Phase II Neoadjuvant trial of Nivolumab in Combination with HF10 Oncolytic Viral Therapy in Resectable Stage IIIB, IIIC, IVM1a Melanoma (Neo-NivoHF10)

Trial ID: HCI102346/ IRB#102346

Drug Manufacturer protocol # CA209-9F3

NCT03259425

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Investigational agent	HF10 Nivolumab (OPDIVO)
IND Number	17693

Historical Protocol Versions

Version 1: 25SEP2017

Version 2: 08AUG2018

Version 3: 06JUN2019

Version 4: 22JUL2019

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation or Term[1]	Definition/Explanation
ACV	Acyclovir
AE	Adverse event
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AV	Atrioventricular
β-HCG	Beta-human chorionic gonadotropin
BID	Twice daily
BLQ	Below limit of quantification
BMI	Body mass index
BP	Blood pressure
BUN	Blood urea nitrogen
Ca^{++}	Calcium
CBC	Complete blood count
CFR	Code of Federal Regulations
CHF	Congestive heart failure
CI	Confidence interval
Cl-	Chloride
CL_{cr}	Creatinine clearance
C_{max}	Maximum observed concentration
C_{min}	Trough observed concentration
CNS	Central nervous system
CR	Complete response
CRF	Case report form

Abbreviation or Term[1]	Definition/Explanation
CT	Computed tomography
CTCAE	Common Toxicity Criteria for Adverse Events
CV	Coefficient of variation
CYP	Cytochrome P450
D/C	Discontinue
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
DLT	Dose Limiting Toxicity
ECG	Electrocardiogram
Eg	Exempli gratia (for example)
FACS	Fluorescence Activated Cell Sorting
FDA	Food and Drug Administration
FDG-PET	Fluorodeoxyglucose (FDG)-positron emission tomography (PET)
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GGT	Gamma glutamyl transferase
GLP	Good laboratory practice
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCO_3^-	Bicarbonate
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Heart rate
hr	Hour or hours
IC_{50}	Half maximal inhibitory concentration
i.e.	Id est (that is)
IEC	Independent ethics committee

Abbreviation or Term[1]	Definition/Explanation
IFN	Interferon
INR	International normalized ratio
IRB	Institutional review board
irRC	Immune-related response criteria
IU	International unit
IV	Intravenous, intravenously
LDH	Lactate dehydrogenase
LLQ	Lower limit of quantitation
MedRA	Medical Dictionary for Drug Regulatory Activities
MRI	Magnetic resonance imaging
MRSD	Maximum recommended starting dose
MTD	Maximum tolerated dose
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect-level
NSCLC	Non-small cell lung cancer
PD	Pharmacodynamic(s)
PFS	Progression Free Survival
PK	Pharmacokinetic(s)
PO	Per os (administered by mouth)
PR	Partial response
PT	Prothrombin time
PTT	Partial thromboplastin time
QC	Quality control
QD	Once daily
QTc	QT interval corrected
QTcF	QT interval corrected using Fridericia equation
RBC	Red blood cell

Abbreviation or Term[1]	Definition/Explanation
RFS	Recurrence-free Survival
SAE	Serious adverse event
SD	Standard deviation or stable disease
$T_{1/2}$	Terminal elimination half-life
T_3	Triiodothyronine
T_4	Thyroxine
T_{max}	Time of maximum observed concentration
TID	Three times daily
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
ULQ	Upper limit of quantitation
UV	Ultraviolet
WBC	White blood cell
WOCBP	Women of childbearing potential
WONCBP	Women of nonchildbearing potential

All of these abbreviations may or may not be used in protocol.

PROTOCOL SIGNATURE

I confirm that I have read this protocol, and I will conduct the study as outlined herein and according to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable ICH guidelines for good clinical practice, and the applicable laws and regulations of the federal government. I will promptly submit the protocol to the IRB for review and approval. Once the protocol has been approved by the IRB, I understand that any modifications made during the course of the study must first be approved by the IRB prior to implementation except when such modification is made to remove an immediate hazard to the subject.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study treatment, the conduct of the study, and the obligations of confidentiality.

This document is signed electronically through submission and approval by the Principal Investigator at Huntsman Cancer Institute in the University of Utah IRB Electronic Research Integrity and Compliance Administration (ERICA) system. For this reason, the Principal Investigator at Huntsman Cancer Institute will not have a hand-written signature on this signature page.

Instructions to multi-site Principal Investigators at locations other than Huntsman Cancer Institute: SIGN and DATE this signature page and PRINT your name. Return the original, completed and signed, to the HCI Research Compliance Office. Retain a copy in the regulatory binder.

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Signature of Principal Investigator **Date**

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Principal Investigator Name (Print)

Name of Institution

STUDY SUMMARY

Title	Phase II Neoadjuvant trial of Nivolumab in Combination with HF10 Oncolytic Viral Therapy in Resectable Stage IIIB, IIIC, IVM1a Melanoma (Neo-NivoHF10)
Short Title	Nivolumab and HF10 for the Neoadjuvant treatment of Advance Melanoma
Protocol Number	IRB #102346
IND	IND #17693
Phase	Phase II
Design	Multi-center, open-label phase II safety, tolerability and efficacy study.
Study Duration	1 year and 3 months for enrollment, 3 years for study completion.
Study Center(s)	Multi-center. This study will be conducted at the Huntsman Cancer Institute, and additional studies to be identified.
Objectives	**Primary objective:** Pathologic response including complete response after 12 weeks of neoadjuvant treatment with Nivolumab and HF10. **Secondary objectives:** Recurrence-free survival, safety, biomarker analysis, overall survival, complete surgical resection.
Number of Subjects	This study is planned to enroll 20 patients with a possibility to increase enrollment based on observed pathologic response.
Eligibility Criteria	**Inclusion** 1. Patients must be >18 years or older. 2. Patients must have stage IIIB, IIIC, or IVM1a (equivalent staging at enrollment via AJCC 7th edition) metastatic melanoma which is eligible for complete surgical resection.

	3. Prior systemic, regional and radiation anticancer therapies must have been completed at least three months prior to enrollment. 4. Patients must be a candidate for intralesional therapy. 5. Patients must have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. 6. Serum LDH level < 1.5 upper limit of normal (ULN) within 28 days prior to enrollment (for stage IIIB and IIIC). 7. Patients have adequate organ function within 28 days prior to enrollment. **Exclusion** 1. Patients with active visceral, central nervous system, or any bone metastases melanoma (Stage IVM1b or IVM1c). 2. Patients whose primary diagnosis was ocular melanoma. 3. Patients receiving anti-herpes medication (i.e., acyclovir, famciclovir, or valacyclovir) within 1 week prior to initiating HF10 treatment. Patients may not require intermittent or chronic systemic (intravenous or oral) treatment with an antiherpetic drug other than intermittent topical use. 4. Patients who have an active herpetic skin lesion(s) or prior complications of HSV-1 infection. 5. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements, as determined by the investigator. 6. Medical history of autoimmune disease (e.g. Crohn's disease, ulcerative colitis) or other disease requiring systemic glucocorticoid or immunosuppressive therapy. 7. Patients with clinically evident Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), or Epstein-Barr Virus (EBV) infection are excluded.
Study Product, Dose, Route, Regimen	Neoadjuvant Nivolumab dose will be 240mg IV Q 2wks, and HF10 will be $1x10^7$ $TCID_{50}$/mL intratumoral injection

	for a maximum of 5mL per injection on days 0, 7, 14, 21, 28, 42, 56, 70, 84 for a total of 9 injections. Adjuvant Nivolumab dose will be 480 mg IV Q4wks.
Duration of administration	A total of 7 IV infusions of Nivolumab and 9 intratumoral injections of HF10 will be administered neoadjuvantly. After surgery, patients will receive adjuvant Nivolumab every 4 weeks for one year.
Statistical Methodology	The primary endpoint of pathologic response including pCR is expected to be seen in 30% of patients, hence 6 of the planned 20 patients are expected to have a pathologic response including pCR.

1 OBJECTIVES

1.1 Primary Objectives and Endpoint

1.1.1 Assess pathologic response rate (including complete response) after completion of 12 weeks of neoadjuvant treatment with nivolumab and HF10.

Endpoint: tumor viablitity will be assessed at surgery to assess pathologic response.

1.2 Secondary Objectives and Endpoint

1.2.1 Assess recurrence-free survival

Endpoint: recurrence after surgery will be assessed by radiologic scans scheduled per section 8 and confirmed by biopsy.

1.2.2 Assess overall survival

Endpoint: patients will be followed for survival for one year after completion of adjuvant nivolumab.

1.2.3 Assess the response of tumors prior to surgery based on RECIST 1.1

Endpoint: patients will be assessed radiographically with CT or MRI scan or assessed clinically.

1.2.4 Assess complete surgical resection

Endpoint: patients will be assessed at surgery to determine if complete surgical resection was achievable after neo-adjuvant treatment with nivolumab and HF10.

1.2.5 Assess the safety and tolerability of neoadjuvant nivolumab and HF10 with adjuvant nivolumab in patients with metastatic melanoma.

Endpoint: patients will be monitored for adverse events (AEs) and serious adverse events (SAEs) related to either or both nivolumab and HF10.

1.3 Exploratory Objective and Endpoint

1.3.1 Assess tissue and blood biomarkers

Endpoint: tissue and blood collection will occur throughout study treatment to assess changes in tumor markers, environment, immune cell population, cytokines and cell free (cf) DNA.

1.3.2 Assess concordance between irRC and RECIST 1.1 response assessments

Endpoint: radiographic response assessment will be performed using RECIST 1.1, in addition to irRC, to explore concordance between the two response criteria.

2 BACKGROUND

2.1 Introduction

Patients with resectable stage IIIB, IIIC, or IVM1a melanoma still represent a patient population with significant unmet medical need as up to 60% have disease recurrence at 18 months following surgical resection.[1-3] Current adjuvant therapies in these patients benefit less than 30% of patients.[4-9] Given the advances in the metastatic melanoma treatment landscape and the potential removal of antigen for immune system priming in the adjuvant setting, a key question remains: Does it make sense to treat these patients in the adjuvant setting or is neoadjuvant therapy more appropriate? Utilizing immunotherapies such as nivolumab combined with an intralesional oncolytic virus such as HF10 in the neoadjuvant treatment environment is likely to yield more clinical benefit because:

1. Nivolumab and HF10 are well tolerated and have been shown to have efficacy in unresectable metastatic melanoma patients.[10-13]
2. Tumor Antigen is present as an immune system target. The increased tumor burden in patients undergoing neoadjuvant treatment compared to patients who have had their tumor resected and are receiving adjuvant treatment may provide a more robust immune activation.
3. There is potential synergy for added efficacy with an oncolytic virus in combination with a checkpoint inhibitor. Indeed, the precedent for the combination of Nivolumab and HF10 (an oncolytic virus) in melanoma has been established via other immune-oncology oncolytic virus studies (e.g. ipilimumab + HF10, ipilimumab + talimogene laherparepvec and pembrolizumab+talimogene laherparepvec) displaying initial robust efficacy and tolerable safety signals.[13,14]
4. Nivolumab neoadjuvant clinical trials are currently on-going in non-small cell lung cancer and genitourinary cancers, while no such trials have started in resectable metastatic melanoma.

