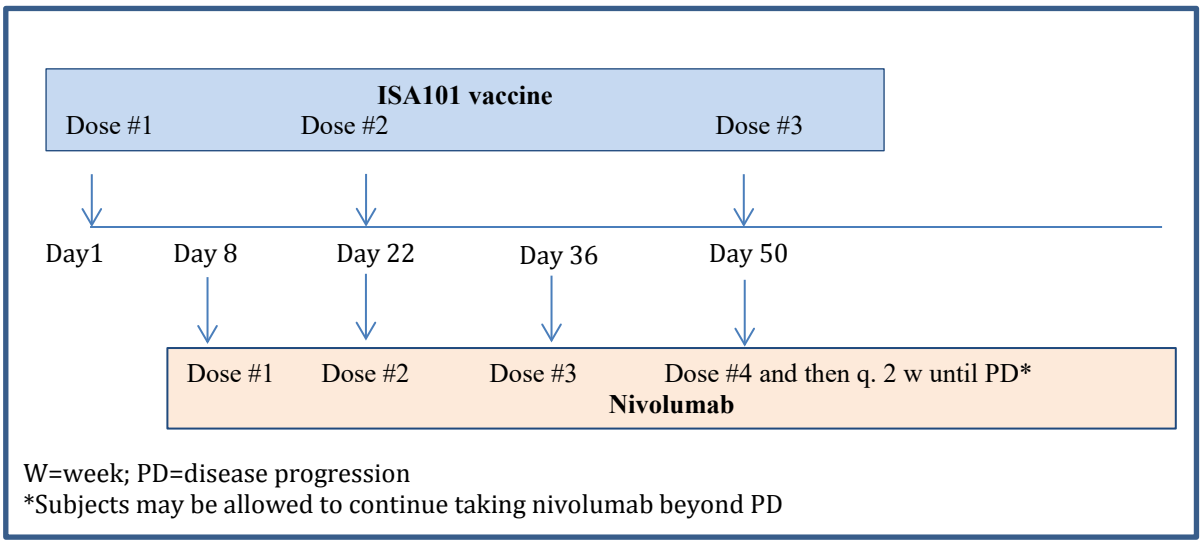
To assure safety for subjects enrolled onto this clinical trial, we will continuously assess toxicity in cohorts of 4 subjects (section 6.3.4) throughout the conduct of the trial.

2.0 INVESTIGATIONAL PLAN

2.1 Study Design and Duration

This is a single arm Phase 2 study in adult (≥ 18 years old) male and female subjects with recurrent/ metastatic HPV-16 positive oropharyngeal, cervical, vulvar, vaginal, penile and anal cancers treatment naïve or after progression during or after one prior chemotherapy regimen. Subjects will undergo screening evaluations to determine eligibility within 28 days prior to inclusion into the trial. A cycle is 2 weeks in duration with the exception that the first cycle is 3 weeks.

STUDY SCHEMA



Treatment will be initiated with the administration of the ISA101 vaccine on day 1, 1 week before starting nivolumab IV infusion to facilitate migration of HPV-specific TILs to the microenvironment. Cycle 1 therefore will last 3 weeks instead of 2 weeks.

This study will consist of 3 phases: screening (Table 5), treatment (Table 6), and follow-up (Table 7).

This study will end when analysis of survival is complete. The duration of study will be approximately 3 years (36 months).

2.2 Study Population

For entry into the study, the following criteria MUST be met.

2.2.1 Inclusion Criteria

1) Signed Written Informed Consent

a) Subjects must have signed and dated an IRB/IEC approved written informed consent form in accordance with regulatory and institutional guidelines. This must be obtained before the performance of any protocol related procedures that are not part of normal subject care.

b) Subjects must be willing and able to comply with scheduled visits, treatment schedule, laboratory tests and other requirements of the study.

2) Target Population

a. Men and women ≥ 18 years of age

b. Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 1

c. Subjects with histologically- or cytologically-documented incurable Human Papillomavirus (HPV)-16 positive solid tumors including oropharyngeal squamous cell carcinoma (OPSCC), cervical, vulvar, vaginal, anal, penile cancer. Incurable HPV-16

solid tumors are defined as tumors which are not curable by salvage approaches including resection and/or re-irradiation. HPV-16 serotype will be assessed by Cervista assay (Table 5)

Subjects can be treatment naïve for metastatic or incurable locally advanced HPV-16 positive solid tumors or can have one prior line of treatment. Patients are eligible upon progression after definitive local treatment (usually concurrent chemoradiation) if they are not candidates for salvage surgery or re-irradiation. Patients are also eligible after progression on first line chemotherapy for recurrent disease. d. Subjects must have measurable disease by CT or MRI per RECIST 1.1 criteria; Radiographic Tumor Assessment performed within 28 days of study inclusion. e. Target lesions may be located in a previously irradiated field if there is documented (radiographic) disease progression in that site. f. Subject entering the study will need to consent for mandatory biopsy at study entrance and as an optional procedure at Week 11 and at progression for biomarker evaluation, as described in Table 5. Biopsy should be excisional, incisional or core needle. Fine needle aspiration is insufficient.

g. Prior radiotherapy or radiosurgery must have been completed at least 2 weeks prior to start

h. All baseline laboratory requirements will be assessed and should be obtained within -14 days of study registration. Screening laboratory values must meet the following criteria

i) WBCs $\geq 2000/\mu L$

ii) Neutrophils $\geq 1500/\mu L$

iii) Platelets $\geq 100 \times 10^3/\mu L$

iv) Hemoglobin ≥ 9.0 g/dL

v) Serum creatinine of ≤ 1.5 X ULN or creatinine clearance > 40 mL/minute (using Cockcroft/Gault formula)

Female CrCl= (140- age in years) x weight in kg x 0.85

72 x serum creatinine in mg/ dL

Male CrCl= (140- age in years) x weight in kg x 1.00

72 x serum creatinine in mg/ dL

vi) AST $\leq$ 1.5X ULN

vii) ALT $\leq$ 1.5X ULN

viii) Total bilirubin $\leq$ ULN (except subjects with Gilbert Syndrome who must have total bilirubin <3.0 mg/dl)

3) Age and Reproductive Status

a) Women of childbearing potential (WOCBP) must use method(s) of contraception for 30 days + 5 half-lives (60 days) of the study drugs. For a teratogenic study drug and/or when there is insufficient information to assess teratogenicity (preclinical studies have not been done), a highly effective method(s) of contraception (failure rate of less than 1% per year) is required. Highly effective birth control in this study is defined as a double barrier method. Examples include a condom (with spermicide) in combination with a diaphragm, cervical cap, or intrauterine device (IUD). The individual methods of contraception should be determined in consultation with the investigator.

b) WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of investigational product.
c) Women must not be breastfeeding
d) Men who are sexually active with WOCBP must use any contraceptive method with a failure rate of less than 1% per year. The investigator shall review contraception methods and the time period that contraception must be followed.
Men that are sexually active with WOCBP must follow instructions for birth control for a period of 90 days plus the time required for the investigational drug to undergo 5 half-lives (60 days).

2.2.2 Exclusion Criteria

1) Target Disease Exceptions
a. Subjects with active CNS metastases are excluded. Subjects are eligible if CNS metastases are adequately treated and subjects are neurologically returned to baseline (except for residual signs or symptoms related to the CNS treatment) for at least 2 weeks prior to enrollment. In addition, subjects must be either off corticosteroids, or on a stable or decreasing dose of ≤ 10 mg daily prednisone (or equivalent) for 2 weeks.
b. Subjects with carcinomatous meningitis.

2) Medical History and Concurrent Diseases
a. Subjects with active, known or suspected systemic autoimmune disease. Subjects with vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune thyroiditis only requiring hormone replacement, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.
b. Subjects with a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of start. Inhaled or topical steroids, and adrenal replacement steroid doses > 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease.
c. Prior therapy with anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-CTLA-4 antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).
d. Subjects with a history of interstitial lung disease.
e. Other active malignancy requiring concurrent intervention.
f. Subjects with previous malignancies (except non-melanoma skin cancers, and the following in situ cancers: bladder, gastric, colon, endometrial, cervical/dysplasia, melanoma, or breast) are excluded unless a complete remission was achieved at least 2 years prior to study entry AND no additional therapy is required during the study period
g. Subjects with toxicities attributed to prior anti-cancer therapy other than alopecia and fatigue that have not resolved to grade 1 (NCI CTCAE version 4) or baseline before administration of study drug.
h. Subjects who have not recovered from the effects of major surgery or significant traumatic injury at least 14 days before the first dose of study treatment.
i. Treatment with any investigational agent within 28 days of first administration of study treatment

3) Physical and Laboratory Test Findings
a) Known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS).
b) Positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV RNA) indicating acute or chronic infection.

4) Allergies and Adverse Drug Reaction
a) History of severe hypersensitivity reactions to other monoclonal antibodies.
b) History of allergy or intolerance (unacceptable adverse event) to study drugs components..

5) Sex and Reproductive Status
a) WOCBP who are pregnant or breastfeeding
b) Women with a positive pregnancy test at enrollment or prior to administration of study medication

6) Prohibited Treatments and/or Restricted Therapies
a) Ongoing or planned administration of anti-cancer therapies other than those specified in this study
b) Use of corticosteroids or other immunosuppressive medications as per Exclusion Criteria 2b

7) Other Exclusion Criteria
a) Any other serious or uncontrolled medical disorder, active infection, physical exam finding, laboratory finding, altered mental status, or psychiatric condition that, in the opinion of the investigator, would limit a subject's ability to comply with the study requirements, substantially increase risk to the subject, or impact the interpretability of study results.
b) Prisoners or subjects who are involuntarily incarcerated.
c) Subjects who are compulsorily detained for treatment of either a psychiatric or physical (eg, infectious disease) illness.
Eligibility criteria for this study have been carefully considered to ensure the safety of the study subjects and to ensure that the results of the study can be used. It is imperative that subjects fully meet all eligibility criteria.

2.2.3 Women of Childbearing Potential
A Woman of Childbearing Potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over age 45 in the absence of other biological or physiological causes.

