Masonic Cancer Center
University of Minnesota

A Phase I Study of HCW9218, a Bifunctional TGF-β Antagonist/IL-15 Protein Complex, in Select Advanced Solid Tumors After Failing at Least Two Prior Therapies

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Revision History

Revision #	Version Date	Summary Of Changes	Consent Changes
	Dec 16 2021	Original to the FDA	n/a
	Jan 12 2022	Full response to FDA clinical deficiencies and non-hold comments received Jan 11 2022. Other minors edits for consistency including Section 13.1 Original to CPRC	yes
	Feb 4 2022	Edits and clarifications since the CCPM, original to IRB • Title page – add Qing Cao as the study statistician replacing Todd DeFor who has left the University • Synopsis and Section 1.3 – delete correlative objective to characterize blood cDNA, cytokine, chemokine and inflammatory marker levels (should have been deleted when a study expansion cohort was deleted prior to FDA submission) • Synopsis Study Design and Enrollment Plan reorganized for clarity (not tracked but available upon request) • Synopsis and Section 4.1.2 – only permit persons with measurable disease to be enrolled (delete evaluable as not relevant in RECIST 1.1) – add Schwartz 2016 to references • Synopsis and Section 4.1 – delete inclusion criteria regarding prior RT to target lesion – move content to Section 4.1.2 (Section 4.2.5 of exclusion already addresses no prior RT in previous 14 days) • Synopsis and Section 4.1 – delete life expectancy of at least 12 weeks as too subjective - ECOG PS of 0 or 1 is required eligibility • Synopsis and Section 4.2. 9 – after querying intent with HCW Biologics, rewrote for clarity as intent is to prohibit St. John's wort and and/or other herbal CYP modulators • Synopsis and Section 4.1 – other minor edits to inclusion criteria for clarity • Schema and Section 6.3 – add any patient experiencing a DLT at any time is discontinued from study treatment, delete last line regarding DLT events only reported in the dose finding phase (should have been deleted when a study expansion cohort was deleted prior to FDA submission) • Synopsis and Section 6.3 – removed language in parentheses (due to treatment related re-distribution of immune cells) as felt undefinable per study clinical staff upon review • Section 4.2.3 and Section 6.1.1 – expand language around cardiac function and monitoring, add a footnote to table in Section 8.1 • Section 4.2.6 – correct wording regarding prior malignancy to reflect schema • Section 5.2 – add language that if a patient does not start study treatment within 14 days of baseline bloodwork for eligibility they must be repeated and continue to meet criteria set in Section 4.1.6. • Section 6.1 and Section 9.4 – HCW9218 is administered as 2-3 injections not 2-4 injections • Section 6.1 – simplify 1st bullet under may delay HCW9218 for 1 week to read ongoing Grade 3 toxicity that is not a DLT – any occurrence of a DLT at any time permanently discontinues HCW9218 • Section 6.1.2 – reiterate pre/post meds are as needed (prn). • Section 6.1.3 – add a reminder that during Cycle 1 and Cycle 2 Day 1 a targeted toxicity worksheet is completed per the schedule in Section 10.2.2.	yes