2.2 The Investigational Product

2.2.1 HF10

HF10 is an attenuated spontaneous mutant oncolytic HSV-1 strain that has not been modified by genetic recombination and contains no external genes. The genome structure of HF10 has numerous deletions and insertions, resulting in the lack of the functional expression of UL43, UL49.5, UL55 and UL56, when compared to HSV-1 strain 17, a reference strain of HSV-1.[15] Additionally, HF10 has amino acid changes in the sequence of the protein encoded by UL1, which is involved in the regulation of syncytium formation and exhibits a relatively high divergence. The attenuation of neurovirulence in HF10 is likely attributable to the lack of the UL56 gene. Although the exact underlying mechanism of attenuation of neurovirulence is not clear, it has been reported that the lack of the UL56 gene decreases HSV-1 pathogenicity without affecting the ability of the virus to

replicate in most types of cultured cells.[16] UL56 belongs to the tail-anchored type II membrane protein that can associate with the kinesin motor protein KIF1A, which is involved with axonal transport.[17,18] Some defective mutations have also been detected in the other three genes. A 3,832 bp deletion at the UL and UL/IRL junction and a 2295 bp deletion and extensive rearrangement at the left end of the genome are also observed. Figure 1 shows the major differences in structure between HF10 and wild type HSV-1.

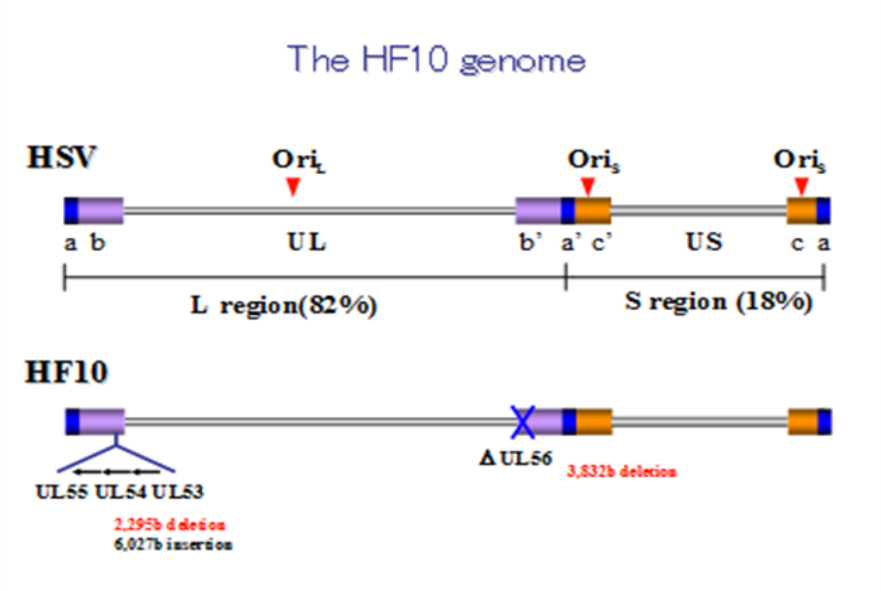

Figure 1.

2.2.2 Nivolumab

Immune checkpoint blockade is a rapidly advancing therapeutic approach in the field of immuno-oncology, and treatment with investigational agents targeting this mechanism has induced regressions in several types of cancer. Cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) receptor are two important cellular targets that play complementary roles in regulating adaptive immunity. Whereas PD-1 contributes to T-cell exhaustion in peripheral tissues, CTLA-4 inhibits at earlier points in T-cell activation. Nivolumab is a fully human monoclonal immunoglobulin (Ig) G4 antibody that binds to the PD-1 cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Inhibition of the interaction between PD-1 and its ligands promote immune responses and antigen-specific T cell responses to both foreign and self-antigens. PD-1 receptor blockade by nivolumab is a new approach for immunotherapy of tumors. Results from several clinical trials indicate that nivolumab is active in multiple tumor types. OPDIVO (nivolumab) is approved in the US for treatment of previously treated, unresectable or metastatic melanoma and previously treated, metastatic squamous non-small cell lung cancer, advanced renal cell carcinoma, classical Hodgkin

lymphoma, and recurrent or metastatic squamous cell carcinoma of the head and neck.

2.3 Nonclinical and Preclinical Studies

2.3.1 Nonclinical Pharmacology Studies

In Vitro Studies

In vitro studies using tumor and normal cell lines of human, mouse, and rat origin have shown that HF10 is capable of infection, replication, and cytotoxicity in a wide range of cell types. *In vivo* antitumor effects of HF10 were investigated using disseminated peritoneal tumor models and solid tumor models in immunocompetent mice. The mice treated with HF10 survived longer than control mice. Moreover, HF10-treated surviving mice that received a subsequent inoculation of the same tumor cell line survived longer than control mice, suggesting that HF10 contributed to acquisition of immunity against the tumors.

Mechanisms of Action Studies

As an attenuated, but replication-competent strain of HSV-1, HF10 has been shown to be capable of infecting a variety of mouse and human tumor cell lines. The virus replicates well in infected cells resulting in cell lysis. It was previously observed that the replication of HF10, unlike wildtype HSV-1 such as strain KOS and SP23, is highly restricted in murine macrophage RAW264 cells. Importantly, addition of anti-IFN α/β antibody results in complete lytic destruction of RAW264 cells.[19] These results indicate that differences in the IFN-pathway activation between normal cells and cancer cells are involved in the selectivity of HF10. It is well known that most cancer cells have a defective IFN pathway and thereby exhibit no or little response to IFN. The replication of HF10 was not inhibited at a concentration of 1-100 units/mL of IFN-β in a variety of tumor cells tested. In contrast, normal skin fibroblast cells were drastically protected by relatively low concentrations of IFN-β. These results indicate that HF10 infection may induce IFN *in vivo* and protect normal tissues from virus lysis, whereas tumor cells are selectively killed due to the lack of IFN responsiveness.

2.3.2 *In Vivo* Studies – In Support of HF10 Single Agent Antitumor Activity

In Vivo Efficacy of Repeat Intratumoral Administration of HF10 under Multiple Dose Levels against Mouse Melanoma in Immunocompetent DBA/2 Mice

The efficacy of repeated injections of HF10 at multiple dose levels against melanoma was evaluated. DBA/2 mice were subcutaneously inoculated with Clone M-3 melanoma cells (1 x 10^6 cells) and injected with 1 x 10^7, 1 x 10^6, or 1 x 10^5 $TCID_{50}$ /animal of HF10 on Days 5, 7, 9, 12, 14 and 16 after tumor inoculation. Results indicate that repeat intratumoral administration of multiple dose levels of HF10 in immunocompetent DBA/2 mice with the Clone M-3 melanoma cells resulted in significantly smaller tumor volumes compared with

control mice. Every HF10 treatment group showed statistically significant survival compared to that of vehicle treatment group. It is notable that the tumor mass completely disappeared in 7 of 10 mice in the HF10 group (1×10^7 $TCID_{50}$), 1 of 10 mice in the HF10 group (1×10^6 $TCID_{50}$) and 5 of 10 mice in the HF10 group (1×10^5 $TCID_{50}$).

In Vivo Antitumor Activity of HF10 against Mouse Cancer in Immunocompetent C3H and BALB/c Mice

Nagoya University in Japan previously investigated the in vivo antitumor effects of single and repeated intratumoral, intraperitoneal, and intravesical HF10 injections in immunocompetent mice with disseminated peritoneal tumors or solid tumors including: NfSa sarcoma [20,21], Colon-26 colorectal [22], MM102-TC breast [23] and MBT-2 bladder [24]. The results showed that mice treated with HF10 survived longer and had smaller tumor volumes than control mice. Moreover, when surviving HF10-treated mice were inoculated again with the same tumor cell lines, they survived longer than control mice, suggesting that infection with HF10 may have resulted in acquisition of antitumor immunity in the HF10-treated mice.

In Vivo Studies – in Support of Combination Treatment with HF10 + Nivolumab Antitumor Activity

The safety and efficacy of HF10 in combination with anti-PD-1 therapy has been evaluated in a preclinical Mouse M3 melanoma model. The M3 mouse melanoma cell line has a high sensitivity to HF10 and a moderate sensitivity to mouse anti-PD-1 therapy. Tumor cells were inoculated to both flanks of mice to examine a systemic anti-tumor effect of the combination treatment. Seven days after tumor inoculation, HF10 (1×10^5 $TCID_{50}$) was then intratumorally injected into a tumor on the left side. On day 8 and 11, 250 μg anti-mouse PD-1 antibody was administered intraperitoneally. Efficacy and survival was compared in 4 groups: controls, HF10 only, anti-mouse PD-1 only, and combination HF10 and anti-mouse PD-1. All treatments had a significant reduction in tumor volume compared to controls. The greatest reduction in tumor volume was seen with the combination of HF10 and anti-mouse PD-1. Additionally, the combination therapy of HF10 and anti-mouse PD-1 antibody exerted an enhancement of the anti-tumor effect not only in the HF10-treated side but also in the non-treated side. Survival was also significantly improved in mice treated with combination compared to both control and monotherapy treated animals.

In a study that investigated the effect of CD4+ T or CD8+ T cells the anti-tumor effect of the combination therapy was completely abrogated in the CD8+ depleted mice, while a trend towards an additive anti-tumor effect was observed in CD4+T cell depleted mice. These results were supported by the survival results. Taken together, it suggests that CD8+T cells are necessary for the anti-tumor effect in the combination therapy.

2.3.3 Nonclinical Toxicology Studies of HF10

In Vivo Toxicity Studies

A comprehensive battery of toxicity and biodistribution studies (intratumoral, s.c., i.p., and i.v.) were performed in both normal and tumor-bearing mice aimed at characterizing the HF10 tissue distribution and defining a NOAEL in mice.