2.3 Concomitant Treatments
2.3.1 Prohibited and/or Restricted Treatments

The following medications are prohibited during the study (unless utilized to treat a drug-related adverse event):
• Immunosuppressive agents
• Immunosuppressive doses of systemic corticosteroids (except as stated in this Section 2.3.3).
• Any concurrent antineoplastic therapy (ie, chemotherapy, hormonal therapy, immunotherapy, extensive, non-palliative radiation therapy, or standard or investigational agents

Palliative and supportive care for disease related symptoms (including local radiotherapy, bisphosphonates and RANK-L inhibitors) may be offered to all subjects prior to first dose of study therapy (prior radiotherapy must have been completed at least 2 weeks prior to start).

2.3.2 Other Restrictions and Precautions
Subjects with active, known or suspected systemic autoimmune disease. Subjects with vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune thyroiditis only requiring hormone replacement, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.

Subjects are excluded if they have a condition requiring systemic treatment with either corticosteroids (>10mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of start. Inhaled or topical steroids, and adrenal replacement steroid doses > 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease.

2.3.3 Permitted Therapy
Subjects are permitted the use of topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids (eg, prednisone ≤ 10 mg/day) are permitted. A brief (less than 3 weeks) course of corticosteroids for prophylaxis (eg, contrast dye allergy) or for treatment of non-autoimmune conditions (eg, delayed-type hypersensitivity reaction caused by a contact allergen) is permitted.

The potential for overlapping toxicities with radiotherapy and nivolumab or ISA 101 currently is not known. Therefore, palliative radiotherapy is not recommended while receiving the study drugs. If palliative radiotherapy is required, then the study drugs should be withheld for at least 1 week before, during, and 1 week after radiation. Subjects should be closely monitored for any potential toxicity during and after receiving radiotherapy, and AEs should resolve to Grade ≤ 1 prior to resuming the study drugs.
Only non-target bone lesions that do not include lung tissue in the planned radiation field may receive palliative radiotherapy. Details of palliative radiotherapy should be documented in the source records. Details in the source records should include: dates of treatment, anatomical site, dose administered and fractionation schedule, and adverse events. If warranted, symptoms requiring palliative radiotherapy should be evaluated for objective evidence of disease progression.

2.4 Discontinuation of Subjects from Treatment

Subjects MUST discontinue investigational products at the discretion of the investigator for any of the following reasons:

• Withdrawal of informed consent (subject's decision to withdraw for any reason)
• Any clinical adverse event (AE), laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
• Pregnancy
• Termination of the study
• Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (eg, infectious disease) illness
• Additional protocol-specific reasons for discontinuation (See Section 3.3.5**)**

All subjects who discontinue should comply with protocol specified follow-up and survival procedures as outlined in section 4.2.2. The ONLY exception to this requirement is when **a subject withdraws consent** for all study procedures or loses the ability to consent freely (ie, is imprisoned or involuntarily incarcerated for the treatment of either a psychiatric or physical illness).

If a subject was withdrawn before completing the study, the reason for withdrawal must be entered on the appropriate case report form (CRF) page.

3.0 TREATMENTS

3.1 Study Treatments

Nivolumab 100 mg (10 mg/mL) will be packaged in an open-label fashion.
Five or ten 10 mL vials will be packaged within a carton. Vial assignments by subject will be tracked on specific forms. See the product information table for more information.

Table 4. Product Information Table

Product Description:(Other names = MDX-1106, ONO-4538, anti-PD-1

Product Description and Dosage Form	**Potency**	**Primary Packaging (Volume)/ Label Type**	**Secondary Packaging (Qty) /Label Type**	**Appearance**	**Storage Conditions (per label)**
nivolumab (BMS-936558-01)* Injection drug product	100 mg/Vial (10 mg/mL).	Carton of 5 or 10 vials	10-cc Type 1 flint glass vials stoppered with butyl stoppers and sealed	Clear to opalescent, colorless to pale yellow liquid. May	**BMS-936558-01 Injection** must be stored at 2 to 8 degrees C (36 to 46 degrees F)

is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/mL	with aluminum seals.	contain particles	and protected from light and freezing

*nivolumab may be labeled as BMS-936558-01 Solution for Injection

ISA101 vaccine will be packaged in an open-label fashion. The ISA101 vaccine contains six HPV-16 E6 and seven HPV-16 E7 SLP. For technical details reference is made to the IB (42). The peptides are dissolved in dimethylsulfoxide and subsequently diluted in WFI and emulsified with Montanideas detailed in the pharmacy manual. The final ratio of dimethylsulfoxide / WFI / Montanide is 20/30/50. The vaccine will be injected in two s.c. injections, one containing HPV-16 E6 and one containing HPV-16 E7.

Treatment should be initiated after registration. Each subject will be dosed at a frequency according to the treatment schedule until disease progression (or until discontinuation of study therapy in patients receiving nivolumab beyond progression), discontinuation due to toxicity, withdrawal of consent, or the study ends.

No premedications are recommended for initiation of dosing for nivolumab or ISA101.

Nivolumab and ISA 101 should be stored in a secure area according to local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations.

In this protocol, investigational products are:
• nivolumab
• ISA 101

Nivolumab will be provided by BMS. ISA101 and Montanide will be provided by ISA. The sites will also procure IV bags, diluents, and micron in-line filters (ie 0.2/ 0.22 micron; see current nivolumab Investigator Brochure for required filter details). Additional information can be found in the ISA101 Investigator Brochure and pharmacy manual.

3.2 Handling and Dispensing

The product storage manager should ensure that the study drugs are stored in accordance with the environmental conditions (temperature, light, and humidity) as determined by BMS (for nivolumab) and ISA (for ISA101). If concerns regarding the quality or appearance of the study drug arise, do not dispense the study drugs and contact BMS (for

nivolumab) or ISA (for ISA101) immediately. Nivolumab vials must be stored in the refrigerator at 2-8°C, protected from light freezing and shaking. If stored in a glass front refrigerator, vials should be stored in the carton.

ISA101 is a lyophilized powder of the two peptides/drug products which must be stored in glass vials in the dark at -20°C as specified in the pharmacy manual.

Investigational product documentation must be maintained that includes all processes required to ensure study drug is accurately administered. This includes documentation of study drug storage, administration and, as applicable, storage temperatures, re-constitution, and use of required processes (e.g. required diluents, administration sets).

Recommended safety measures for preparation and handling of nivolumab and ISA101 include laboratory coats and gloves.

After nivolumab has been prepared for administration, the total storage time (combination of refrigeration and room temperature) is not to exceed 24 hours. For details on prepared drug storage and use time under room temperature/light and refrigeration, please refer to the current nivolumab Investigator Brochure (29).

The maximum recommended hold time between ISA101 vaccine preparation and administration is 2 hours. The final formulation for s.c. administration consists of 20/30/50 v/v/v-% DMSO/WFI/Montanide.

Care must be taken to assure sterility of the prepared solution as the product does not contain any anti-microbial preservative or bacteriostatic agent.

Nivolumab is to be administered as a 60 minute IV infusion. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline or 5% dextrose. For details regarding preparation and administration of nivolumab, please refer to the current Investigator Brochure (29).

The ISA101 preparation is administered as two separate injections in two anatomically distinct locations (e.g. one injection in an upper extremity and the other in a lower extremity).

3.3 Selection and Timing of Dose for Each Subject

Nivolumab will be given at 3mg/kg as a 60 minute IV infusion, on Day 1 of a treatment cycle every 2 weeks. The first dose of nivolumab will be one week following first vaccination with ISA 101, that is, day 8 of treatment on the trial. Dosing calculations should be based on the body weight assessed at the start of each cycle as per Table 6. All doses should be rounded to the nearest milligram. There will be no dose escalations or reductions allowed. Subjects may be dosed no less than 12 days from the previous dose. There are no premedications recommended for nivolumab on the first cycle. If an acute infusion reaction is noted, subjects should be managed according to Section 3.3.6.

ISA101 will be administered at 3 specific time points, on days 1, 22 and 50. On each occasion it will be administered as two s.c. injections, one injection for HPV-DP-6P (containing 6 peptides) and one injection for HPV-DP-7P (containing 7 peptides) in two anatomically distinct locations (e.g. one injection in an upper extremity and the other in a lower extremity or one in each upper extremity with different sites used for each at each specified ISA101 vaccination time point). Dose of ISA 101 will be 100 mcg per peptide per injection.

Subjects will be monitored continuously for AEs while on study. Treatment modifications (eg, dose delay, or discontinuation) will be based on specific laboratory and adverse event criteria.

In some cases, the natural history of immunotherapy-related AEs of special interest can differ and be more severe than AEs caused by other therapeutic classes. Early recognition and management may mitigate severe toxicity. Evaluation and Management Guidelines were developed to assist investigators and can be found in Appendix 4:
• Suspected Pulmonary Toxicity
• Diarrhea and Colitis
• Suspected Hepatotoxicity (including asymptomatic LFT elevations)
• Suspected Endocrinopathy

3.3.1 Dose Delay Criteria
Tumor assessments for all subjects should continue as per protocol even if dosing is interrupted.

3.3.1.1 Investigational drugs Dose Delay Criteria
nivolumab and/or ISA101 administration should be delayed for the following:
• Any Grade ≥ 2 non-skin, drug-related adverse event, with the following exceptions:
− Grade 2 drug-related fatigue that does not require a treatment delay
• Any Grade 3 skin, drug-related adverse event
• Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leukopenia, AST, ALT, or total bilirubin:
− Grade 3 lymphopenia or leukopenia does not require dose delay
− Asymptomatic Grade 3/4 increase in amylase/lipase does not require dose delay
− If a subject has a baseline AST, ALT or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity
− If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity
• Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

3.3.2 Dose Reductions

3.3.2.1 Nivolumab Dose Reductions
There will be no dose modifications of nivolumab.

3.3.2.2 ISA101 Dose Reductions
There will be no dose modifications of ISA101.

3.3.3 Criteria to Resume Dosing
3.3.3.1 Criteria to Resume Treatment with nivolumab
Subjects may resume treatment with nivolumab when the drug-related AE(s) resolve(s) to Grade ≤ 1 or baseline, with the following exceptions:
• Subjects may resume treatment in the presence of Grade 2 fatigue
• Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
• Subjects with baseline AST/ALT or total bilirubin in the Grade 1 toxicity range who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin
• Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters (Section 3.3.5.1) should have treatment permanently discontinued
• Drug-related pulmonary toxicity, diarrhea, or colitis must have resolved to baseline before treatment is resumed. Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment
• If treatment is delayed > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in Section 3.3.5.