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		• Section 6.1.4 – expand (repeat) dose delay language from Section 6.1 and add a participant is discontinue from treatment if experiences a DLT at any time. • Section 6.4 (supportive care) – move language for management of injection site reaction from Section 7.2 • Section 6.5 (prohibited concomitant therapy), Section 6.6 (duration of treatment), Section 6.7 (duration of study participation – expand sections for clarity and content as included in other sections of the protocol • Section 7.2 – relabel as Most Common and Most Serious Side Effects Seen in Drugs Similar to HWE9218 and expand the section to include the AE events in the consent form sorted by common, occasional and rare. Reiterate that the Table in Appendix II should continue to be used when assigning for event documentation and reporting requirements. • Section 8 – further define acceptable windows, move all lab tests and ECG charged to research to the research activities table (Section 8.2), rework footnotes in both tables. • Section 9.7 – potential side effects – rework section referring reader to Section 7.2 and Appendix II. • Section 10 – Copy language from Section 10.2 to introduction regarding the AE monitoring period. • Section 10.2 – make two new sub sections (exceptions to documentation and targeted toxicity) to allow hyperlinking from other protocol sections. edit table for clarity • Section 10.3 – update table for clarity • Section 11.1 – delete reference to REDCap as will not be used. • Section 12.2 – add “In a 3 patient cohort at least 14 days must separate the 1st and 2nd patients with no staggering required between the 2nd and 3rd patient. A minimum of 21 days must separate each 3 patient cohort.” • Section 14.1 – add protocol deviations must be report per Section 10.3 • Appendix IV – delete pruritus and rash rows as skin covered by other skin/subcutaneous	
1	October 4 2022	• Schema and Section 4.1.6 inclusion criteria – require adequate organ function within 14 days prior to “Cycle 1 Day 1” rather than “study enrollment” • Synopsis and Section 6 – edit the DLT regarding inability to start Cycle 2 due to treatment related toxicity • Synopsis, Section 6.1 - Clarify weight calculation as done on weight taken Cycle 1 Day 1 or within 14 days prior as currently uses vague term of baseline weight • Section 6.1 - add details of how and when to recalculate the HCW dose based on a ≥10% weight change from the weight used for Cycle 1 Day 1. If a 2nd dose recalculation is needed patient can either continue treatment (if weight is returning to Cycle 1 Day 1 weight) or discontinue treatment as a ≥20% weight change is a Grade 3 toxicity. • Section 6.1.4, Section 6.6 – Removed references from original protocol language that a patient who experiences a DLT or unable to receive treatment after a 1 week delay is taken off study. • Section 6.3 – Expand Definition of Dose Limiting Toxicity section and remind reader about fast track pre-defined events that do not meet the definition of DLT but trigger a change in the enrollment plan and remind the reader of monitoring for excessive toxicity by the study statistician regular review of DLT reported events. • Section 6.3, Section 8 - clarify the window for Cycle 2 Day 1 is +3 days and that “the DLT period does not end until the patient is	yes

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		assessed on Cycle 2 Day 1 (+3 days) and the results of lab work drawn on Cycle 2 Day 1 (+3 days) confirms no new DLTs since the previous assessment and/or no ongoing DLT events". (After cycle 2, the window for Day 1 is ±3 days.) • Section 6.4 - Permit palliative radiation therapy to non-target lesions (as defined by RECIST) • Section 8.1 SOC activities - add a row for eligibility assessment to prompt completion of the relevant checklist/eCRF, expand footnote one to permit consent signing at any time prior to the start of study related activities, permit disease assessments to be done within 42 days of Cycle 1 Day 1 if not intervening anti-cancer therapy. Add x's to the vitals, assessment and other activities during Cycles 1 and 2 as not otherwise performed in CRU. Increased window for imaging studies from ± 1 week to ± 14 days. • Section 8.2 – Update schedule of research sample collection and tube type to match the administrative letter dated March 2022 previously submitted to the IRB. Added language to optional biopsy footnote to indicate it is exempt from 28 day limit. Other minor edits and clarifications including: • Synopsis, Schema, Section 3 Expand HCW9218 is administered by subcutaneous injection in the outpatient setting once every 3 weeks for a minimum of 2 cycles adding unless medically contraindicated or patient refusal – a minimum of 2 cycles is felt needed to see any effect, but study uses intent-to-treat for efficacy and toxicity. • Synopsis - Expanded exclusion criteria regarding use of St John's Wort or other herbals CYP modulators to match Section 4.29 - Replace "courses" with "cycles" for consistency through-out the protocol • Synopsis, Section 6.6 Section 8 – End of Treatment visit – add flexibility to the EOT visit in the event decision to stop treatment occurs outside of the window from last dose and/or patient isn't returning to the U. • Section 8.2 - add reference to the HCW provided IIT manual for sample collection, processing and shipping. • Section 9 - update HCW information based on the IIT manual provided by HCW • Section 10.2.2 - add a Day 2 assessment, but otherwise reduce the frequency for completion of the targeted toxicity worksheet to end at Day 5 • Previous Section 10.3 regarding reporting death due to disease as an SAE as QA suggests adding a CRF for death reporting (date and cause) • Reformat the definition of DLT into table format for ease of reading • Synopsis - Refer to the study agent as an investigational product (IP), add IP to key abbreviations table. Other updates to match the MCC protocol template	
2	February 14 2023	In response to IRB stipulations • Synopsis and Schema – remove remaining references that a participant is discontinued from treatment if they experience a DLT at any time.	no
3	March 29 2023	• Synopsis, and Sections 4.2 and 6.5 – Added an exception for use of prednisone in persons with CT contrast allergies as it is necessary for persons with CT contrast allergies to receive high-dose prednisone prior to a CT.	yes