A single intratumoral dose study demonstrated that HF10 was overall well tolerated (1.51×10^8, 6.76×10^4, and 5.89×10^3 $TCID_{50}$/mouse), and promoted a dose-related extension of tumor cell death in mice with M3 melanoma tumors. Although some adverse pathology was noted in the high-dose (1.51×10^8 $TCID_{50}$/mouse) treated mice in ovaries, adrenal gland, and bone marrow, these findings did not extend to the lower dose levels of the study, and thus intratumoral administration of 5.89×10^3 $TCID_{50}$/mouse and 6.76×10^4 $TCID_{50}$/mouse were well tolerated. A NOAEL was established for HF10 at a dose of 6.76×10^4 $TCID_{50}$/mouse for intratumoral administration, the same route of the administration used in the clinical studies. This dose level equates to a level of 1.89×10^8 $TCID_{50}$/70 kg patient based on body weight. Therefore, the HF10 dose at 1×10^7 $TCID_{50}$/mL up to 5 mL planned for the administration in the Phase II trial, is below the NOAEL established in mice for intratumoral administration.

A single s.c. dose study in normal mice demonstrated that HF10 of up to 1.51×10^8 $TCID_{50}$ was well tolerated. Minor degenerative and/or inflammatory changes occurred at the injection site on Days 3 and 15, however these changes were recovered thereafter. On Day 3, most of the treated mice showed extramedullary hematopoiesis in the spleen and increased cellularity of the paracortex of axillary lymph nodes. These findings in the spleen and lymph node are considered to be secondary to the findings at the injection site. There was no treatment-related histopathology in any tissue on Day 30, thus demonstrating complete recovery.

In the i.p. toxicity studies, adrenal necrosis was seen in mice given highest dose of HF10 (1.51×10^8 $TCID_{50}$/mouse). Body weight losses at all doses tested (5.89×10^3, 4.79×10^5 and 1.51×10^8 $TCID_{50}$/mouse) were recoverable by 14 days post dose. Changes in hematology and blood chemistry composition reflected the expected response to viral infection and were considered to be non-adverse. The NOAEL was considered to be 4.79×10^5 $TCID_{50}$/mouse.

In the i.v. toxicity studies representing a worst-case scenario of complete systemic exposure to the virus, mice dosed with the high level of HF10 (1.51×10^8 $TCID_{50}$/mouse) showed a number of degenerative and/or inflammatory changes in the adrenals, pituitary, ovaries, uterus, spleen, and bone marrow.

At middle dose levels of HF10 (6.76×10^4 and 5.89×10^3 $TCID_{50}$/mouse), mortality and adverse treatment-related effects were observed. In the toxicity study, one female receiving 6.76×10^4 $TCID_{50}$ was sacrificed and presented with significant pathology in the gastrointestinal tract, adrenal gland, and liver. Unscheduled deaths also occurred in the biodistribution study between Day 8 and 10 and considered to be related to treatment. Marked levels of HF10 DNA were detected in those pathologically affected organs (e.g., adrenals, liver) and ovaries.

At lower dose levels of 15.1 and 63.1 $TCID_{50}$/mouse, HF10 did not elicit any adverse effect in any of the parameters investigated. There was no mortality, clinical signs or any adverse pathology or distribution associated with HF10 administration. Thus, a NOAEL was established for HF10 at a dose of 63.1 $TCID_{50}$. A dose of 63.1 $TCID_{50}$/mouse equates to a dose of nearly 2.5×10^3 $TCID_{50}$/kg based on body weight, or 1.77×10^5 $TCID_{50}$/70 kg patient.

It should be noted that HF10 is susceptible to acyclovir (ACV) to a similar extent as wild-type HSV-1 in vitro, and therefore anti-herpes drugs can be used as rescue therapy when the virus needs to be quickly controlled.

2.3.4 Nonclinical Toxicology Studies of Nivolumab

Programmed death receptor-1 (PD-1, CD279), a 55 kD type I transmembrane protein, is

a member of the CD28 family of T-cell costimulary receptors that also includes CD28, CTLA-4, ICOS, and BTLA.[5] PD-1 contains an intracellular membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal immunoreceptor tyrosine-based switch motif (ITSM). Two ligands specific for PD-1 have been identified: PD-L1 (B7-H1/CD274) and PD-L2 (B7-DC/CD273). PD-L1 and PD-L2 have been shown to down-regulate T-cell activation upon binding to PD-1 in both murine and human systems.[25,26] PD-1 delivers a negative signal by the recruitment of a protein tyrosine phosphatase SHP-2 to the phosphorylated tyrosine residue in the ITSM in its cytoplasmic region.[27,28] PD-1 is primarily expressed on activated T cells, B cells and myeloid cells.[29]

Further evidence for a negative regulatory role of PD-1 comes from studies of PD-1-deficient mice. PD-1-deficient mice develop various autoimmune phenotypes, including dilated cardiomyopathy, a lupus-like syndrome with arthritis and nephritis, and accelerated diabetes mellitus.[30-32] The emergence of these autoimmune phenotypes is dependent upon the genetic background of the mouse strain and many of these phenotypes emerge at different times and show variable penetrance. In addition to the phenotypes of null mutations, PD-1 inhibition by antibody-mediated blockade in several murine models has been found to play a role in the development of autoimmune diseases such as encephalomyelitis, graft-versus-host disease, and type I diabetes.[33,34] Taken together, 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.

Preclinical animal models of tumors have shown that blockade by PD-1 by monoclonal

antibodies (mAbs) can enhance the anti-tumor immune response and result in tumor rejection. Antitumor activity by PD-1 blockade functions in PD-L1+ tumors as well as in tumors that are negative for the expression of PD-L1.[33,35-39]

This suggests that host mechanisms (i.e., expression of PD-L1 in antigen-presenting cells) limit the antitumor response. Consequently, both PD-L1 positive and negative tumors may be targeted using this approach. In humans, constitutive PD-L1 expression is normally limited to macrophage-lineage cells, although expression of PD-L1 can be induced on other hematologic cells as well, including activated T cells. However, aberrant expression of PD-L1 by tumor cells has been reported in a number of human malignancies.[40-46] PD-L1 expressed by tumor cells has been shown to enhance apoptosis of activated tumor-specific T cells in vitro.[30] Moreover, the expression of PD-L1 may protect the tumor cells from the induction of apoptosis by effector T cells.[47] Retrospective analyses of several human tumor types suggest that tumor over-expression (as measured by immunohistochemistry (IHC)) of PD-L1 may permit immune evasion by tumors. In renal cell carcinoma, high surface expression levels of PD-L1 on tumor cells are related to tumor aggressiveness.[42,45] Subjects with high tumor and/or lymphocyte PD-L1 levels are 4.5 times more likely to die from their cancer than subjects exhibiting low levels of PD-L1 expression. In addition, in multivariate analysis, high expression of PD-L1 is correlated to have a worse overall survival rate compared to low expression levels of PD-L1.[48]

Nivolumab is a fully human, IgG4 (kappa) isotype, mAb that binds PD-1. Blockade of the PD-1 pathway by nivolumab was studied using the mixed lymphocyte reaction (MLR). PD-1 blockade resulted in a reproducible enhancement of both proliferation and IFN-γ [49] The effect of nivolumab on antigen-specific recall response was investigated using a cytomegalovirus (CMV) -restimulation assay with human peripheral blood mononuclear cells (PBMCs), and was evaluated by an enzyme-linked immunosorbent assay (ELISA).

These data indicated that nivolumab, versus an isotype-matched control antibody, augmented IFN-γ. Nivolumab is therefore considered a promising immunotherapeutic option.

2.4 Clinical Studies with HF10

Completed Phase I Studies of HF10 in Japan

HF10 was studied previously in patients with breast cancer (N=6)[50], head and neck cancer (N=3)[51], and unresectable pancreatic cancer (N=8)[52] in three investigator-initiated open-label studies conducted in Japan. The only adverse event reported was low-grade fever in two of the three treated patients with head and neck cancer. This adverse event is consistent with adverse events reported previously in other clinical studies of oncolytic viruses (e.g. flu-like symptoms such as low grade fever, chills, fatigue, nausea/vomiting, headache, diarrhea, and hypotension).[53]

Phase I Study of HF10 in Patients with Refractory Head and Neck Cancer or Solid Tumors with Cutaneous and/or Superficial Lesion in the US (Protocol No. M06-10083, under BB-IND 13342)

This was an open label, non-randomized, multicenter, two-stage, dose escalation Phase I study evaluating single and repeated intratumoral injections of the oncolytic

virus, HF10, in patients with refractory head and neck cancer, or solid tumors with cutaneous and/or superficial lesions (e.g., squamous cell carcinoma of the skin, carcinoma of the breast, and malignant melanoma).

Stage 1 of the study investigated dose escalation of a single intratumoral injection of HF10 over the following dose levels: 1 x 10^5 $TCID_{50}$, 3 x 10^5 $TCID_{50}$, 1 x 10^6 $TCID_{50}$, and 1 x 10^7 $TCID_{50}$. In Stage 1, 3 patients were to be enrolled per single dose cohort. Within each single dose cohort, accrual would temporarily be suspended after the first patient was entered and the patient was followed for safety and for viral distribution and elimination. In the single dose cohorts, after the first patient in a cohort showed no significant increase of virus DNA in comparison to baseline in two consecutive weekly samples of each specimen (whole blood, saliva, and urine), safety considerations permitting, entry of two additional patients would occur concurrently**.** Escalation to the next dose level required confirmation of no significant change of virus DNA, as described for the first patient in each cohort. All patients in a cohort completed the first cycle of treatment (with a 28-day safety observation period), before escalation to the next cohort was initiated. The patients in Stage 1 were required to be seropositive for HSV-1. Stage 1 was completed.

Stage 2 evaluated repeated intratumoral injections of HF10 at dose levels of 1 x 10^6 $TCID_{50}$/dose and 1 x 10^7 $TCID_{50}$/dose. Three patients were enrolled in each of the repeated dose cohorts.

In Stage 2, the first patient treated in each repeated dose cohort was required to be seropositive for HSV-1. Then, if no safety issue had occurred as a result of HF10 treatment of the first patient in a cohort, the cohort was to be opened to enrollment of HSV-1 seropositive and seronegative patients. Accrual on the cohort was temporarily suspended for seronegative patients only, i.e., after enrollment of the first seronegative patient in a cohort, a second seronegative patient could not be enrolled in the cohort until after the first seronegative patient completed the safety observation period for 2 cycles of treatment. If all of three patients were seropositive, one seronegative patient could additionally be enrolled.

Twenty-eight patients were enrolled in the study and a total of 26 patients were treated in the study (15 patients in Stage 1 and 11 patients in Stage 2). Two patients were not evaluable for safety because they were not treated with HF10; one withdrew consent before HF10 treatment and one was not treated with HF10 due to hospitalization for pneumonia. Another patient, enrolled in Stage 2 (repeated HF10 administration portion of the study), was not evaluable for dose limiting toxicities (DLTs) per protocol because the patient received only one HF10 injection; Stage 2 patients were required to receive two HF10 injections to be considered evaluable for DLTs.