3.3.3.2 Criteria to Resume Treatment with ISA101
Subjects may resume treatment with ISA101 when the drug-related AE(s) resolve(s) to Grade ≤ 1.

3.3.4 Treatment beyond Disease Progression
Accumulating evidence indicates a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of PD.
Therefore, subjects enrolled in the clinical trial will be permitted to continue treatment beyond initial irRC and RECIST 1.1 defined PD as long as they meet the following criteria:
1. Investigator-assessed clinical benefit, and do not have rapid disease progression
2. Continue to meet all other study protocol eligibility criteria
3. Tolerance of study drug
4. Stable performance status
5. Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (eg, CNS metastases)

A radiographic assessment/ scan should be performed within six (6) weeks of original PD to determine whether there has been a decrease in the tumor size, or continued PD. The assessment of clinical benefit should be balanced by clinical judgment as to whether the subject is clinically deteriorating and unlikely to receive any benefit from continued treatment with nivolumab and ISA101 (if <3 doses have been administered).

If the investigator feels that the subject continues to achieve clinical benefit by continuing treatment, the subject should remain on the trial and continue to receive monitoring according to the Time and Events Schedule on Table 6.

For the subjects who continue nivolumab study therapy and ISA101 (if <3 doses have been administered) beyond progression, further progression is defined as an additional 10% increase in tumor burden volume from time of initial PD. This includes an increase in the sum of all target lesions and/ or the development of new measurable lesions.

New lesions are considered measureable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). Any new lesion considered non-measureable at the time of initial progression may become measureable and therefore included in the tumor burden volume if the longest diameter increases to at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm).

Global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective progression (ie radiographic confirmation) even after discontinuation of treatment.

3.3.5 Treatment Discontinuation Criteria

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

3.3.5.1 Nivolumab Dose Discontinuation

Nivolumab treatment should be permanently discontinued for the following:

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

• Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for laboratory abnormalities, drug-related bronchospasm, hypersensitivity reactions, and infusion reactions:

− Grade 3 drug-related bronchospasm, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation

− Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:

♦ Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation

♦ Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:

• AST or ALT > 8xULN

• Total bilirubin > 5x ULN

• Concurrent AST or ALT > 3x ULN and total bilirubin > 2x ULN

• Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:

− Grade 4 neutropenia ≤ 7 days
− Grade 4 lymphopenia or leukopenia
− Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
• Any dosing interruption lasting > 6 weeks with the following exceptions:
− Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Tumor assessments should continue as per protocol even if dosing of study drug(s) is interrupted.
− Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed. Tumor assessments should continue as per protocol even if dosing is interrupted.
• Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued nivolumab.

Subjects may remain on study and take the other study drug when the drug-related AE(s) resolve(s) to Grade ≤ 1.

3.3.5.2 ISA101 Dose Discontinuation
ISA101 will be discontinued in case of severe systemic allergic reaction, characterized by dyspnea, urticarial, severe generalized skin rash or other signs of systemic allergy, particularly if not controllable by epinephrine and/or anti-histamines.

3.3.6 Treatment of nivolumab-Related Infusion Reactions
Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthalgias, hypotension, hypertension, bronchospasm, or other allergic-like reactions. All Grade 3 or 4 infusion reactions should be reported within 24 hours to BMS and reported as an SAE if it meets the criteria. Infusion reactions should be graded according to NCI CTCAE (Version 4.0) guidelines.

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

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

• Stop the infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or acetaminophen 325 to 1000 mg PO; remain at bedside and monitor subject until resolution of symptoms. Corticosteroid and/or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur, then no further nivolumab will be administered at that visit.
• For future infusions, the following prophylactic premedications are recommended: diphenhydramine 50 mg (or equivalent) and/or acetaminophen/paracetamol 325 to 1000 mg PO should be administered at least 30 minutes before nivolumab infusions. If necessary, corticosteroids (up to 25 mg i.v.of SoluCortef or equivalent) may be used.
For Grade 3 or 4 symptoms: (Severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [eg, renal impairment, pulmonary infiltrates]. Grade 4: Life-threatening; pressor or ventilatory support indicated).
• Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline and treat the subject as follows: Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the Investigator is comfortable that the symptoms will not recur.

Nivolumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery of the symptoms.

In case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine or corticosteroids).

3.3.7 Treatment of ISA101 Related Systemic Reactions

Systemic allergic reactions up to Grade 2 have been reported in approximately 7% of patients and have been controlled with antihistamines and/or epinephrine. Patients receiving HPV-16-SLP or ISA101 in Montanide should be closely monitored for the first 3 hours after vaccination. Immediate treatment of severe allergic reactions should be available, including staff well-trained in resuscitation, intravenous access for administration of fluids, antihistamines and corticosteroids, and epinephrine for intramuscular injection.

3.4 Treatment Compliance

Treatment compliance will be monitored by drug accountability as well as the subject's medical record.

3.5 Destruction and Return of Study Drug

3.5.1 Destruction of Study Drug

For this study, study drugs (those supplied by BMS or ISA) such as partially used study drug containers, vials and syringes may be destroyed on site.

Any unused study drugs can only be destroyed after being inspected and reconciled by the responsible pharmaceutical company unless study drug containers must be immediately destroyed as required for safety, or to meet local regulations
On-site destruction is allowed provided the following minimal standards are met:
• On-site disposal practices must not expose humans to risks from the drug.
• On-site disposal practices and procedures are in agreement with applicable laws and regulations, including any special requirements for controlled or hazardous substances.
• Written procedures for on-site disposal are available and followed. The procedures must be filed with the site's SOPs and a copy provided to BMS or ISA (as appropriate) upon request or termination of the study, all unused and/or partially used study drug that was supplied by BMS or ISA must be returned to BMS or ISA, respectively.

It is the investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

4.0 ASSESSMENT FOR SAFETY AND EFFICACY

4.1 Safety Assessments

Safety assessments will be monitored at Screening (Baseline visit), during treatment according to the frequency for each treatment starting on Cycle 1 Day 1 and will continue at the specified frequency until discontinuation from the study. (See Tables 5, 6, 7)

Table 5. Flow Chart/Time and Events Schedule

Procedure	Screening Visit	Notes
Eligibility assessment		
Informed Consent	**X**	
Inclusion/Exclusion Criteria	**X**	
Medical History	**X**	
Safety Assessment		
Vital Signs and Oxygen Saturation	**X**	Temperature, BP, HR, RR, O2 saturation by pulse oximetry (also monitor amount of supplemental oxygen if applicable) Obtain vital signs at screening visit (within 14 days of registration) and within 72 hours of first dose
Physical Exam	**X**	Review of systems including

		measurements of Height and Weight, and ECOG Performance status
Laboratory Tests	**X**	Labs performed: CBC with differential including neutrophil and lymphocyte count, Serum chemistry (BUN or serum urea level, serum creatinine, sodium, potassium, calcium, magnesium, phosphate, chloride, and bicarbonate, glucose), AST, ALT, total bilirubin, alkaline phosphatase, albumin, LDH, TSH, free T3, free T4, HBV sAg, HCV RNA, HIV1/2 immunoassay
HPV-16 Confirmation	**X**	
Pregnancy Test	**X**	Performed within **24** hours of registration (serum or urine for WOCBP only)
Procedure		
Assessment (of signs and symptoms)	**X**	
Concomitant Medication collection	**X**	
Efficacy assessments		
Radiographic Tumor Assessment (chest, abdomen, pelvis)	**X**	Should be performed within 28 days of start of treatment. CT/MRI of brain (with contrast) should only be performed in subjects with a known history of treated brain metastases. Additional sites of known or suspected disease (including CNS) should be imaged at the screening visit and at subsequent on-study assessments
Biomarker Assessment		
Mandatory tumor biopsy	**X**	

4.1.1 Cervista HPV Assay

Tumors are required to be HPV-16 positive in order to be eligible for this clinical trial. Cervista HPV-16/18 assay will be conducted according to previously published data (48). This assay is not PCR-based but uses proprietary Invader chemistry, a signal-amplification method for the detection of specific nucleic acid sequences (Hologic, Inc.). The Cervista HPV-16/18 assay uses two oligonucleotide mixtures containing probes specific for the L1, E6, and E7 genomic regions of either HPV-16 or HPV-18. In both assays, oligonucleotides targeting the human histone 2 gene (H2be, HIST2H2BE) were used to serve as an internal control for detection of cellular DNA. A signal-to-noise value, referred to as fold over zero (FOZ), was determined for each specimen. The FOZ cutoff value for a positive result was 2.13.