Revision #	Version Date	Summary Of Changes	Consent Changes
		• Synopsis, Section 13 – Removed language requiring 14 days between the 1st and 2nd patients in a cohort of 3 patients as no DLTs have been observed from dose level 1 to dose level 4. • Sections 4.1.2 and 8.2 (footnote 10) – Added language to indicate if a biopsy is obtained prior to treatment start (any time after consent) and a patient is later deemed ineligible to receive treatment, the tumor sample will not be used for research purposes since the sample cannot determine the effect of HCW9218. • Section 8.2 – Updated text in footnotes 5-8 regarding the 3 hour post dose blood draw window to read “30 minutes” instead of “0.5 hour” to be consistent with how all other windows are written throughout the protocol. Removed blood volume and tube type content as that is captured in the study lab manual.	

Table of Contents

Key Abbreviations

Acronym	Definition
ADCC	antibody-dependent cellular cytotoxicity
ADL	activities of daily living
AE	adverse event
AGC	absolute granulocyte count
AIDS	acquired immunodeficiency syndrome
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ALT-803	IL-15 Mutein/IL-15RαSu-Fc Complex
ANC	absolute neutrophil count
AST	aspartate aminotransferase
ATPP	activated partial thromboplastin time
BUN	blood urea nitrogen
C1D1	Cycle 1 Day 1
C2D1	Cycle 2 Day 1
CBC	complete blood count
CMP	complete metabolic panel
CNS	central nervous system
CR	complete response
CRF	Code of Federal Regulations
CRF/eCRF	case report form / electronic case report form
CRP	C Reactive Protein
CT	CT Computed Tomography
dL	decaliter
DL	dose level
DLT	dose limiting toxicity
DNA	deoxyribonucleic acid
DOR	duration of response
DSMP	data and safety monitoring plan
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
FACT-G	The Functional Assessment of Cancer Therapy - General
FDA	Food and Drug Administration
FEV1	forced expiratory volume
FFPE	formalin-fixed, paraffin embedded
GCP	Good Clinical Practice
G-CSF	granulocyte-colony stimulating factor
GFR	glomerular filtration rate
GLP	good laboratory practice
GM-CSF	granulocyte-macrophage colony-stimulating factor
HCW9228	TGF-β Antagonist/IL-15-Null Protein Complex
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IFN	interferon
IL	interleukin
IL-15RαSu	Soluble (Sushi) Domain of IL-15 Receptor α
IP	Investigational Product

Acronym	Definition
IRB	Institutional Review Board
IV or i.v.	intravenous
kg	kilogram
L	liters
mg	milligram
MI	myocardial infraction
Min	minute
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NK	natural killer
ORR	objective response rate
OS	overall survival
PBS	phosphate buffered saline
PD	progressive disease
PET	positron emission tomography
PFS	progression free survival
PFT	pulmonary function test
PK	pharmacokinetic
PR	partial response
PS	performance status
QOL	Quality of Life
RECIST	Response Evaluation Criteria in Solid Tumors Committee
S/I	Sponsor/Investigator
SAE	Serious Adverse Event
SC, s.c. or SQ	subcutaneous
SD	stable disease
SOC	standard of care
SOP	standard operating procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TGF	transforming growth factor
TIS	therapy induced senescence
TME	tumor microenvironment
TNF	tumor necrosis factor
TTP	time to progression
ULN	upper limit of normal
UMN	University of Minnesota