Twenty-four (92.3%) of the 26 patients treated in this study experienced adverse events.

Treatment-emergent adverse events (TEAEs) were reported for ≥ 20% of patients in the following system organ classes, in descending order: general disorders and administration site condition (13 patients; 50.0%), gastrointestinal disorders (8;

30.8%), and metabolism and nutrition disorders (8; 30.8%). The most frequently reported TEAEs were chills, fatigue, and nausea, each reported for 3 patients (11.5%).

Nine patients (34.6%) reported HF10-related TEAEs. The most frequent related TEAEs were chills, experienced by 3 patients (11.5%), and fatigue, which was reported for 2 patients (7.7%). Regarding the repeat dosing cohorts, fewer patients (33.3%) in the lower dose cohort (1 x 10^6 $TCID_{50}$/mL/dose) experienced HF10-related TEAEs compared to patients (50.0%) in the 1 x 10^7 $TCID_{50}$/mL/dose cohort, suggesting a dose-response. It is notable that the frequent HF10-related TEAEs (chills and fatigue) were generally mild and are symptoms reported commonly and easily managed in viral infections and treatment with other oncolytic viruses.[53] All other HF10-related TEAEs were each reported for one patient. Overall, 8 patients (30.8%) reported severe (≥ Grade 3) TEAEs. No patients reported an event that was both HF10-related and Grade 3 or greater in severity. No HF10-related allergic events were reported. Also, there were no differences in toxicity between patients who were HSV-1-positive and HSV-1-negative at enrollment. No HF10-related serious adverse events were reported in this study.

Viral clearance was investigated following intratumoral injection of HF10. Viral clearance was assessed in blood, saliva, and urine samples by quantitative polymerase chain reaction (qPCR). Patients showed rapid clearance of virus in blood, saliva, and urine. In both stages of the study, HF10 DNA was not detected in any of the biological samples in the majority of patients. For the few patients in whom HF10 DNA was detected, it was almost exclusively restricted to saliva samples and was only transiently detected in samples. Two patients in Stage 1 had transient virus detection in saliva only. In Stage 2, 6 patients had transient virus detection in saliva only. Two patients in Stage 2 had transient virus detection in blood, and one of these patients also had transient virus detection in urine.

All 26 patients are off-study. The reasons for off-study are as follows: thirteen (50.0%) patients total, including 7 (46.7%) patients in Stage 1 and 6 (54.5%) patients in Stage 2, were off-study due to progressive disease per RECIST evaluation. Ten (38.5%) patients completed the study per protocol (including 5 patients (33.3%) in Stage 1 and 5 patients (45.5%) in Stage 2 [3 patients in the 1 x 10^6 $TCID_{50}$/mL cohort and 2 in the 1 x 10^7 $TCID_{50}$/mL cohort]). Two (7.7%) patients were off-study due to unrelated death; both patients were treated in Stage 1. One (3.8%) patient in Stage 1 was removed from the study due to symptomatic deterioration.

Clinical Studies with HF10 + Ipilimumab in unresectable melanoma

Phase 2 multicenter trial to evaluate efficacy and safety of HF10 oncolytic virus immunotherapy and ipilimumab in patients with unresectable stage IIIB-IV melanoma in the US (Protocol T14-10682, IND No 13,342)

An ongoing phase 2 trial of HF10 and ipilimumab in unresectable metastatic melanoma patients is assessing whether the antitumor effect of HF10 is enhanced by concurrent ipilimumab treatment. Ipilimumab naïve adults with Stage IIIB, IIIC or IV unresectable melanoma with measurable non-visceral lesion(s) received HF10

injections into single or multiple tumors ($1x10^7$ $TCID_{50}$/mL, up to 5mL/dose); 4 injections every week; then up to 15 injections every 3 weeks. Ipilimumab infusions (3 mg/kg) were given every 3 weeks for 4 doses. Tumor responses were assessed at 12, 18, 24, 36, and 48 wks. Best Overall Response Rate (BORR) was determined at 24 wks. Of 46 pts treated, 20% were stage IIIB, 43% stage IIIC, and 37% stage IV. Most HF10-related AEs were ≤ Grade 2, similar to HF10 monotherapy. No DLTs were reported; 3 Grade 4 AEs reported, all not treatment related. 30.4% had Grade 3 AEs. HF10-related Grade 3 AEs (n=3) were left groin pain, thromboembolic event and lymphedema, hypoglycemia, and diarrhea. Twenty-one (46%) patients had received at least 1 prior therapy for metastatic melanoma. Of 43 efficacy evaluable patients, preliminary BORR at 24 weeks per irRC was 41.8% (11.6% CR, 30.2% PR), disease stability rate 67.4% (25.6% SD). Eight responders (53%) were Stage IV. Overall study BORR, including those after 24 weeks, by irRC was 47.7% (15.9% CR, 31.8% PR), disease stability was 65.9% (18.2% SD). In summary, HF10 in combination with ipilimumab treatment does not appear to exacerbate ipilimumab toxicity, is safe, well tolerated and has both local and systemic antitumor activity. The efficacy appears greater than that seen with ipilimumab monotherapy. Since the clinical trial is on-going, the final results of the trial are still pending.

Clinical Studies with Nivolumab

One Phase 1 study (CA209003) has contributed much to the clinical experience with nivolumab monotherapy in subjects with melanoma and other solid malignancies.[14] CA209003 is an ongoing Phase 1 open label, multiple dose escalation study in 304 subjects with select previously treated advanced solid tumors, including melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), colorectal cancer, and hormone-refractory prostate cancer. Subjects received nivolumab at doses of 0.1, 0.3, 1, 3 or 10 mg/kg intravenously every 2 weeks, up to a maximum of 2 years of total therapy. As of 03-Jul-2012, a total of 107 melanoma subjects were treated with nivolumab in the dose range of 0.1 - 10 mg/kg.

No maximal tolerated dose was identified in CA209003. The incidence, severity and relationship of AEs were generally similar across dose levels and tumor types. Nivolumab related AEs of any grade occurred in 72.4% of subjects. The most frequent nivolumab related AEs occurring in 5% of subjects included: fatigue (25.7%), rash (13.5%), diarrhea (11.8%), pruritus (10.2%), nausea (7.9%), decreased appetite (7.9%), hemoglobin decreased (5.9%) and pyrexia (5.3%). The majority of events were low grade, with Grade 3 - 4 drug related AEs observed in 14.8% of subjects. The most common Grade 3 - 4 drug-related AEs occurring in 1% of subjects were: fatigue (1.6%), lymphopenia (1.3%), abdominal pain (1%), diarrhea (1%), hypophosphatemia.

3 POTENTIAL RISKS AND BENEFITS

3.1 HF10

Potential risks of investigational intratumoral injections include the following reported adverse events:

Occurring in more than 10% of subjects – chills

Occurring in 1%-10% of subjects - injection site disorders, fatigue, malaise, pyrexia, nausea, dehydration, pruritus, and/or hypotension

One patient with anal and scrotal tumors injected with HF10 experienced swelling in the genital area, skin breakage and scab formation in the area on/near the scrotum.

The results of previous and ongoing clinical trials of HF10 in Japan, and the Phase I clinical trial of HF10 conducted under BB-IND 13,342, suggest that HF10 is safe and tolerable. Few HF10- related adverse events have been reported in any of the trials and are similar across the trials. They include: flu-like symptoms such as fever, chills, fatigue, nausea/vomiting, and hypotension. Such events are consistent both with symptoms of viral infections and adverse events reported in trials of other investigational oncolytic viruses. No HF10-related serious adverse events have been reported in these trials.

In the event that HF10-treated patients experience unexpected viral toxicity, patients may be treated by the anti-herpes drugs acyclovir, famciclovir, or valacyclovir, to which HF10 is sensitive.

The Investigator Brochure may be updated during the course of this study with additional risks and benefits. Please see the current Investigator Brochure for further details about the potential risks and benefits associated with this study.

Treatment with HF10 may offer potential benefits. Preliminary evidence suggests that intratumoral injection with HF10 may result in an antitumor response.

3.2 Nivolumab

Overall, the safety profile of nivolumab monotherapy is manageable and generally consistent across completed and ongoing clinical trials with no MTD reached at any dose tested up to 10 mg/kg. There was no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. Most AEs were low-grade (Grade 1-2) with relatively few drug-related high-grade (Grade 3-4) AEs. The most common adverse events ($\geq$20%) in patients with melanoma were fatigue, rash, musculoskeletal pain, pruritus, diarrhea and nausea. The safety profile of nivolumab combination therapy varies with the agent combined with nivolumab, but is generally consistent with the safety profiles observed with either agent alone and, in some cases, both frequency and severity of AEs were greater than that observed with either agent alone.

The Investigator Brochure may be updated during the course of this study with additional risks and benefits. Please see the current Investigator Brochure for further details about the potential risks and benefits associated with this study.

Treatment with nivolumab offers patients potential benefits. In a significant proportion of nivolumab-treated patients, durable tumor responses were achieved, as well as a significant increase in overall survival.

4 STUDY DESIGN

4.1 Description

This is a single-arm, open label, Phase II study evaluating the safety and efficacy of neoadjuvant Nivolumab and HF10 in resectable stage IIIB, IIIC, and IVM1a melanoma.

4.2 Number of Patients

The total number of subjects for all sites is initially planned as 20 patients. The primary endpoint of pathologic response rate, including pCR, is expected to be seen in 20% of patients, hence 4 of the planned 20 patients are expected to have a pathologic response including pCR. Only patients who receive the combination neo-adjuvant treatment, nivolumab and HF10, and have a surgical resection according to the study calendar will be considered evaluable. Patients who discontinue the study before surgery then will be considered non-evaluable and will be replaced per principal investigator discretion.

4.3 Number of Study Centers

This study will open at the Huntsman Cancer Institute. If additional recruitment is required this will become a multi-center trial.

4.4 Study Duration

1 year and 3 months for enrollment, 3 years for study completion.

5 ELIGIBILITY CRITERIA

This eligibility checklist is used to determine patient eligibility and filed with the enrolling investigators signature in the patient research chart.

Patient No. _____

Patient's Initials: (L,F,M) _____

5.1 Inclusion Criteria

Yes/No (Response of "no" = patient ineligible)

5.1.1 _____ Patients must be ≥18 years or older.

5.1.2 _____ Patients must have stage IIIB, IIIC, or IVM1a (equivalent staging at time of enrollment via AJCC 7th edition) metastatic melanoma which is eligible for complete surgical resection.

5.1.3 _____ Prior systemic, regional and radiation anticancer therapies must have been completed at least three months prior to enrollment. Prior therapies (including anti-PD-1 inhibitors) are allowed provided three months have elapsed from last dose.

5.1.4 _____ Patients must be a candidate for intralesional therapy.