Table 6. On –study assessment

Procedure	**C1D1 (±7 days)**	**C1D8 (±7 days)**	**C2D1 (±7 days)**	**Each cycle (every 2 weeks) on Day 1 (±7 days for every clinic visit)**	**Notes**
Tumor Biopsy for response evaluation					At Week 11 and at progression (optional)
Safety Assessment					
Vital Signs and Oxygen saturation	**X**	**X**	**X**	**X**	Temperature, BP, HR, RR, O2 saturation by pulse oximetry (also monitor amount of supplemental oxygen if applicable) prior to dosing and at any time a subject has any new or worsening respiratory symptoms
Adverse Events (AE) and Serious Adverse Events (SAE) Assessment	**…….Continuously……..**				
Physical Exam	**X**	**X**	**X**	**X**	Review of systems including measurements of Height and Weight, and ECOG Performance status
Complete blood count (CBC) (Results obtained prior to dosing on nivolumab infusion days)	**X**	**X**	**X**	**X**	Includes WBC count with differential, ANC, lymphocyte count, hemoglobin, hematocrit, and platelet count
Serum Chemistry	**X**	**X**	**X**	**X**	Serum chemistry

Tests					(BUN or serum urea level, serum creatinine, sodium, potassium, calcium, magnesium, phosphate, chloride, and bicarbonate, glucose), LDH
Liver Function Testing (Results obtained within **72** hours prior to dosing on nivolumab infusion days)	**X**	**X**	**X**	**X**	
Thyroid Function Testing	**X**	**X**	**X**	**X**	
Whole blood for research and immunology studies[a]					
Review of Concomitant Medications	**X**	**X**	**X**	**X**	Concomitant medications taken throughout the study duration should be recorded within the eCRF.
Pregnancy Test	**C1D1 and then monthly**				
Efficacy Assessments					
Radiographic Tumor Assessment[b]					**Week 11 (±5 days) and every 6 weeks (±5 days) thereafter**
Clinical Drug Supplies					
nivolumab		**X**	**X**	**X**	
ISA101 vaccine	**X**				**Only 3 doses: C1D1, C2D1 and C4D1 (±3 days)**

a. Whole blood will be collected pre-treatment and pre-vaccination at weeks 3 and 7, pre-nivolumab at weeks 9 and 11, then every 12 weeks for biomarker testing.

b. Subjects will be evaluated for response according to RECIST 1.1 and immune related response criteria.

Table 7. Follow-up and Survival Procedures

Procedure	Initial Follow-up phase: Follow-up #1 to occur 30 days (±5 days) after last dose. follow-up #2 to occur approximately 70 days (±5 days)	Further Follow-up Phase (beyond follow-up #2)	Notes
Radiographic Tumor Assessment[a]	**X**	**X**	For subjects who discontinue study treatment for reasons other than PD, follow up scans should be performed every 6 weeks (± 5 days) until PD, withdrawal of consent, death, lost to follow-up, or start of a subsequent anticancer therapy
Tumor Biopsy for response evaluation			At progression (optional)
Safety Assessment			
Vital Signs	**X**	**X**	
Physical Exam	**X**	**X**	Review of systems including measurements of Height and Weight, and ECOG Performance status
Adverse Events (AE) and Serious Adverse Event (SAE) Assessment[b]	**X**	**X**	
Laboratory Tests	**X**	**X**	
Review of Concomitant Medications	**X**		
Collection of Survival Information[c]	**X**	**X**	**Direct contact (office visit) or phone call**

a. *Radiographic assessments for subjects who have not experienced PD **must** be obtained every 6 weeks (±5 days), and **not** delayed until follow-up #1 or #2. Patients with PD by RECIST 1.1 criteria at any point may be allowed to remain on study drugs unless they are demonstrating evidence of clinical progression. If the*

investigator feels the patient is benefiting and should continue on study, this should be documented in the medical record.

b. *Subjects will have two follow-up visits for safety within the first 100 days from the last dose of study therapy. Beyond 100 days from the last dose of study therapy, subjects will be followed for ongoing drug-related adverse events until resolved, return to baseline or deemed irreversible, or until lost to follow-up, withdrawal of study consent, or start of a subsequent anti-cancer therapy.*
c. *All subjects will be followed for overall survival every 3 months until death, being lost to follow-up, or withdrawal of study consent, for up to 3 years (36 months).*

4.2 Efficacy Assessments

4.2.1 Screening (Baseline visit) and On-Study Efficacy Assessments

Study evaluations will take place in accordance with Table 5, and according to irRC and, RECIST 1.1 (Appendix 1). High resolution CT with PO/IV contrast or contrast-enhanced MRI are the preferred imaging modalities for assessing radiographic tumor response. If a subject has a known allergy to contrast material, use local prophylaxis standards to obtain the assessment with contrast if at all possible, or use the alternate modality. In cases where contrast is strictly contraindicated, a non-contrast scan will suffice. In addition to chest, abdomen and pelvis, all known or suspected sites of disease (including CNS) should be assessed at screening and at subsequent assessments using the same imaging method and technique. If more than one method is used at screening, then the most accurate method according to RECIST 1.1 should be used when recording data, and should again be used for all subsequent assessments. Bone scan, PET scan, or ultrasound are not adequate for assessment of RECIST response. In selected circumstances where such modalities are the sole modality used to assess certain non-target organs, those non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected. Subjects with a history of brain metastasis should have surveillance MRI approximately every 12 weeks or as per local standard of care, or sooner if clinically indicated. Radiographic tumor assessments will be conducted at Week 11(± 5 days) and every 6 weeks from Week 11 (± 5 days) until disease progression (or until discontinuation of study therapy in patients receiving nivolumab beyond progression), lost to follow-up, withdrawal of study consent, or start of a subsequent anti-cancer therapy. Tumor assessments for all subjects should continue as per protocol even if dosing is interrupted. Tumor measurements should be made by the same investigator or radiologist for each assessment whenever possible. Changes in tumor measurements and tumor responses to guide ongoing study treatment decisions will be assessed by the investigator using irRC and RECIST 1.1 (see Appendix 1-2).

4.2.2 Follow-up and Survival Procedures

Subjects who discontinue study treatment prior to progression, and subjects being treated beyond disease progression, will be followed with radiographic tumor assessments every 6 weeks (± 5 days) until documented or further disease progression, withdrawal of study consent, lost to follow-up, or beginning of a subsequent anti-cancer treatment.

Radiographic assessments should be performed according to Section 4.1. All radiographic assessments performed for study purposes during the follow-up phase will be submitted to the IRC for adjudication of the primary and secondary efficacy endpoints. Survival will be followed for up to 3 years (36 months) after progression, either by direct contact (office visits) or via telephone contact, according to Table 6 until death, withdrawal of study consent, or lost to follow-up.

4.2.3 Primary Efficacy Assessment

This study has primary endpoint of ORR See section 6.3 for definitions of ORR. All subjects will be monitored by radiographic assessment on an every-6-week schedule [beginning from the first on-study assessment on Week 11 (±5 days)] to determine changes in tumor size according to Section 4.2.1. irRC and RECIST 1.1 criteria will be used for the assessment (see Appendix 1). The PI will determine efficacy assessment and it will be documented on the CRF.

4.2.4 Secondary Efficacy Assessments

For secondary efficacy analyses (response rate by irRC, RECIST, PFS, Immune-related PFS, subjects will be monitored by radiographic assessment on an every-6-week schedule beginning from the first on-study assessment on Week 11 (±5 days)], as for the primary efficacy assessment and according to Section 4.2.1. RECIST 1.1 criteria will be used for the assessment (see Appendix 1). The PI will determine efficacy assessment and it will be documented on the CRF. Survival information will be collected as for the primary efficacy assessment.

4.3 Other Assessments

4.3.1 Exploratory Biomarker Assessments

Tumor tissue and blood samples will be collected for immune monitoring as follows:

4.3.1.1 Peripheral Blood Markers

The treating physician or designee will have the option to cancel the laboratory protocol collection for patient safety without protocol deviation.

a) 100 mL of peripheral blood will be collected for evaluation of HPV-specific immune response in immune cell populations, including but not limited to CD4 and CD8 T cells in pre and post therapy samples at the following time points: pre-treatment and pre-vaccination at weeks 3 and 7, pre-nivolumab at weeks 9 and 11, then every 12 weeks. In the event of poor recovery of functional lymphocytes from frozen cells, selected patients may have phlebotomy performed earlier than every 12 weeks on one occasion.

HPV-specific immune responses will include IFN-γ ELISPOT assay, flow cytometry to characterize HPV-specific T cell populations, and T cell receptor sequencing to track the expansion of HPV specific T cells in the blood, (Dr. Chantale Bernatchez's lab).

4.3.1.2 Tumor Markers

Tumor tissue will be obtained at baseline, and as an optional procedure, at Week 11 (response evaluation), and at progression (as possible). Where possible tumor biopsy samples pre- and post- treatment will be analyzed for expression of costimulatory and co-inhibitory ligands on tumor cells as well as the corresponding costimulatory / coinhibitory receptors, phenotypic differentiation and activation markers on effector cells and immune cell subset analysis using a panel of antibodies previously established by Dr. Wistuba, who directs the proposed HPV-Related Cancers Pathology Core. This will inform the immune response in the context of the tumor microenvironment and may be used to predict response to therapy, and evaluate for any correlation with peripheral blood analyses and clinical history.

Pathologists led by Dr. Ignacio Wistuba, will conduct standardized IHC studies to define and characterize infiltrating immune cell populations with markers including CD3, CD4, FoxP3, CD8, CD68, CD57, CD45RO, granzyme, PD-1, PD-L1, PD-L2, CTLA-4. Peritumoral versus intratumoral infiltrates will be scored, as these staining patterns have been shown by others to correlate with clinical outcomes (49-53). An intratumoral and peritumoral immune cell infiltrate grade of (0) none, (1) rare lymphocytes (2) focal lymphohistocytic aggregates or (3) severe diffuse infiltration will be assigned (54). Three representative fields will be evaluated by image analysis allowing for the data to be reported as a percentage of area with positive staining. Digital images and numerical data will be captured and stored in a secure database which will be accessible to team members for review. IHC analysis of exploratory markers may include: the B7 family ligands, PD-L2 (B7-DC), B7-H3 and B7-x/H4, as well as inhibitory receptors on lymphocytes, including 2B4, LAG-3, BTLA, and Tim-3; these cell surface molecules are candidates for therapeutic antibody blockade. Expression of the ligands for Tim-3, BTLA and 2B4 (galectin 9, HVEM, and CD48, respectively)may also be assessed. These studies will provide a comprehensive view of cellular subsets and checkpoint molecule expression in tumors from untreated patients and how cellular subsets and key immune regulatory molecules are impacted intratumorally after treatment with anti-PD-1 and the ISA101_vaccine. Determining which inhibitory molecules/pathways are present despite treatment with anti-PD-1 and vaccine should inform selection of rational combination therapy in the future. Importantly, all of these parameters of the immune microenvironment will be correlated with clinical outcome.