Study Synopsis

A Phase I Study of HCW9218, a Bifunctional TGF-β Antagonist/IL-15 Protein Complex, in Select Advanced Solid Tumors After Failing at Least Two Prior Therapies

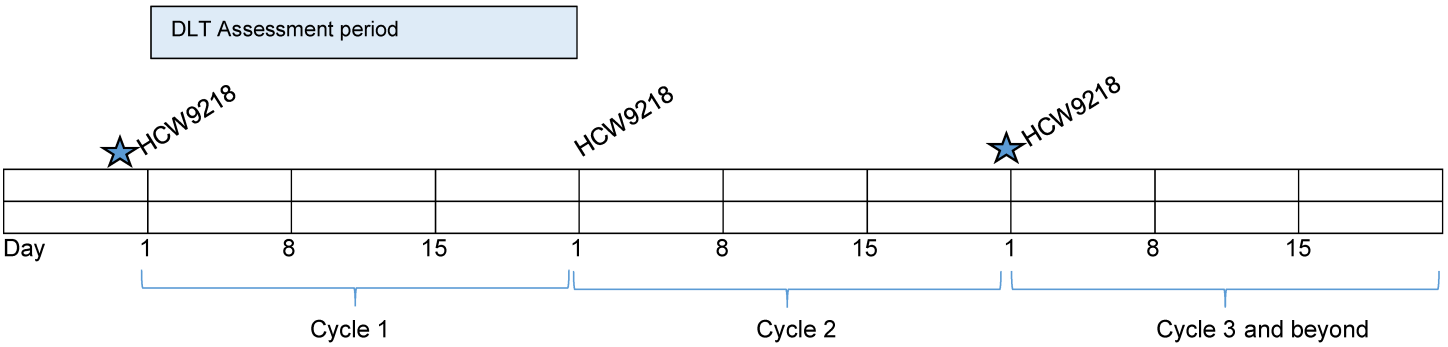
Study Overview:	This is a single center, Phase I dose finding study of HCW9218 for the treatment of select advanced solid tumor cancers, including, but not limited to breast, ovarian, prostate and colorectal. Pancreatic cancer is excluded due to a competing HCW9218 protocol. Primary brain tumors are not eligible. HCW9218 is a novel bi-functional fusion protein complex administered by subcutaneous (SC) injection. It is comprised of a soluble fusion of two human TGFβRII domains, human tissue factor, and human IL-15, and a second soluble fusion of two human TGFβRII domains and a sushi domain of human IL-15Rα. HCW9218 activates IL-15R signaling on effector immune cells and the dimeric TGFβRII functions as a "trap" for all three human TGF-β isoforms. HCW9218 is administered by subcutaneous injection in the outpatient setting once every 3 weeks for a minimum of two cycles unless medically contraindicated or the participant wishes to discontinue. Treatment may continue until disease progression, unacceptable toxicity, patient refusal, or treatment is felt to be no longer of benefit. Patients are followed for 1 year from the 1st dose of HCW9218 to determine progression free survival (PFS) and overall survival (OS). The primary analysis will be intent-to-treat from the point of 1st injection of HCW9218 both for toxicity and efficacy. Up to 5 dose levels of HCW9218 will be tested (including a safety (-1) level in the event of unexpected toxicity) to identify the maximum tolerated dose (MTD) with a small expansion cohort of the MTD or Dose Level 4 if no dose limiting toxicity is encountered.
Investigational Product (IP):	HCW9218 provided by HCW Biologics, Inc.
Study Rationale:	HCW9218 potently activates natural killer (NK) cells and $CD8^+$ T cells in vitro and in vivo to promote their proliferative and metabolic activities and enhance their cytotoxicity against tumor targets. The fusion complex also exhibits TGF-β neutralizing activity in vitro and sequesters plasma TGF-β in vivo. It is hypothesized that HCW9218 may serve as a novel therapeutic to simultaneously provide immunostimulation and lessen immunosuppression associated with solid tumors.
Primary Objective:	The primary objective of this study is to determine the maximum tolerated dose (MTD) of HCW9218 as monotherapy in advanced solid tumor cancers except pancreatic cancer and primary brain tumors.
Secondary Objectives:	Secondary objectives are: • Determine safety and feasibility of repeat dosing of HCW9218 as outlined by incidence of adverse events (AEs) based on CTCAE v5. • Estimate response rate at 3 months, 6 months and 12 months, progression-free survival (PFS) and overall survival (OS) at 6 months and 1 year from 1st dose of HCW9218.
Correlative Objectives:	Correlative objectives include: • Characterize the immunogenicity and pharmacokinetic (PK) profiles of HCW9218 • Evaluate lymphocyte number, phenotype and function • Flow cytometry analysis to include surface markers that define lymphocyte subsets (NK, NKT, B, and T cells, both CD4 and CD8), as well as intracellular markers that define regulatory T cells (Foxp3) and proliferating cells (Ki67) • Immunohistochemistry analysis and genomic, transcriptomic and proteomic molecular profiling on available tumor biopsies • Evaluate effect on quality of life using patient completed FACT-G and skin condition questionnaires