- At least 1 injectable cutaneous, subcutaneous, or nodal melanoma lesion ≥ 10 mm in longest diameter

OR

- Multiple injectable melanoma lesions which in aggregate have a longest diameter of ≥ 10 mm

AND

- Must have no known bleeding diathesis or coagulopathy that would make intratumoral injection unsafe.

5.1.5 _____ Patients must have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.

5.1.6 _____ Serum LDH level ≤ 1.5 upper limit of normal (ULN) within 28 days prior to enrollment.

5.1.7 _____ Patients have adequate organ function within 28 days prior to enrollment, as defined in table 1 listed below.

Table 1: Adequate Organ Function Laboratory Values:

	System	Laboratory Value
☐	**Hematological:**	
	Absolute Neutrophil Count ANC	≥ 1,500/uL
	Platelets	≥ 100,000/uL
	Hemoglobin	≥ 9 g/dL
☐	**Renal**	
	Serum Creatinine OR measured calculated creatinine clearance (GFR may also be used in place of creatinine or CrCl)	≤ 1.5 upper limit of normal (ULN) **OR** ≥ 60 mL/min for subject with creatinine levels ≥ 1.5 X institutional ULN
☐	**Hepatic**	
	Serum total bilirubin	≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin ≥ 1.5 ULN
	AST or ALT	≤ 2.5 X ULN OR ≤ 5 X ULN for subjects with liver metastases

5.1.8 _____ Men and women of childbearing potential must agree to use adequate contraception from the time of consent through 7 months after final nivolumab study treatment.

5.1.9 _____ Females of childbearing potential must have a negative urine or serum pregnancy test within 1 week prior to the start of treatment.

5.1.10 _____ Patients must be able to provide informed consent and willing to sign an approved consent form that conforms to federal and institutional guidelines.

5.2 Exclusion Criteria

Yes/No (Response of "yes" = patient ineligible)

5.2.1 _____ Patients with active visceral, central nervous system, or any bone metastases melanoma (Stage IVM1b or IVM1c).

5.2.2 _____ Patients whose primary diagnosis was ocular melanoma

5.2.3 _____ Patients receiving anti-herpes medication (i.e., acyclovir, famciclovir, or valacyclovir) within 1 week prior to initiating HF10 treatment. Patients may not require intermittent or chronic systemic (intravenous or oral) treatment with an antiherpetic drug other than intermittent topical use.

5.2.4 _____ Patients who have an active herpetic skin lesion(s) or prior complications of HSV-1 infection.

5.2.5 _____ Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements, as determined by the investigator.

5.2.6 _____ Medical history of autoimmune disease (e.g. Crohn's disease, ulcerative colitis) or other disease requiring systemic glucocorticoid or immunosuppressive therapy. Subjects who receive daily steroid replacement therapy serve as an exception to this rule. Daily prednisone equivalent at doses up to 10 mg would qualify.

5.2.7 _____ Patients with clinically evident Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), or Epstein-Barr Virus (EBV) infection are excluded

5.2.8 _____ Pregnant or breast feeding women; women desiring to become pregnant within the timeframe of the study are also excluded.

I certify that this patient meets all inclusion and exclusion criteria for enrollment onto this study.

_____ _____ _____

Investigator Signature **Date** **Time**

6 TREATMENT PLAN

6.1 Administration Schedule

The order in which the patient receives Nivolumab and HF10 is not specified and will be determined based on clinic scheduling.

6.1.1 Neo-adjuvant treatment

Nivolumab

Nivolumab 240 mg, IV will be administered over 60 minutes (±15 minutes) as tolerated by the patient, every 14 days starting on day 0. The rate of infusion may be increased to 30 minutes as tolerated. A total of 7 infusions will be administered prior to surgery.

HF10

HF10 1 x 10^7 $TCID_{50}$/mL will be administered by intratumoral injection to a single or multiple eligible tumors. HF10 will be administered on days 0, 7, 14, 21, 28, 42, 56, 70, and 84 for a total of 9 injections. The volume to be injected will be determined by lesion size (see table below for injection volume by tumor mass) with a maximum volume of 5 mL to be administered on a single treatment day. All eligible tumors except one will be treated with HF10 up to the maximum volume allowed. The untreated tumor will be used as an untreated control lesion.

Lesion Size#	**Injection Volume†(Dose*)**
>5.0 cm	≤ 5.0 mL (5x10^7 $TCID_{50}$)
>2.5 cm to 5.0 cm	≤ 2.0 mL (2x10^7 $TCID_{50}$)
>1.5 cm to 2.5 cm	≤ 1.0 mL (1x10^7 $TCID_{50}$)
> 0.5 cm to 1.5 cm	≤ 0.5 mL (5x10^6 $TCID_{50}$)
≤ 0.5 cm	0.1 mL (1x10^6 $TCID_{50}$)

#Per longer perpendicular diameter

†If the dose level is decreased to 1x10^6 $TCID_{50}$/mL, the recommended injection volume**s** stated in this table are not to be changed.

*Administration of maximum volume at 1x10^7 $TCID_{50}$/mL.

6.1.2 Surgery

Surgery will be performed within 28 days of completing neo-adjuvant therapy with nivolumab and HF10. Patients who cannot proceed to surgery will not be evaluable and will be replaced. Patients may be allowed to remain on study treatment at the discretion of the principal investigator.

6.1.3 Adjuvant treatment

Nivolumab

Adjuvant nivolumab should be initiated within 90 days after definitive surgery. Nivolumab will be administered at a flat dose of 480 mg IV every 28 days (every 4 weeks) for one year. We have chosen the 480mg IV dose every 4 weeks in accordance with the application made by Bristol-Myers Squibb to the FDA on July 24, 2017. This dose will be similar to the current FDA approved dose of 240 mg IV every 2 weeks with fewer infusions for the patient.

6.2 Nivolumab

6.2.1 Dosage Form

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

6.2.2 Preparation and Administration

Nivolumab is to be administered as an IV infusion through a 0.2-micron to 1.2-micronpore size, low-protein binding (polyethersulfone membrane) in-line filter at the protocol specified doses and infusion times. It is not to be administered as an IV push or bolus injection. Nivolumab can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 0.35 mg/mL. When the dose is fixed (e.g., 240 mg, 360 mg, or 480 mg flat dose), nivolumab can be infused undiluted or diluted so as not to exceed a total infusion volume of 120 mL.

During drug product preparation and handling, vigorous mixing or shaking is to be avoided. Dilution and infusion of nivolumab will be conducted per the package insert for the adjuvant treatment of melanoma. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent. Nivolumab infusions are compatible with polyvinyl chloride (PVC) or polyolefin containers and infusion sets, and glass bottles.

6.2.3 Storage and Use Conditions

The administration of nivolumab infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored under refrigeration conditions (2°C to 8°C, 36°F to 46°F) for up to 24 hours, and a maximum of 8 hours of the total 24 hours can be at room temperature (20°C to

25ºC, 68ºF to 77ºF) and room light. The maximum of 8-hour period under room temperature and room light conditions includes the product administration period.

6.3 HF10

6.3.1 Preparation

Preparations of the HF10 product will be provided in vials for injection in aqueous buffer comprised of 20 mmol/L Tris, pH 7.0-7.5, and 10% glycerol. HF10 will be stored at < -70ºC. The HF10 product will be diluted with physiological saline just before use to meet the target dose level. Specific instructions will be provided in the Pharmacy Manual.

6.4 Prohibited Concomitant Medications

No investigational agents other than the study therapy may be administered. No anticancer therapy other than the study therapy may be administered. Any medication that is considered necessary for the patient's welfare, and that will not interfere with the study medication, may be given at the discretion of the Investigator.

It is recommended that patients who routinely receive warfarin anticoagulant therapy should receive modified anticoagulant treatment with low-molecular-weight heparin (LMWH) during study treatment. Such patients will undergo continued monitoring of coagulation function by PT/INR for the duration of study treatment.

Following are recommendations for providing alternative anticoagulant therapy in warfarin receiving patients during HF10 treatment. However, actual management of anticoagulant therapy in each patient will be at the discretion of the study Investigator and the practice of the treatment center and treating physician.

Patients should be switched from warfarin to low-molecular-weight heparin (LMWH) at least 4 to 5 days prior to HF10 injection.

LMWH treatment should be stopped 24 hours prior to HF10 injection.

LMWH treatment should be reinitiated 24 hours after HF10 injection.

Warfarin should be reinitiated 24 hours after HF10 injection in conjunction with LMWH treatment.

LMWH treatment would then be stopped approximately 3 days later (according to INR monitoring), allowing warfarin approximately 3 days to achieve a therapeutic level of anticoagulation.

Continued INR monitoring is necessary for the duration of study treatment.

Patients should receive anti-viral medication (e.g., acyclovir, famciclovir, or valacyclovir) only in the event of viremia caused by HF10 treatment.

Immunosuppressive agents and immunosuppressive disease of system corticosteroids are also prohibited. Inhaled or topical steroids, and adrenal replacement steroid doses

> 10 mg daily prednisone equivalent, are permitted in the absence of autoimmune disease.

6.5 Duration of Therapy

Subjects must be withdrawn from the study treatment for the following reasons:

- Subject withdraws consent from the study treatment and/or study procedures. A subject must be removed from the trial at his/her own request or at the request of his/her legally acceptable representative. At any time during the trial and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.
- Adverse events incurred from intra-tumoral administration of the study agent including but not limited to grade 3 or greater persistent infection, delayed healing, necrosis or ulcer formation.
- Subject is lost to follow-up.
- Death.

Subjects may be withdrawn from the study for the following reasons:

- The subject is non-compliant with study drug, trial procedures, or both; including the use of anti-cancer therapy not prescribed by the study protocol.
- If, in the investigator's opinion, continuation of the trial would be harmful to the subject's well-being.
- The development of a second cancer.
- Development of an intercurrent illness or situation which would, in the judgment of the investigator, significantly affect assessments of clinical status and trial endpoints.
- Deterioration of ECOG performance status to 4.

7 TOXICITIES AND DOSEAGE MODIFICATION

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 4.0 for adverse event and serious adverse event reporting. A copy of the CTCAE Version 4.0 can be downloaded: (**http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx**).

7.1 Dose Modifications

7.1.1 Nivolumab

Recommendations for nivolumab dose modifications are listed in the table 1 below.