5.0 SAFETY MONITORING AND REPORTING

5.1 ADVERSE EVENTS: Definitions and Reporting

An ***Adverse Event (AE)*** is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a subject or 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 (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

The causal relationship to either or both study drugs is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:
Related: There is a reasonable causal relationship between study drug administration (either ISA101, nivolumab or both) and the AE.
Not related: There is not a reasonable causal relationship between administration of either of the two study drugs (or both) and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship to either or both investigational agents.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

5.1.1 Serious Adverse Events Reporting

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate 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. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures

outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Serious Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.
- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.
- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.
- Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Potential drug induced liver injury (DILI) is also considered an important medical event. (See Section 5.5 for the definition of potential DILI.)
Suspected transmission of an infectious agent (eg, any organism, virus or infectious particle, pathogenic or non-pathogenic) via the study drug is an SAE.
Although pregnancy, overdose, cancer, and potential drug induced liver injury (DILI) are not always serious by regulatory definition, these events must be handled as SAEs. (See Section 5.3 for reporting pregnancies).

The following reasons for hospitalization or prolongation of existing hospitalization are not considered SAEs:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (specify what this includes)
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
- Social reasons and respite care in the absence of any deterioration in the patient's general condition

Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event.

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

5.1.2 Communication between Investigator and BMS and ISA
If applicable, SAEs must be collected that relate to any later protocol-specified procedure (eg, a follow-up skin biopsy). An SAE report should be completed for any event where doubt exists regarding its status of seriousness.

If the investigator believes that an SAE is not related to either study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy, or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS and ISA within 24 hours. SAEs must be recorded on the SAE Report Form; pregnancies on a MD Anderson SAE Form (electronic or paper forms). When using paper forms, the reports are to be transmitted via email or confirmed facsimile (fax) transmission to:
SAE Email Address: BMS: Worldwide.Safety@BMS.com; **ISA**: Visscher@isa-pharma.com.
SAE Facsimile Number: BMS: 609-818-3804; **ISA**: +31 71 33 22 311.
SAE Telephone Contact (required for SAE and pregnancy reporting): See Contact Information list.

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

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

All SAEs should simultaneously be faxed or e-mailed to BMS at:
Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

And to ISA at: ISA pharmaceuticals
Attention to Sonja Visscher

Fax Number: +31 71 33 22 311
Email: Visscher@isa-pharma.com.

5.2 Laboratory Test Abnormalities

The following laboratory abnormalities should be captured:

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

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

5.3 Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives (72 days) after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for subject safety). Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (eg, x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated.

The investigator must immediately notify the BMS and ISA of this event and complete and forward a MD Anderson SAE form to BMS and ISA within 24 hours and in accordance with SAE reporting procedures described in Section 5.1.2.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the MD Anderson SAE form.

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS and ISA. Information on this pregnancy will be collected on the MD Anderson SAE Form.

5.4 Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE (see Section 5.1.1 for reporting details).

5.5 Potential Drug Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential

DILIs, meeting the defined criteria, must be reported as SAEs (see Section 5.1.1. for reporting details).

Potential drug induced liver injury is defined as
1) AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)
AND
2) Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase),
AND
3) No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

5.6 Other Safety Considerations

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

For recommendations regarding suspected pulmonary toxicity, diarrhea and colitis, suspected hepatotoxicity (including asymptomatic LFT elevations), or suspected endocrinopathy, please see the Evaluation and Management Guidelines found in the IB-nivolumab.

ISA101, which includes the adjuvant Montanide, may be associated with ulceration of abscess formation up to 2 years after the last vaccination. In addition, up to Grade 2 systematic allergic reactions may be expected in approximately 7% of subjects receiving ISA101. Please refer to the Investigator Brochure for ISA101 regarding additional information on safety and risk management.

For the purpose of this study at MD Anderson Cancer Center, all patients will be registered in the Clinical Oncology Research System (CORE). All study related data will be captured in the Protocol Data Management System (PDMS). All adverse events, regardless of grade or attribution, will be documented in CORE.

6.0 STATISTICAL CONSIDERATIONS

6.1 Sample Size Determination

We will use Simon's two-stage MiniMax design (55) targeting an alternative hypothesis response rate of 0.3 versus a null hypothesis response rate of 0.10 with 80% power and a one-sided 0.05 significance level. A response is defined as ORR (CR +PR) by the RC at 11 weeks from the start of treatment. This design requires a maximum of 25 evaluable patients. Fifteen patients will be treated in the first stage. A response summary will be submitted to the IND Medical Monitor after the 15^{th} subject and at the end of the study. If < 2 responses are seen, the trial will be stopped for futility. If there are at least 2 responses, 10 additional patients will be enrolled into the second stage. If there are more

than 5 responses out of 25, the combination will be deemed worthy of further study. Enrollment will be suspended at the end of the first stage after 15 patients have been accrued for response evaluation if less than 2 response were observed.

A total sample size (N) of 28 is planned to obtain 25 evaluable patients.

Dose-limiting toxicity (DLT) is defined for non-hematologic toxicity as a grade 3-4 adverse event or grade 2 or greater ocular adverse event within 12 weeks of treatment. Grade 3 infusion reactions are exempt from the DLT definition. All grade 3 immune-related adverse events that resolve to grade 1 or less within 28 days are exempt from the DLT definition excluding: pancreatitis, colitis, and ocular, hepatic and endocrine toxicities. DLT for hematologic toxicity is defined as grade 4 neutropenia > 7 days duration or grade 3-4 neutropenia accompanied by neutropenic fever, grade 3-4 thrombocytopenia > 7 days duration or grade 3-4 thrombocytopenia accompanied by bleeding, and grade 3-4 anemia.

Stopping Rules for Toxicity:

DLT will be monitored continuously in cohorts of 4 patients for 28 all patients to ensure safety using the method by Thall et al (56. The trial will be stopped early for toxicity if Prob(DLT> 30%) > 0.8 using a prior of beta (0.4, 1.6). Stopping boundaries corresponding to this probability criterion are to terminate the trial if (# of patients with DLT) / (# patients evaluated) >= 3/4, 4/8, 6/12, 7/16, 8/20, and 10/24. If the true DLT rates are 0.1, 0.2, 0.3, 0.4 and 0.5, the probabilities of early stopping are 0.01, 0.09, 0.35, 0.7 and 0.92, receptively, and the average numbers of patients treated under these three scenarios are 28, 26, 22, 16 and 11, respectively (Multc Lean version 2.1.0).

Accrual rate is estimated at 2 patients per month.

Analysis plan: Summary statistics will be provided to summarize response, toxicity, and other categorical variables. Overall response rate by the RECIST 1.1 criteria will be estimated with 95% confidence interval. Progression free survival and overall survival will be summarized using the method of Kaplan and Meier) and Cox proportional hazards model.

6.2 Populations for Analyses

- All enrolled subjects: All subjects who signed an informed consent form and were registered.
- All treated subjects: All subjects who received at least one dose of ISA101 and/or one dose of nivolumab. This is the primary dataset for dosing and safety.
- Evaluable subjects: All subjects who receive at least one dose of ISA 101 and one dose of nivolumab and have had repeat imaging.
- Biomarker subjects: All treated subjects with pre-treatment tumor biopsy and blood biomarkers available

6.3 Endpoint Definitions

6.3.1 Primary Endpoint

The primary objective in the study will be measured by ORR defined as the sum of subjects with a CR and PR divided by the number of evaluable subjects at 11 weeks from start of treatment. Response can be identified on any date between start of treatment and date of progression. For the purposes of determining response in the first 15 evaluable pts (per the mini-max design), only patients who have completed at least one restaging assessment will be included. If there are < 2 responses at that time, accrual of new patients will be held while active patients are further followed and declared to be responding or progressing.

-CR is defined as disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
-PR: is defined as at least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
-PD is defined as at least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. Note: the appearance of one or more new lesions is also considered progression.
-SD is defines as neither sufficient shrinkage from the baseline study to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Best overall response (BOR) is defined as the best response designation, recorded between the date of start of treatment and the date of objectively documented progression per RECIST 1.1 (55) or the date of subsequent anticancer therapy, whichever occurs first. For subjects without documented progression or subsequent anti-cancer therapy, all available response designations will contribute to the BOR determination. For subjects who continue nivolumab beyond progression, the BOR should be determined based on response designations recorded up to the time of the initial RECIST 1.1-defined progression.

Response can be identified on any date between start of treatment and date of progression. For the purposes of determining response in the first 15 evaluable pts (per the mini-max design), only patients who have completed at least one restaging assessment will be included. If there are < 2 responses at that time, accrual of new patients will be held while active patients are further followed and declared to be responding or progressing. This has been added to section 6.3.1

6.3.2 Secondary Endpoints

- The first secondary endpoint is safety and tolerability which will be measured by the incidence of adverse events, serious adverse events, deaths, and laboratory abnormalities. Adverse event assessments and laboratory tests are performed at baseline, and continuously throughout the study at the beginning of each subsequent cycle.
- The second secondary endpoint is HPV-specific immune responses

- The third secondary endpoint is immune-related ORR (ir-ORR) (ir CR + irPR) at 11 weeks from the start of treatment by the irRC (56). ORR using the irRC is derived from time-point response assessments (based on tumor burden) as follows:

- irCR, complete disappearance of all lesions (whether measurable or not, and no new lesions) with confirmation by a repeat, consecutive assessment no less than 4 wk from the date first documented
- irPR, decrease in tumor burden ≥50% relative to baseline confirmed by a consecutive assessment at least 4 wks after first documentation
- irSD, not meeting criteria for irCR or irPR, in absence of irPD
-irPD, increase in tumor burden ≥25% relative to nadir (minimum recorded tumor burden) with confirmation by a repeat, consecutive assessment no less than 4 wk from the date first documented.
-Patients are considered to have irPR or irSD even if new lesions are present, as long as they meet the respective thresholds of response as described above. Furthermore, patients are not considered to have irPD if new lesions were present and the tumor burden of all lesions did not increase by ≥25%. In contrast to irCR, irPR, and irPD, a response of irSD does not require confirmation. Refer to Appendix 1 for more details about irRC.