<table>
<tr><td>Treatment Overview:</td><td>Upon signing of consent and confirmation of eligibility, the patient is assigned to the currently enrolling dose level of HCW9218.
HCW9218 at the assigned dose level is administered as a subcutaneous injection once every 3 weeks for a minimum of 2 treatment cycles unless medically contraindicated or participant wishes to discontinue. Patients may continue until disease progression, unacceptable toxicity (an AE meeting the definition of a DLT) or other reason detailed in Section 6.6.
Dosing is initially calculated on the patient's weight taken on Cycle 1 Day 1 or within 14 days prior to Cycle 1 Day 1. The dose is recalculated in subsequent cycles in the event of a weight change of ±10% or greater.
An End of Treatment (EOT) visit occurs approximately 3 weeks after the last dose of HCW9218; however refer to Section 6.6 for exceptions. The patient transitions to the study required follow-up for disease response (if discontinued treatment for a reason other than disease progression) and survival for 1 year from the 1st dose of HCW9218.</td></tr>
<tr><td>Study Design:</td><td>
<table>
<tr><th>Dose Level (DL) Cohort</th><th>HCW9218 Dose (mg/kg)</th><th>Enrollment Plan</th></tr>
<tr><td>-1</td><td>0.1</td><td rowspan="5">Administer HCW9218 as monotherapy at assigned dose by SC injection once every 3 weeks.
All patients are assessed for AEs and dose limiting toxicities (DLT). A minimum of 21 days must separate each dose cohort.
Refer to enrollment plan below.</td></tr>
<tr><td>1 (start)</td><td>0.25</td></tr>
<tr><td>2</td><td>0.5</td></tr>
<tr><td>3</td><td>0.8</td></tr>
<tr><td>4</td><td>1.2</td></tr>
</table>
</td></tr>
<tr><td>Enrollment Plan:</td><td>Dose limiting Toxicity (DLT) is defined in the Schema and Section 6.3.
STAGE 1 STEP 1 Fast-track design (1 patient per dose level)
Start at DL1, enroll 1 patient per DL separated by a minimum of 21 days (DLT period) until the:
• 1st pre-defined adverse event defined as any of the following treatment emergent Grade 2 events lasting >72 hours within 5 days of 1st dose of HCW9218: fatigue, flu-like symptoms or injection site reaction
• and the patient completes the 21 day DLT period with no DLT- Activate Stage 1 Step 2
OR
• 1st DLT event within 21 days after the 1st dose of HCW9218 as defined in the Schema and in Section 6.3 - Activate Stage 2 (Stage 1 Step 2 is not used)
OR
• 9 patients are treated at the DL4 (This completes enrollment - Stage 1 Step 2 and Stage 2 are not used)
STAGE 1 STEP 2 Expand current DL and subsequent DLs to 3 patients
The cohort size increases from 1 to 3 patients with 2 additional patients added to the current cohort. A minimum of 21 days must separate each dose cohort. Continue dose escalation until:
• 1st DLT event as defined in the Schema and Section 6.3 - Activate Stage 2
OR
• 9 patients are treated at the DL4 (This completes enrollment and Stage 2 is not used.)
STAGE 2 Continual Reassessment Method (CRM)
The study design changes to an application of the continual reassessment method (CRM). Enrollment occurs in cohorts of 3. Within a 3 patient dose cohort, a minimum of 14 days must separate the 1st and 2nd patient with no staggering of enrollment required between and 2nd and 3rd patient. Once the 3rd patient in a cohort reaches Day 21 (DLT period), a new cohort of 3 patients is sequentially assigned to the most appropriate dose by the study statistician based on the updated toxicity probabilities. The goal will be to identify one of the 4 dose levels corresponding to the desired maximum toxicity rate of ≤25%. The MTD will be identified by the minimum of the following criteria: (1) the total Stage 2 sample size of 24 is exhausted or (2) 9 consecutive patients are enrolled at the same dose.</td></tr>
<tr><td>Key Inclusion Criteria:</td><td>• Histologically or cytologically confirmed advanced/metastatic solid tumor cancer (except pancreatic and primary brain cancers), has failed at least 2 prior lines of therapy given either in the recurrent or metastatic setting and must be refractory to or intolerant of existing therapy(ies) known to provide clinical benefit for their condition.</td></tr>
</table>