Table 1: Recommended Dose Modification for Nivolumab

Adverse Reaction	**Severity***	**Dose Modification**
Colitis	Grade 3 diarrhea or colitis	Withhold dose[a]
	Grade 4 diarrhea or colitis	Permanently discontinue
Pneumonitis	Grade 2 pneumonitis	Withhold dose[a]
	Grade 3 or 4 pneumonitis	Permanently discontinue
Hepatitis	Grade 2 AST or ALT or total bilirubin increased	Withhold dose[a]
	Grade 3 or 4 AST or ALT or total bilirubin	Permanently discontinue
Hypophysitis	Grade 2 or 3 hypophysitis	Withhold dose[a]
	Grade 4 hypophysitis	Permanently discontinue
Adrenal Insufficiency	Grade 2 adrenal insufficiency	Withhold dose[a]
	Grade 3 or 4 adrenal insufficiency	Permanently discontinue
Type 1 Diabetes Mellitus	Grade 3 hyperglycemia	Withhold dose[a]
	Grade 4 hyperglycemia	Permanently discontinue
Nephritis and Renal Dysfunction	Grade 2 or 3 Serum creatinine	Withhold dose[a]
	Grade 4 Serum creatinine	Permanently discontinue
Skin	Grade 3 rash or suspected Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)	Withhold dose[a]
	Grade 4 rash or confirmed SJSor TEN	Permanently discontinue
Encephalitis	New-onset moderate or severe	Withhold dose[a]

	neurologic signs or symptoms	
	Immune-mediated encephalitis	Permanently discontinue
Other	Other Grade 3 adverse reaction First occurrence Recurrence of same Grade 3 adverse reactions	Withhold dose[a] Permanently discontinue
	Life-threatening or Grade 4 adverse reaction	Permanently discontinue
	Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks	Permanently discontinue
	Persistent Grade 2 or 3 adverse reactions lasting 12 weeks or longer	Permanently discontinue
* Toxicity was graded per National Cancer Institute Common Terminology Criteria for Adverse Events. Version 4.0 (NCI CTCAE v4). [a] Resume treatment when adverse reaction returns to Grade 0 or 1.		

If nivolumab is withheld for more than 12 weeks, the treatment will be permanently discontinued.

In the event of a nivolumab dose delay, the concurrent HF10 dose will be similarly delayed; the HF10 dose will then be administered on the same day that nivolumab is administered.

7.1.2 HF10

In the event that the patient is unable to tolerate HF10 (grade 2 intolerable or higher chills, injection site disorders, fatigue, malaise, pyrexia, nausea, dehydration, pruritus, and/or hypotension which do not respond to optimal management) at the discretion of the treating provider, the dose will be reduced to 1×10^6 $TCID_{50}$/mL. The HF10 product will be diluted with physiological saline just before use to meet the target dose level. Specific instructions will be provided in the Pharmacy Manual.

If the patient is unable to tolerate the reduced dose the patient will be withdrawn from treatment.

7.2 Subject Compliance

Both Nivolumab and HF10 will be dispensed from the Huntsman Cancer Institute Investigational Pharmacy and administered by a study investigator, thus ensuring compliance with therapy parameters.

7.3 Patient Precautions for Minimizing Virus Exposure to Others

The HSV-1 virus, from which the study drug HF10 is derived, is very widespread in the environment, and many people in the United States have been exposed to HSV-1. HF10 is a weakened strain of HSV-1 and is believed to pose minimal health risk. No association of HF10 with any human illness has been established. However, given the investigational nature of HF10 therapy, it is appropriate that HF10-treated patients follow some precautions for minimizing HF10 exposure to persons with whom they have close contact. Appendix I provides guidance for the patient for minimizing exposure of HF10 to others.

7.4 Healthcare Provider Precautions

HF10 is a replication-competent virus. Although it is believed that the risk of inadvertent infection to study staff is extremely low, infectious disease precautions should be implemented to minimize viral exposure to healthcare providers. In the event of an accidental spill of HF10 containing solution, standard biohazard waste procedures should be employed, including use of gloves, gown, and mask, by healthcare workers coming in contact with or disposing of the study drug product. Contaminated surfaces should be first washed with a 10% sodium hypochlorite solution, followed by rinsing with water, and finally with 70% ethanol. All tubing and other disposables that may have been in contact with HF10 must be discarded as biohazardous waste.

In the event of an accidental needle stick, the healthcare provider should report the incident and follow the institutional inadvertent needle stick exposure policy implemented at the study site. In the event of inadvertent contact with HF10 (e.g., accidental spill, body fluid contact, or needle stick), exposed individuals may be treated, if warranted, with acyclovir, famciclovir, or valacyclovir. HF10 has been demonstrated to be sensitive to these anti-herpes agents. Treatment recommendations would follow those for acute wild type HSV infection.

An additional precaution recommended in this clinical trial is that pregnant healthcare providers be excluded from participating in the administration of HF10 or the care of HF10-treated patients. Although preclinical data indicate that HF10 does not undergo germline genomic integration, potential risks of HF10 to the developing human fetus have not been determined.

7.5 Supportive Care

All supportive measures consistent with optimal patient care will be given throughout the study.

Management of immune mediated events (irAEs) will follow the package inserts for the Nivolumab products.

8 STUDY CALENDAR

Examination	**Pre-study[1]**	**D0**	**D7±4 and D21±4**	**D14±4 and D28±4**	**D42±4**	**D56±4 and D70±4**	**D84±4**	**Surgery[8]**	**Every 4 wks ±2 wks for one year[9]**	**EOT[10] or Progression**	**Follow-up[11]**
Informed consent	X										
Medical history	X										
Concomitant Medications	X	X	X	X	X	X	X		X	X	X
Eligibility criteria	X										
Vital signs[3]	X	X	X	X	X	X	X		X	X	
Height	X										
Weight	X	X		X	X	X	X		X	X	
Physical examination	X	X[2]	X	X	X	X	X		X	X	
Lesion Photography[18]	X			X	X		X				
CBC/differential platelet count	X	X[2]	X	X	X	X	X		X	X	
Chemistry[4]	X	X[2]	X	X	X	X	X		X	X	
Pregnancy Test (serum or urine)	X[15]	X[2]					X		X[15]		
Thyroid function tests[17]	X		X		X		X				
PT/INR in patients receiving warfarin anticoagulation therapy	X	X	X	X	X	X	X				
LDH	X						X			X	
Genomic testing[16]	X										
Adverse Event Assessment[7]		X	X	X	X	X	X	X	X	X	X
Radiologic Evaluation[5]	X						X		X[12]	X	
irRC[19]	X						X				
RECIST 1.1[19]	X						X				
Biopsy for correlative studies[6]		X			X			X		X	
Blood for correlative studies[14]		X	X	X	X	X	X		X		
Nivolumab		X		X	X	X	X		X		
HF10 injections		X	X	X	X	X	X				
Surgery								X[13]			

Study visits during neoadjuvant nivolumab are scheduled in days. Windows are ± business days.

1 ALL Pre-study/Screening procedures should be completed within 28 days of study enrollment

2. Does not need to be done if occurred 7 days before D0.

3. Includes temperature, blood pressure, and pulse rate.

4. Chemistry includes: Albumin, Alkaline Phosphatase, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Total Bilirubin, Calcium, Carbon Dioxide, Creatinine, Chloride, Glucose, Potassium, Protein, Sodium, Urea Nitrogen. Fasting Glucose will be tested as clinically indicated.

5. A scan of the chest abdomen and pelvis with extension to the extremities if applicable. The same method (CT scans or possibly PET/CT scan) should be used for all assessments.

6. Biopsies are mandatory at day 0 and surgery. Biopsies are optional at day 42 and progression. See section 13.1 for details about the collection.

7. Adverse events will be followed until resolution while the patient remains on treatment. Events will be followed for 90 post treatment for SAEs and events thought to be related to the study medication will be followed until resolution or stabilization of the adverse event, or until the patient starts a new treatment regimen.

8. Surgery should occur no later than 28 days after D84.

9. Adjuvant nivolumab should be initiated within 90 days after definitive surgery.

10. EOT visit should occur 28 days (+-14 days) after the completion of therapy.

11. Follow-up should occur every three months (+-or 14 days) for 1 years post EOT visit.

12. Surgery will reset the scan schedule and should occur approximately every 12 weeks after surgery as standard of care.

13. Pathology at surgery will be used to determine pathological response.

14. Collection of blood samples for correlatives is optional and will be collected prior to HF10 injection during the neo-adjuvant (days 0,7, 14, 21, 28, 42, 56, 70, 84) and prior to nivolumab infusion during the adjuvant nivolumab administration.

15. Men and women of childbearing potential must agree to use adequate contraception from the time of consent through 7 months after nivolumab study treatment (neoadjuvant and adjuvant). A pregnancy test will be conducted w/in 72 hours prior to beginning adjuvant nivolumab.

16. Next generation sequcencing is required at screening as per standard of care. Testing will include, but is not limited to BRAF mutational analysis.

17. Thyroid Tests will include TSH and free T4. In the event of hypo/hyper thyroidism, appropriate support will be provided in accordance with institutional guidelines.

18. Lesion photography should be completed at each time point AND when a new lesion is identified.

19. irRC and RECIST should be done prior to surgery. Only RECIST will be used as clinical response criteria and used to make treatment decisions

9 CRITERIA FOR EVALUATION AND ENDPOINT

9.1 Pathological response

The primary objective of this study will be to identify the pathologic response rate at surgery, including complete response, after 12 weeks of neoadjuvant treatment with nivolumab and HF10. Pathologic response rate will be defined based on the quantity of residual viable tumor in the surgical resection specimen at the time of definitive surgery. A percent viable tumor will be assessed semi-quantitatively in the pre treatment biopsy, on study biopsy at day 42 and the definitive surgical resection specimen by estimating the proportion of residual tumor in relation to the total tumor area and reported as percentage viability (Ribero et al 2007) as per institutional practice. A pathologic complete response will be defined as no viable residual melanoma cells in the surgical specimen. A major pathologic response will be defined as <50% viable tumor cells. A minor pathologic response will be defined as 50% or greater viable tumor cells, including specimens that have 100% viability at surgery. Pathologic response rate (pRR) will be defined as frequency of either pCR or a pathologic major response. This will be compared to the viablitiy values of the tumor at baseline. Biopsies will be required at Day 0 and at surgery to perform this analysis. A patient must complete the neoadjuvant phase of the trial and complete surgery to be considered evaluable for pathological response.

9.2 Safety

Routine safety and tolerability will be evaluated from the results of reported signs and symptoms, scheduled physical examinations, vital sign measurements, and clinical laboratory test results. More frequent safety evaluations may be performed if clinically indicated or at the discretion of the investigator.

Physical Examination

Complete and symptom-directed physical examinations will be performed by a licensed physician (or physician's assistant or nurse practitioner).

Vital Signs

Vital signs will include (blood pressure, respiratory rate, pulse rate and temperature).

Safety Laboratory Determinations

Laboratory evaluations will be performed as noted in the study calendar.

9.3 Recurrence Free Survival

Secondary objectives for this study include recurrence free survival, which is defined as the time from complete surgical resection to the date of the first confirmation of

disease reoccurance or death (whichever comes first). Progressive disease will be based on irRC response criteria.