- The fourth secondary endpoint is PFS. It is defined as the time from first day of treatment to the date of the first documented tumor progression (per RECIST 1.1), or death due to any cause. Subjects who die without a reported prior progression will be considered to have progressed on the date of their death. Subjects who did not progress or die will be censored on the date of their last evaluable tumor assessment. Subjects who did not have any on study tumor assessments and did not die will be censored on the date they were randomized. Subjects who started any subsequent anti-cancer therapy without a prior reported progression will be censored at the last evaluable tumor assessment prior to initiation of the subsequent anti-cancer therapy.
- The fifth secondary endpoint is irPFS. It is defined as the time from treatment to the date of the first documented tumor progression (per irRC), or death due to any cause.
- The sixth secondary endpoint is OS. It is defined as the time from treatment to the date of death.

6.3.3 Exploratory Endpoints

- Exploratory endpoints are correlative markers of immune response in the tumor and peripheral blood that are discussed in detail in Section 4.3.1.

6.3.4 Safety Analyses

The safety analysis will be performed in all treated subjects. Descriptive statistics of safety will be presented using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. All treatment emergent AEs, drug-related AEs, SAEs and drug-related SAEs will be tabulated using worst grade per NCI CTCAE v 4.0 criteria by system organ class and preferred term.

On-study lab parameters including hematology, chemistry, liver function, thyroid function and renal function will be summarized using worst grade per NCI CTCAE v 4.0 criteria. Toxicity will be monitored continuously in cohorts of 4 patients.

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APPENDIX 1

RECIST 1.1 CRITERIA

This Appendix has been excerpted from the full RECIST 1.1 criteria. For information pertaining to RECIST 1.1 criteria not contained in the study protocol or in this Appendix, please refer to the full publication.1

1 ASSESSMENT OF OVERALL TUMOR BURDEN AND MEASURABLE DISEASE

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Measurable disease is defined by the presence of at least one measurable lesion.

1.1 Measurability of tumor

At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.
Measurable lesions must be accurately measured in at least one dimension (longest diameter in the plane of the measurement to be recorded) with a minimum size of:

• 10 mm by CT scan - (CT scan slice thickness no greater than 5 mm)

• 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable)

• 20 mm by chest x-ray

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

All measurements should be recorded in metric notation, using calipers if clinically assessed.

Special considerations regarding lesion measurability

Bone lesions:

• Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

• Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as

CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.

• Blastic bone lesions are non-measurable.

Cystic lesions:

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

• 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above.
However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

• Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

Non-measurable lesions are all other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), as well as non-measurable lesions. Lesions considered non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

1.2 Method of assessment

The **same method of assessment and the same technique should be used** to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be performed rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical examination.
CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. Measurability of lesions on CT scan is based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.
Chest x-ray: Chest CT is preferred over chest x-ray, particularly when progression is an important endpoint, since CT is more sensitive than x-ray, particularly in identifying new lesions. However, lesions on chest x-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.
Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and ≥10 mm diameter as assessed using calipers. For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may be reviewed at the end of the study.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised.
Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised.
Tumor markers: Tumor markers alone cannot be used to assess objective tumor response.

2 BASELINE DOCUMENTATION OF 'TARGET' AND 'NONTARGET' LESIONS

Target lesions: When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.
Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, and should lend themselves to reproducible repeated measurements.
Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. Pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. All other pathological nodes (those with short axis ≥10 mm but < 15 mm) should not be considered non-target lesions. Nodes that have a short axis <10 mm are considered non-pathological and should not be recorded or followed.
A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.
Non-target lesions: All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or 'unequivocal progression'. In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (eg, 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

3 TUMOR RESPONSE EVALUATION AND RESPONSE CRITERIA

3.1 Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if

that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. Note: the appearance of one or more new lesions is also considered progression.
Stable Disease (SD): Neither sufficient shrinkage from the baseline study to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.
Special notes on the assessment of target lesions

• **Lymph nodes:** Lymph nodes identified as target lesions should always have the actual short axis measurement recorded and should be measured in the same anatomical plane as the baseline examination, even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of <10 mm.

• **Target lesions that become 'too small to measure':** All lesions (nodal and nonnodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (eg, 2 mm). If the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm.
However, when such a lesion becomes difficult to assign an exact measure to then:
(i) if it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm.
(ii) if the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned (note: in case of a lymph node believed to be present and faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness).
Lesions that split or coalesce on treatment: When non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

3.2 Evaluation of non-target lesions

While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively at the time points specified in the protocol.
Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).
Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
Progressive Disease (PD): Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

• The concept of progression of non-target disease requires additional explanation as follows:

• *When the patient also has measurable disease*: To achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status.

• *When the patient has only non-measurable disease*: To achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening such that the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: ie, an increase in tumor burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion).
Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from localized to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point.

3.3 New lesions

The appearance of new malignant lesions denotes disease progression. The finding of a new lesion should be unequivocal: ie, not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.
A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to constitute PD even if he/she did not have brain imaging at baseline.
If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents new disease. If repeat scans confirm that there is a new lesion, then progression should be declared using the date of the initial scan.

3.4 Tumor markers

Tumor markers alone cannot be used to assess objective tumor responses. If markers are initially above the upper normal limit, however, they must normalize in order for a patient to be considered as having attained a complete response.

4 EVALUATION OF BEST OVERALL RESPONSE

4.1 Time point response

A response assessment should occur at each time point specified in the protocol.
For patients who have measurable disease at baseline Table 1 provides a summary of the overall response status calculation at each time point

Appendix Table 1. **Summary of the Overall Response Status Calculation [Time point response: patients with target (+/-) non-target disease]**

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

4.2 Missing assessments and inevaluable designation

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD.

4.3 Special notes on response assessment

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (<10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of 'zero' on the case report form (CRF).
Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective

progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease as shown in Appendix Table 1.
For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

5 ADDITIONAL CONSIDERATIONS

5.1 Duration of response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study).
The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.
Duration of stable disease: Stable disease is measured from the start of the treatment (in randomized trials, from date of start of treatment) until the criteria for progression are met, taking as reference the smallest sum on study (if the baseline sum is the smallest, this is the reference for calculation of PD).

5.2 Lesions that disappear and reappear

If a lesion disappears and reappears at a subsequent time point it should continue to be measured. However, the patient's response at the point in time when the lesion reappears will depend upon the status of his/her other lesions. For example, if the patient's tumour had reached a CR status and the lesion reappeared, then the patient would be considered PD at the time of reappearance. In contrast, if the tumour status was a PR or SD and one lesion which had disappeared then reappears, its maximal diameter should be added to the sum of the remaining lesions for a calculated response: in other words, the reappearance of an apparently 'disappeared' single lesion amongst many which remain is not in itself en ough to qualify for PD: that requires the sum of all lesions to meet the PD criteria. The rationale for such a categorisation is based upon the realisation that most lesions do not actually 'disappear' but are not visualised because they are beyond the resolving power of the imaging modality employed.

5.3 Use of FDG-PET

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:
a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion. Confirmatory CT is recommended.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up:

• If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.

• If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan).
If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

Reference:
1 Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. (2009); 45:228-247.

APPENDIX 2

Immune-related Response Criteria (irRC)

Antitumor response based on total measurable tumor burden

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 (five lesions per organ, up to 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:

$$\text{Tumor Burden} = \text{SPD}_{\text{index lesions}} + \text{SPD}_{\text{new, measurable lesions}}$$

Appendix Table 2. Comparison between WHO criteria and the irRC

	WHO	irRC
New, measurable lesions (i.e., ≥5 × 5 mm)	Always represent PD	Incorporated into tumor burden
New, nonmeasurable lesions (i.e., <5 × 5 mm)	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two	Disappearance of all lesions in

	WHO	irRC
	consecutive observations not less than 4 wk apart	two consecutive observations not less than 4 wk apart
PR	≥50% decrease in SPD of all index lesions compared with baseline in two observations at least 4 wk apart, in absence of new lesions or unequivocal progression of non-index lesions	≥50% decrease in tumor burden compared with baseline in two observations at least 4 wk apart
SD	50% decrease in SPD compared with baseline cannot be established nor 25% increase compared with nadir, in absence of new lesions or unequivocal progression of non-index lesions	50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point)	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 wk apart

Time-point response assessment using irRC

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 irPD). 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 (Table 3). However, the irRC response categories have been modified from those of WHO criteria as detailed in Tables 2 and 3.

Table 3. Derivation of irRC overall responses

Measurable response	Nonmeasurable response		Overall response
Index and new, measurable lesions (tumor burden),[*] %	Non-index lesions	New, nonmeasurable lesions	Using irRC
↓100	Absent	Absent	irCR[‡]
↓100	Stable	Any	irPR[‡]
↓100	Unequivocal progression	Any	irPR[‡]
↓≥50	Absent/Stable	Any	irPR[‡]
↓≥50	Unequivocal progression	Any	irPR[‡]
↓<50 to <25↑	Absent/Stable	Any	irSD
↓<50 to <25↑	Unequivocal	Any	irSD

Measurable response	Nonmeasurable response		Overall response
Index and new, measurable lesions (tumor burden),* %	Non-index lesions	New, nonmeasurable lesions	Using irRC
	progression		
≥25?	Any	Any	irPD‡

- *Decreases assessed relative to baseline, including measurable lesions only (>5 × 5 mm).
- †Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart.