	• Measurable disease per RECIST v 1.1. • Acute effects of any prior therapy must have resolved to baseline or Grade ≤1 NCI CTCAE v5 except for AEs not constituting a safety risk in the opinion of the enrolling Investigator. • Age 18 years or older at the time of consent. • ECOG Performance Status 0 or 1. • Evidence of adequate organ function within 14 days prior to treatment on Cycle 1 Day 1 as defined in Section 4.1.6. • Adequate pulmonary function with PFTs >50% FEV1 if symptomatic or known impairment. • Sexually active persons of child-bearing potential or persons with partners of childbearing potential must agree to use (or agree to their partners' use of) a highly effective form of contraception (refer to Section 4.1.10 for acceptable methods) for at least 28 days after the last dose of HCW9218. • Provides voluntary written consent prior to the performance of any research related activity.
Key Exclusion Criteria:	• Pregnant or breastfeeding. • History of clinically significant vascular disease, including any of the following within 6 months prior to start of study treatment: MI or unstable angina, percutaneous coronary intervention, bypass grafting, ventricular arrhythmia requiring medication, stroke or transient ischemic attack, symptomatic peripheral arterial disease. • Marked baseline prolongation of QT/QTc interval (e.g., demonstration of a QTc interval greater or equal to 470 milliseconds by Fridericia correction). • Known or suspected untreated CNS metastases. • Anti-cancer treatment including surgery, radiotherapy, chemotherapy, other immunotherapy, or investigational therapy within 14 days before treatment start. • Other prior malignancy except for the following: adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, adequately treated Stage I or II cancer from which the subject is currently in complete remission, or any other cancer from which the subject has been disease-free for 3 years after surgical treatment. • Known hypersensitivity or history of allergic reactions attributed to compounds of similar chemical or biologic composition to the agents used in the study. • Prior therapy with TGF-β antagonist, IL-15 or analogs. • Ongoing use of St. John's Wort and/or other herbal CYP modulators as certain cytokines, including IL-2 and TGF-β, have been found to suppress the activities of cytochrome P450 enzymes. The participant may be considered for the study if they discontinued the herbal CYP modulator at least 7 days before the 1st dose of HCW9218 and agree not resume it until after the EOT visit. • Known autoimmune disease requiring active treatment. Persons with a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of enrollment, with the exception of use for persons with CT contrast allergies. Inhaled or topical steroids, and adrenal replacement steroid doses ≤ 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease. • Active systemic infection requiring parenteral antibiotic therapy. All prior systemic infections must have resolved following optimal therapy. • Prior organ allograft or allogeneic transplantation. • Known HIV-positive or AIDS. • Psychiatric illness/social situations that would limit compliance with study requirements. • Other illness or a medical issue that in the opinion of the Investigator would exclude the subject from participating in this study
Enrollment Goal:	A minimum of 12 patients or a maximum of 24 if DLT is encountered.