9.4 Neo-Adjuvant Therapy Tumor Response

Clincal (radiographic or physical exam) Response to neo-adjuvant therapy will be assessed by RECIST 1.1. The following definitions and criteria should be used for the baseline evaluations of existing disease, and for the ongoing evaluation of tumor responses prior to surgery.

Measurable lesions - lesions that can be accurately measured in at least one dimension with longest diameter (LD) ≥ 10 mm using CT, MRI, or caliper or ruler measurements or ≥20 mm with x-ray. A lymph node must be ≥15 mm in short axis when assessed by CT scan

Non-measurable lesions - all other lesions including small lesions (LD < 10 mm with CT, MRI, or caliper measurements or <20 mm with x-ray).

Documentation of "Target" and "Non-Target" Lesions

- All measurable lesions up to a maximum of two lesions per organ and five lesions in total, representative of all involved organs should be identified as ***target lesions*** and recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinical assessments).
- A sum of the LD for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as the reference by which to characterize the objective tumor response.
- All other lesions (or sites of disease) should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria:

	Evaluation of target lesions
Complete Response (CR)	Disappearance of all target lesions (Must persist for a minimum of four weeks)
Partial Response (PR)	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD (Must persist for a minimum of four weeks)
Progressive Disease (PD)	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

	Evaluation of non-target lesions
Complete Response (CR)	Disappearance of all non-target lesions
Stable Disease (SD)	Persistence of one or more non-target lesion(s)
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Evaluation of Best Overall Response

The best overall response is the best response observed until progression/recurrence and is determined as indicated in the table below:

Target Lesions	**Non-Target Lesions**	**Evaluation of New Lesions**	**Best Overall Response**
CR	CR	No	CR
CR	SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

9.5 irRC and RECIST 1.1 Concordance

Exploratory objectives for this study include review of the concordance between irRC and RECIST 1.1 response assessments. Both irRC and RECIST 1.1 response assessments will be performed at all timepoints prior to surgery.

Immune-Related Response Criteria (irRC)

Clinical response assessments will be performed according to irRC (Wolchok, 2009)

Table 1: Overall Response using irRC:

Response Assessment	**Description**
irCR	Complete disappearance of all lesions (whether measurable or not, and no new lesions) Confirmation by repeat, consecutive assessment ≥4 weeks after first documentation.
irPR	Decrease in tumor burden ≥50% relative to baseline Confirmed by a consecutive assessment ≥4 weeks after first documentation.
irSD	Not meeting criteria for irCR or irPR, in absence of irPD. A change in tumor burden of neither 50% decrease from baseline nor 25% increase from nadir can be determined.
irPD	Increase in tumor burden ≥25% relative to nadir (minimum recorded tumor burden) Confirmation by a repeat, consecutive assessment ≥4 weeks after the first documentation.

Abbreviations: CR= Complete Response; irRC= Immune-Related Response Criteria; PD= Progressive Disease; PR=Partial Response; SD=Stable Disease

Table 2: Derivation of irRC overall responses

Measurable Response	Non-measurable response		Overall irRC Response
Target and new, measurable lesions % change in tumor burden	Non-target lesions	New non-measurable lesions	
↓ 100[1]	Absent	Absent	irCR[3]
↓ 100[1]	Stable	Any	irPR[3]
↓ 100[1]	Unequivocal progression	Any	irPR[3]
↓ ≥50[1]	Absent or Stable	Any	irPR[3]
↓ ≥50[1]	Unequivocal progression	Any	irPR[3]

↓ ≥50 to ↑ <25[1]	Absent or Stable	Any	irSD
↓ ≥50 to ↑ <25[1]	Unequivocal Progression	Any	irSD
↑ ≥25[2]	Any	Any	irPD[3]

[1] Decrease relative to baseline

[2] Increase relative to nadir

[3] confirmed by consecutive scan ≥4 weeks after first documentation

9.6 Overall Survival

Patient survival will be collected for up to one year following the EOT visit. Survival will be calculated by the Kaplan-Meier method.

9.7 Correlative Biomarker Analysis

Tumor and blood samples will be assessed for response and immune activation using: histopathology including immunohistochemistry, flow cytometry, NanoString, Multispectral analysis. Analysis will include: tumor response/viability, cytokine profile changes, T-cell population changes, and RNA level changes.

9.8 Complete Surgical Resection

Patients will be assessed at surgery to determine if complete surgical resection was achievable after neo-adjuvant treatment with nivolumab and HF10. Completeness of surgical resection will be defined by standard definitions as follows: R0 indicates all gross clinical disease resected with microscopic pathologic negative margins; R1 indicates all gross clinical disease resected, but microscopic pathologic margins are positive for residual disease; R2 indicates inability to completely excise all clinical disease (i.e. gross residual tumor unable to be removed at surgery).

9.9 Pathology

Archival Tissue: Archival tissue from tumor of lymph node will be used to confirm disease pathology and next generation sequencing per standard of care practice.

9.10 Stopping Rules

If two subjects experience a grade 4 or 5 toxicity thought to be at least possibly related to the study products the study will be paused. Enrollment will be closed until the safety issue is discussed with the medical monitor and DSMC. Enrollment cannot reopen without written approval from the DSMC chair.

10 STATISTICAL CONSIDERATIONS

The primary endpoint of pathologic response including pCR is expected to be seen in 30% of patients, hence 6 of the planned 20 patients are expected to have a pathologic response, including pCR.

Pathological response will be determined by assessing the tumor viability at baseline and at surgery. Samples will be scored as a percentage viability (0-100%). A pathologic complete response (pCR) will be defined as no viable residual melanoma cells in the surgical specimen. A major pathologic response will be defined as <50% viable tumor cells. A minor pathologic response will be defined as 50% or greater viable tumor cells, including specimens that have 100% viability at surgery. Pathologic response rate (pRR) will be defined as frequency of either pCR or a major pathologic response. Analysis will also be performed of baseline tumor biopsies and on treatment biopsies if available for comparisons with the surgical resection specimen.

The null hypothesis is that the pathologic response rate for patients receiving the treatment is 5% or less. The alternative hypothesis is that the pathologic response rate is 30%. With 20 evaluable patients there will be 59% power using an exact binomial test at the one-side 0.05 significance level if the true response rate is 20%, and 89% power at one-sided alpha = 0.05 if the true response rate is 30%. Four or more pathologic responses in up to 20 patients will be sufficient to reject the null hypothesis. In additional to the statistical test, a 95% exact binomial confidence interval for the pathologic response rate will be calculated.

Kaplan-Meier methods and associated confidence intervals will be used to analyze RFS and OS. All subjects assessed for relapse will contribute to this analysis.

The statistical analysis of the safety data will be descriptive and tabular in nature.

The statistical analysis of pathologic and correlative exploratory endpoints will be descriptive. No hypothesis tests will be performed for exploratory endpoints.

11 REGISTRATION GUIDELINES

Patients must meet all of the eligibility requirements listed in Section 5 prior to registration.

Study related screening procedures can only begin once the patient has signed a consent form. Patients must not begin protocol treatment prior to registration.

Treatment should start within five working days after registration.

To register eligible patients on study, complete a Clinical Trials Office Patient Registration Form and submit to: CTORegistrations@hci.utah.edu.

12 DATA SUBMISSION SCHEDULE

The Case Report Forms (CRFs) are a set of (electronic or paper) forms for each patient that provides a record of the data generated according to the protocol. CRF's should be created prior to the study being initiated and updated (if applicable) when amendments to the protocol are IRB approved. **Data capture should be restricted to endpoints and relevant patient information required for planned manuscripts.** These forms will be completed on an on-going basis during the study. The medical records will be source of verification of the data. During the study, the CRFs will be monitored for completeness, accuracy, legibility and attention to detail by a member of the Research Compliance Office. The CRFs will be completed by the Investigator or a member of the study team as listed on the Delegation of Duties Log. The data will be reviewed no less than annually

by the Data and Safety Monitoring Committee. The Investigator will allow the Data and Safety Monitoring Committee or Research Compliance Office personnel access to the patient source documents, clinical supplies dispensing and storage area, and study documentation for the above-mentioned purpose. The Investigator further agrees to assist the site visitors in their activities.

13 SPECIAL INSTRUCTIONS – CORRELATIVE STUDIES

13.1 Tissue Samples

Fresh core tumor biopsies are mandatory at day 0 and surgery, and optional at day 42 and progression. On day 0, pre-HF10 injection, collect 2-4 core biopsies from one tumor lesion that will be injected (target lesion). If there is a second lesion available collect 2-4 core biopsies from one tumor lesion that will not be injected (non-target lesion). On day 42, surgery and progression collect 2-4 core biopsies from the same lesions as days 0 if the lesions are still present. Tumor samples analysis may include, but is not limited to:

- Tumor response/viability
- Immunohistochemistry
- Multispectral analysis

Details of tissue collection, processing and storage will be found in the Lab Manual.

13.2 Blood Samples

24 to 28 mLs of blood will be collected prior to HF10 injection at Day 0, 7, 14, 21, 28, 42, 56, 70 and 84 of the neoadjuvant treatment period and prior to nivolumab on infusion days during the adjuvant period.

Blood sample analysis may include, but is not limited to:

- Flow cytometry including; CD3+ / CD8, CD3+ / CD4+, CD56+, CD3+/CD8+/CD69+, CD3+/CD8+/(TIM3 or LAG3), CD19+
- NanoString
- Multispectral analysis
- Cytokine/chemokines including; MIP-1 alpha, IL-20, LIF, IL-1 beta, IL-2, IL-4, IL-5, IP-10, IL-6, IL-7, IL-8, IL-10, PECAM, Eotaxin, IL-12p70, IL-13, IL-17A, MCP-2, IL-1RA, SCF, RANTES, IFN-gamma, GM-CSF, TNF-alpha, HGF, MIP-1 beta, IFN-alpha, IL-9, VEGF-D, IL-16, P-Selectin, TNF-RII, BDNF, Gro-alpha/KC, IL-1 alpha, IL-15, IL-21, tPA, OPG, TSLP, PDGF-BB, VEGF-A

Details of blood sample collection, processing and storage will be found in the Lab Manual

14 ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Informed consent

Informed consent will be obtained from all research participants prior to performing any study procedures using the most recent IRB approved version.

14.2 Institutional Review

Study will be approved by the Institutional Review Board of University of Utah.