Overall response using the irRC

The overall response according to the irRC is derived from time-point response assessments (based on tumor burden) as follows:

irCR, complete disappearance of all lesions (whether measurable or not, and no new lesions)

- confirmation by a repeat, consecutive assessment no less than 4 wk from the date first documented

irPR, decrease in tumor burden ≥50% relative to baseline

- confirmed by a consecutive assessment at least 4 wk after first documentation

irSD, not meeting criteria for irCR or irPR, in absence of irPD

irPD, increase in tumor burden ≥25% relative to nadir (minimum recorded tumor burden)

- confirmation by a repeat, consecutive assessment no less than 4 wk from the date first documented

Patients were considered to have irPR or irSD even if new lesions were present, as long as they met the respective thresholds of response as described above. Furthermore, patients were not considered to have irPD if new lesions were present and the tumor burden of all lesions did not increase by ≥25%. In contrast to irCR, irPR, and irPD, a response of irSD does not require confirmation. It is important to note that irCR, irPR, and irSD include all patients with CR, PR, or SD by WHO criteria as well as those patients that shift to these irRC categories from WHO PD. Patients with irSD, particularly those with slow-declining tumor burden ≥25% from baseline at the last tumor assessment, are considered clinically meaningful because they show an objectively measurable reduction in tumor burden without reaching the 50% threshold that defines irPR (it represented an objectively measured reduction not commonly observed in the natural history of advanced melanoma patients).

If a patient is classified as having irPD at a post-baseline tumor assessment, then confirmation of irPD by a second scan in the absence of rapid clinical deterioration is required. The definition of confirmation of progression represents an increase in tumor burden ≥25% compared with the nadir at two consecutive time points at least 4 wk apart. It is recommended that this be done at the discretion of the investigator because follow-up with observation alone may not be appropriate for patients with a rapid decline in performance status. Confirmation of irPD allows for the capture of all observed responses using the irRC (Table 2), as most of these late-responding patients have a trend toward response within 4 wk after initial irPD. Whereas WHO criteria consider any new measurable lesion to indicate PD, determination of immune-related best overall response (irBOR) is based on changes in total tumor burden from the baseline (nadir, for irPD) tumor assessment, regardless of any initial increase in baseline lesions or the appearance of new lesions.

APPENDIX 3

Toxicity Monitoring

1.1 Model Parameters and Stopping Criteria

Maximum sample size: 28

Minimum sample size: 4

Cohort size: 4

	Response		**Toxicity**	
Standard	**Constant Rate c:** 0.1		**Constant Rate c:** 0.3	
Experimental	**Beta a:** 0.2	**Beta b:** 1.8	**Beta a:** 0.6	**Beta b:** 1.4
Stopping	π^* : 1		π_* : 0.8	
Shift	δ_R : 0		δ_T : 0	

Patients (in complete cohorts of 4) (inclusive)	**Stop the trial if there are this many toxicities total:**
	# Toxicities (inclusive)

4	3-4
8	4-8
12	6-12
16	7-16
20	8-20
24	10-24
28	Always stop with this many patients

APPENDIX 4

Toxicity Management Algorithms

These general guidelines constitute guidance to the Investigator and may be supplemented by discussions with the Medical Monitor representing the Sponsor. 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.

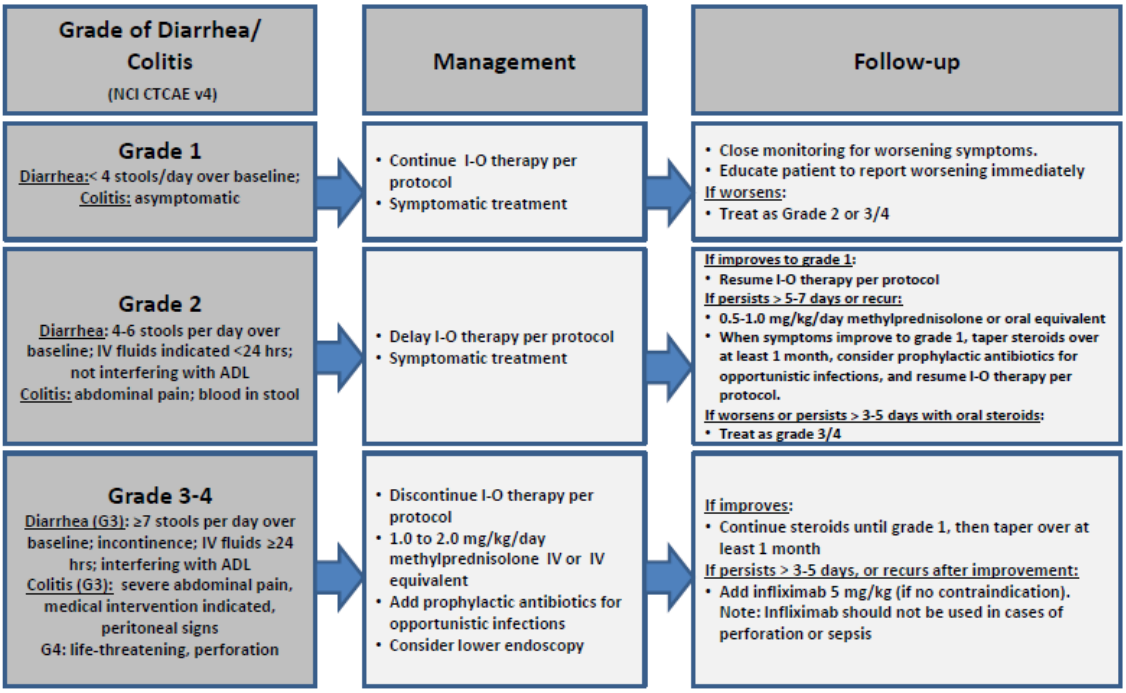
Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the 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

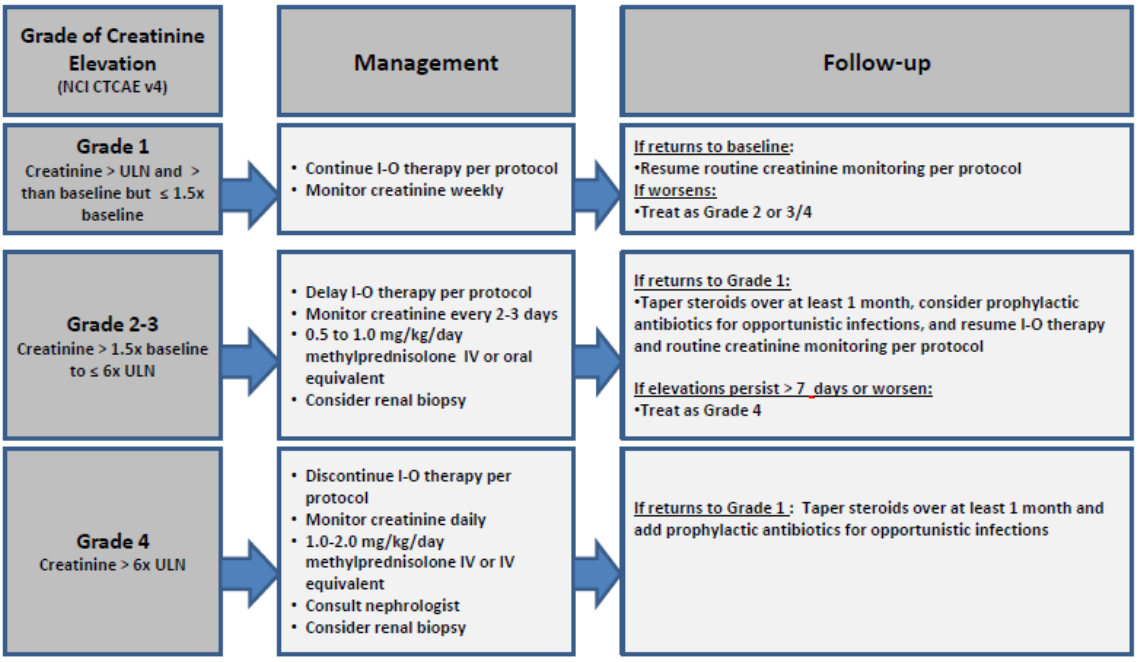
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



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

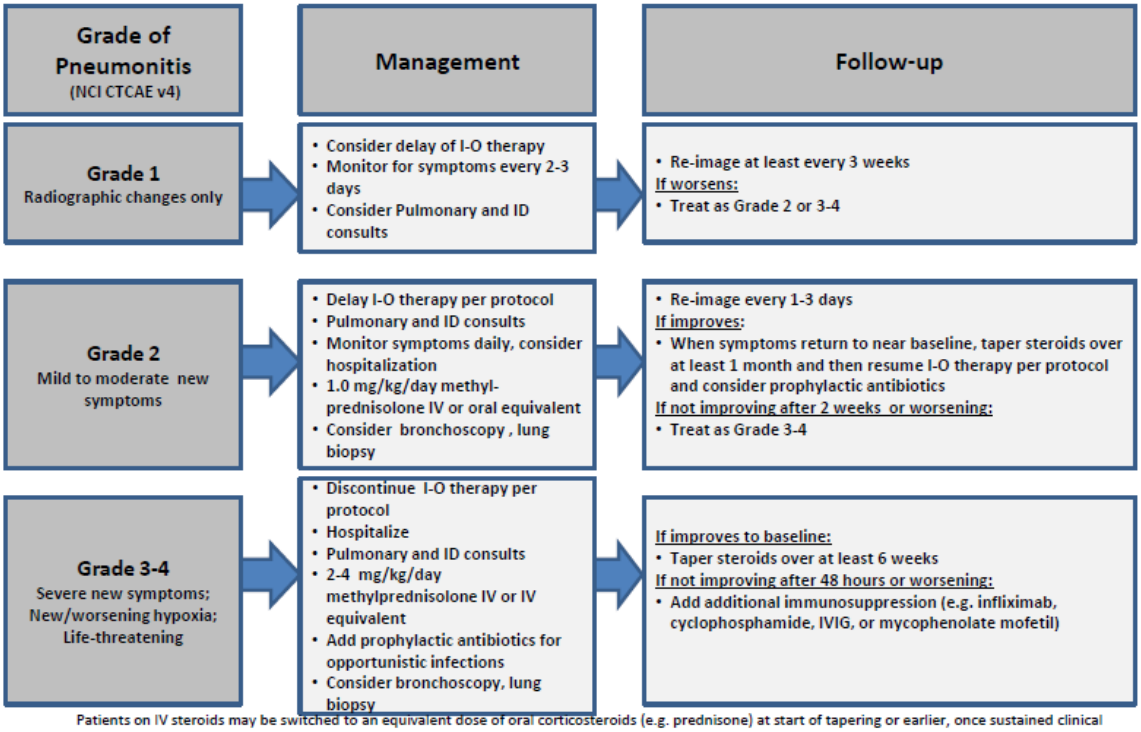
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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