14.3 Data and Safety Monitoring Plan

A Data and Safety Monitoring Committee (DSMC) is established at Huntsman Cancer Institute (HCI) and approved by the NCI to assure the well-being of patients enrolled on Investigator Initiated Trials that do not have an outside monitoring review. Roles and responsibilities of the DSMC are set forth in the NCI approved plan. The activities of this committee include a quarterly review of adverse events including SAEs, important medical events, significant revisions or amendments to the protocol, and approval of cohort/dose escalations. If the DSMC and/or the PI have concerns about unexpected safety issues, the study will be stopped and will not be resumed until the issues are resolved. The DSMC also reviews and approves audit reports generated by the Research Compliance Office.

All **phase II** studies are reviewed by the full committee at each quarterly DSMC meeting. This includes a review of all serious adverse events (SAEs) occurring in patients treated at HCI or its affiliates as well as all grade 3 or greater toxicities for patients on treatment and within 30-day follow-up window (only if possibly, probably or definitely related).

14.4 Adverse Events / Serious Adverse Events

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 4.0 for AE and SAE reporting. An electronic copy of the CTCAE Version 4.0 can be downloaded from:

http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx

14.4.1 Adverse Events (AE)

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study drug even if the event is not considered to be related to study drug. For the purposes of this study, the terms toxicity and adverse event are used interchangeably. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

The collection of AEs will begin after the first dose of study treatment and end 30 days post the last dose of adjuvant study treatment.

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit or phone contact during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. the severity grade based on CTCAE v.4 (grade 1-5)
2. its relationship to the study drug(s) (definite, probable, possible, unlikely, not related) Relationship to both nivolumab and HF10 will both be assessed.
3. its duration (start and end dates or if continuing at final exam)
4. action taken (no action taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization)
5. whether it constitutes an SAE

All adverse events will be treated appropriately. Such treatment may include changes in study drug treatment as listed in the dose modification section of this protocol (see section 7 for guidance). Once an adverse event is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Information about common side effects already known about both Nivolumab and HF10 is described in the Drug Information (section 3) and the most recent investigator brochure. This information will be included in the patient informed consent and will be discussed with the patient during the study as needed.

All adverse events will be immediately recorded in the patient research chart.

14.4.2 Serious Adverse Event (SAE)

Information about all serious adverse events will be collected and recorded. A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity

- is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
- causes congenital anomaly or birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
  - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (procedures such as central line placements, paracentesis, pain control)
  - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
  - 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

Following the subject's written consent to participate, all SAEs must be collected. SAEs will be followed after consent for 90 days post adjuvant nivolumab treatment, or until the patient starts a new a treatment.

Any death from any cause while a patient is receiving treatment on this protocol or up to 30 days after the last dose of protocol treatment, or any death which occurs more than 30 days after protocol treatment has ended but which is felt to be treatment related, must be reported.

Toxicities which fall within the definitions listed above must be reported as an SAE regardless if they are felt to be treatment related or not. Toxicities unrelated to treatment that do NOT fall within the definitions above, must simply be documented as AEs in the patient research chart.

14.5 SAE Reporting Requirements

SAEs must be reported to the DSMC, the FDA, the IRB, BMS and Takara, according to the requirements described below:

A MedWatch 3500A form must be completed and submitted to compliance@hci.utah.edu within 1 business day of first knowledge or notification of event.

DSMC Notifications:

- An HCI Research Compliance Officer (RCO) will process and submit the MedWatch form to the proper DSMC member as necessary for each individual study.
- The RCO will summarize and present all reported SAEs according to the Data

and Safety Monitoring Plan at the quarterly DSMC meeting.

For multisite studies the HCI DSMC will notify all participating sites of all unexpected and related SAEs via the Research Compliance Office. The RCO will also notify all investigators at remote clinical sites participating in a multisite trial of any other safety update, including external safety reports, manufacturer's reports and updates to the investigator's brochure.

FDA Notifications:

- Adverse events occurring during the course of a clinical study that meet the following criteria will be promptly reported to the FDA:
- Serious
- Unexpected
- Definitely, Probably or Possibly Related to the investigational drug
- Fatal or life-threatening events that meet the criteria above will be reported within 7 calendar days after first knowledge of the event by the investigator; followed by as complete a report as possible within 8 additional calendar days.
- All other events that meet the criteria above will be reported within 15 calendar days after first knowledge of the event by the investigator.
- The RCO will review the MedWatch report for completeness, accuracy and applicability to the regulatory reporting requirements.
- The RCO will ensure the complete, accurate and timely reporting of the event to the FDA.
- The Regulatory Coordinator will submit the report as an amendment to the IND application.
- All other adverse events and safety information not requiring expedited reporting that occur or are collected during the course of the study will be summarized and reported to the FDA through the IND Annual Report.

IRB Notification:

- Events meeting the University of Utah IRB reporting requirements (http://www.research.utah.edu/irb/) will be submitted through the IRB's electronic reporting system within 10 working days.
- External sites should abide by local IRB requirements for submission of SAEs

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

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: 609-818-3804

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

Adverse events classified as serious require expeditious handling and reporting to Takara Bio Inc. (Takara) to comply with regulatory requirements.

For any serious adverse event (SAE) that occurs while a patient is on-study; within 30 days of the last study drug administration, regardless of any opinion as to the relationship of the SAE to the study drug; or if any SAE that the Investigator feels is related to the study drug occurs later than 30 days after the last study drug administration, the investigator must notify Takara's Safety Team immediately (within 24 hours of becoming aware of the event) by fax or email. Notification by email is preferred. The fax number and the email address are listed below.

All SAEs require that a Serious Adverse Event Report Form be completed and forwarded either via fax or as a PDF via email to Takara at the fax number or email listed below within 24 hours of becoming aware of the event.

SAEs will be reported to: Takara Safety Team.

Email: trial1ae1info@takara-bio.co.jp

FAX: 077-567-9265

All SAEs should be followed to resolution or stabilization.

14.6 **Reporting of Pregnancy**

Although pregnancy is not considered an adverse event, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject, including the pregnancy of a male subjects' female partner as an SAE. Pregnancies or lactation that occurs during the course of the trial or with 30 days of completing the trial or starting another new anticancer therapy, whichever is earlier, must be reported to the DSMC, IRB, FDA, and the sponsor as applicable. All subjects and female partners who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events.

14.7 **Protocol Amendments**

Any amendments or administrative changes in the research protocol during the period, for which the IRB approval has already been given, will not be initiated without submission of an amendment for IRB review and approval.

These requirements for approval will in no way prevent any immediate action from being taken by the investigator in the interests of preserving the safety of all patients included in the trial.

Any amendments to the protocol that significantly affect the safety of subjects, scope of the investigation, or the scientific quality of the study are required to submit the amendment for FDA review.

14.8 Protocol Deviations

A protocol deviation (or violation) is any departure from the defined procedures and treatment plans as outlined in the protocol version submitted and previously approved by the IRB. Protocol deviations have the potential to place participants at risk and can also undermine the scientific integrity of the study thus jeopardizing the justification for the research. Protocol deviations are unplanned and unintentional events.

Because some protocol deviations pose no conceivable threat to participant safety or scientific integrity, reporting is left to the discretion of the PI within the context of the guidelines below. The IRB requires the **prompt reporting** of protocol deviations which are:

- Exceptions to eligibility criteria.
- Intended to eliminate apparent immediate hazard to a research participant or
- Harmful (caused harm to participants or others, or place them at increased risk of harm - including physical, psychological, economic, or social harm), or
- Possible serious or continued noncompliance

14.9 FDA Annual Reporting

An annual progress report will be submitted to the FDA within 60 days of the anniversary of the date that the IND went into effect. (21 CFR 312.33).

14.10 Clinical Trials Data Bank

The study will be registered on **http://clinicaltrials.gov** and the NCI CTRP (Clinical Trials Reporting Program) by the Clinical Trials Office.

14.11 Record Keeping

Per 21 CFR 312.57, Investigator records shall be maintained for a period of 2 years following the date a marketing application is approved; or, if no application is filed or the application is not approved, until 2 years after the investigation is discontinued and the FDA is notified.

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16 APPENDIX

APPENDIX I: PATIENT INFORMATION SHEET: MINIMIZING VIRUS EXPOSURE TO OTHERS

HF10 is an antitumor medicine in which the active component is a strain of a live virus called Herpes Simplex Virus Type 1 (HSV-1). HSV-1 is very widespread in the environment. In the United States, many people have been exposed to HSV. The HF10 virus used in the study drug is a weakened strain of the "live virus", much like many vaccines children receive. Therefore, exposure to the virus may pose a risk of infection to other people, particularly those with decreased immunity (see list below). If an infection occurs, the most common signs are:

- Fever
- Flu-like symptoms (e.g., chills, fatigue, or headache)
- Nausea, vomiting, or diarrhea

This information sheet addresses questions that some patients may have regarding risks that the virus may pose to people with whom they come in contact. Previous clinical studies with HF10 suggested that the virus poses minimal health risk. HF10 has been studied in three Phase I trials in Japan, in patients with recurrent breast cancer, head and neck cancer, and inoperable pancreatic cancer. The only side effect reported was low-grade fever in two patients in the head and neck cancer study.

The association of HF10 with any human illness has not been established. However, given the investigational nature of this treatment, your physicians believe that it is appropriate to take some precautions to minimize the exposure to HF10 in persons with whom you have close contact. After the administration of HF10, the virus may be present in your saliva and/or other body fluids (e.g., blood or urine). This can begin within a few hours of the treatment and may persist for several days. It is currently not known if, or for how long, HF10 may be found in these fluids following your treatment.

Therefore, your physicians believe it is wise for you and your family to take steps to reduce their exposure to HF10, and especially, to minimize exposure to persons beyond your immediate family. For one week after each injection, you should carry out the following precautions to minimize exposure of others to HF10:

- Stay at home as much as possible for the first five days after receiving your HF10 injection. When you are around others, including members of your family, please wear a mask, provided by the clinic.
- Avoid close contact with possible exposure to body fluids (e.g., kissing and sexual activity) to minimize exposure to your partner. It is also important that patients (or their partners) avoid becoming pregnant during this time.
- If possible, arrange to sleep in a room separate from others.
- Do not share towels or eating utensils with others.
- Isolate your toothbrush from those of others.
- Wash your hands frequently, especially after coughing, blowing your nose, or using a

restroom.

• If a surface comes in contact with body fluids, the area should be cleaned using chlorine bleach.

• As much as possible, avoid public transportation and crowds; be sure to wear a mask when out in public.

• Avoid contact with persons who may have decreased immunity, such as:

- Children 6 years old or younger
- Elderly persons
- Anyone who has had an organ or tissue transplant
- Anyone with AIDS or HIV infection
- Other cancer patients.

• Be sure to wear a mask when visiting the treatment clinic.

You may need to continue to carry out these precautions based on the results of laboratory testing of your saliva, blood, and urine for the detection of virus DNA. If you have any questions or concerns about any of the precautions listed above, please ask your healthcare providers.