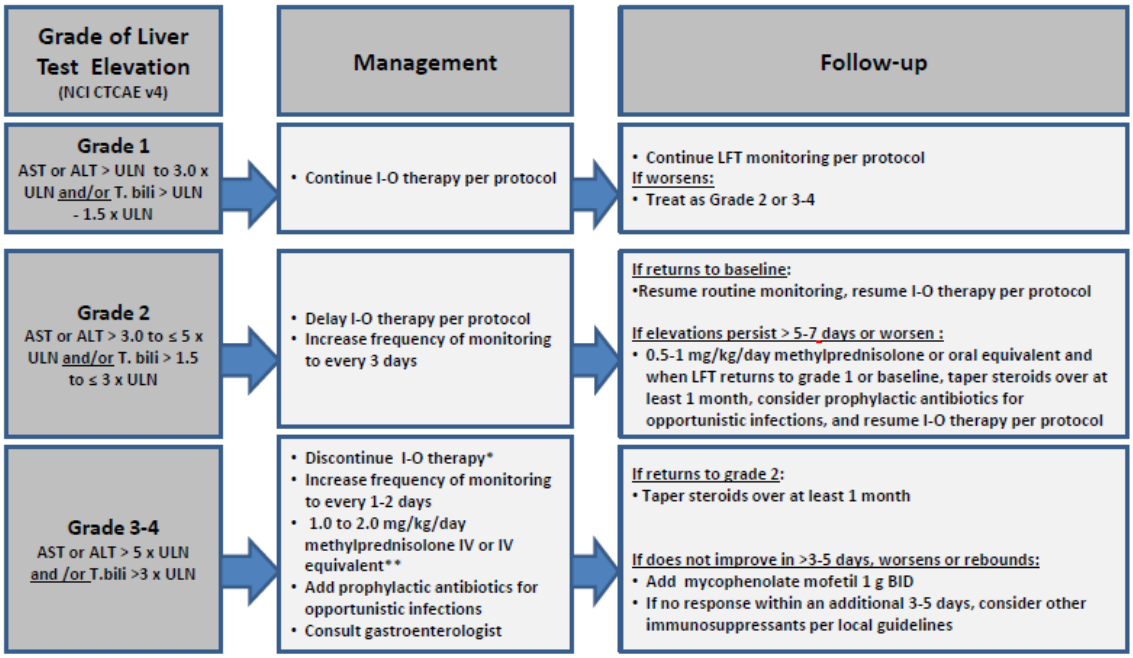
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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.

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.



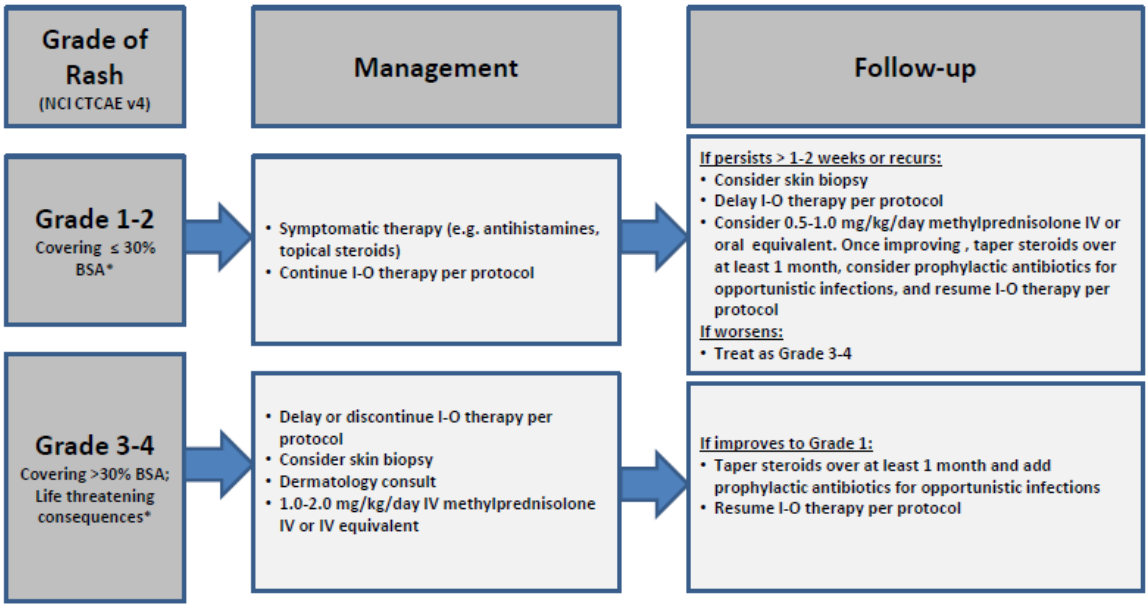
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

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

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

Skin Adverse Event Management Algorithm

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

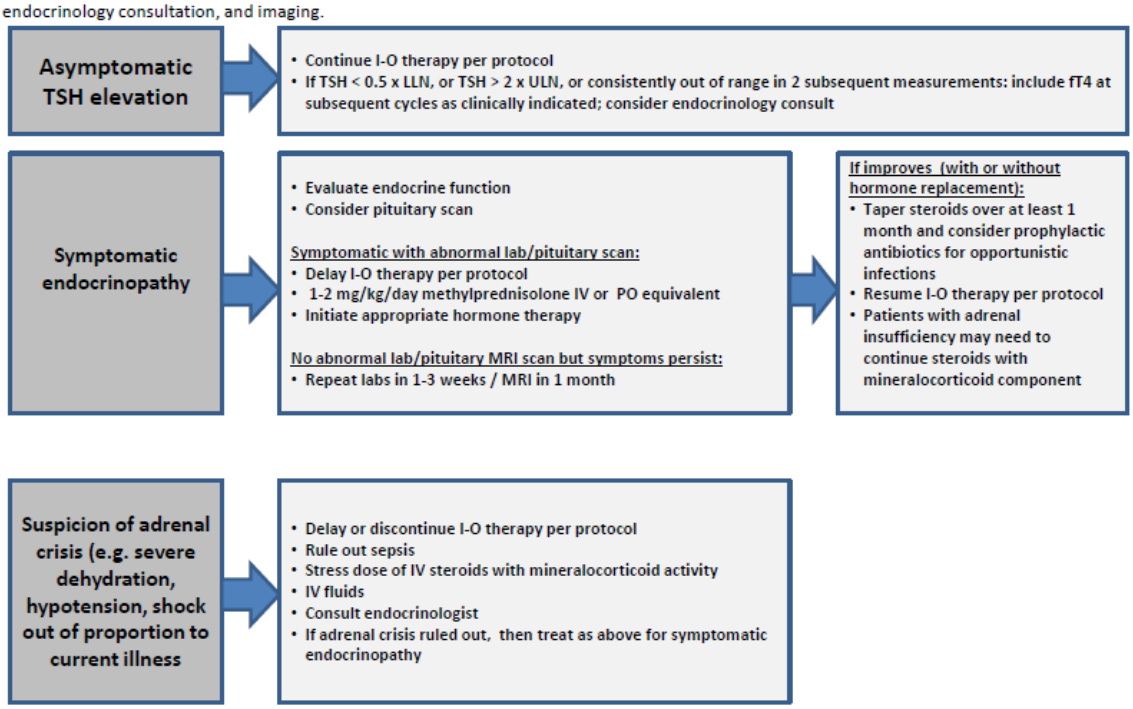


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

*Refer to NCI CTCAE v4 for term-specific grading criteria.

Endocrinopathy Management Algorithm

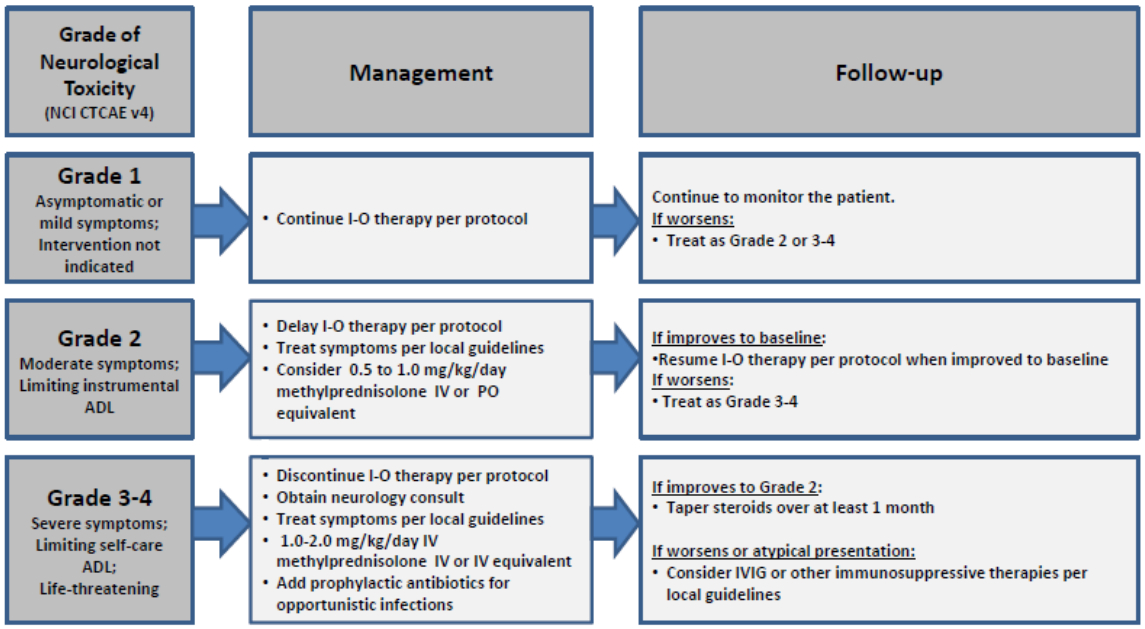
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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.

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.