Study Schema

1 cycle = 3 weeks (21 days) with a 2-cycle minimum unless medically contraindicated.



HCW9218 at assigned dose by subcutaneous (SC) injection once every 3 weeks until disease progression, unacceptable toxicity, patient refusal, or felt to be no longer of benefit
Assess for Dose Limiting Toxicity (DLT) at Cycle 2 Day 1 (defined below and in Section 6.3)
★ tumor biopsy (baseline and prior to Cycle 3) – optional, in participants with accessible tumor who have signed the separate biopsy consent (Tumor used for biopsy should not be a RECIST target lesions unless there are no other lesions suitable for biopsy.)

Disease reassessment per disease specific standard of care (i.e. every 8-12 weeks)

Phase I HCW9218 Dose Levels	
Dose Level (DL)	HCW9218 dose
-1*	0.1 mg/kg
1 (start)	0.25 mg/kg
2	0.5 mg/kg
3	0.8 mg/kg
4	1.2 mg/kg

* use only if excessive toxicity encountered at DL1

Dose limiting toxicity (DLT) is defined as any of the following **treatment emergent events** based on CTCAE v5 within 21 days after the 1st dose of HCW9218 as assessed on Day 22 (Cycle 2 Day 1) per Section 6.3.

DLT event	**Exceptions:**
Any Grade 4 hematologic event	○ Grade 4 lymphopenia that resolves to Grade 1 or lower (or to Baseline or lower, if pre-existing Grade 2 or higher) within 7 days ○ Grade 4 leukopenia that resolves to Grade 1 or lower (or to Baseline or lower, if pre-existing Grade 2 or higher) within 7 days
Any Grade 3 or Grade 4 febrile neutropenia	
Any Grade 3 thrombocytopenia with bleeding	
Any Grade 3 or higher non-hematologic adverse event	○ Grade 3 nausea, vomiting and/or diarrhea that last < 72 hours ○ Grade 3 or higher electrolyte abnormality that lasts < 72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical interventions ○ Isolated Grade 3 elevation of ALT and/or AST that resolves to Grade 1 or lower (or to Baseline or lower, if pre-existing Grade 2 or higher) within 7 days ○ Grade 3 amylase or lipase that is not associated with symptoms or clinical manifestations of pancreatitis ○ Grade 3 fatigue, not due to disease progression, that lasts < 1 week
Hy's Law case (i.e., alanine aminotransferase/ aspartate aminotransferase (ALT/AST) >3 x ULN in combination with total bilirubin level (TBL) > 2 x ULN in the absence of cholestatic injury (ALP < 2 x ULN (Ricart, 2017; FDA, 2009)) and no other cause identified after excluding biliary tract obstruction and liver metastasis	
Inability to receive Cycle 2 dosing due to treatment related toxicity	

Monitoring for Excessive Toxicity Stage 2 Only: A stopping rule is in place during this study to stop the trial in case there are excessive DLTs. At the end of the 21-day evaluation period after each cohort of 3 patients is enrolled, the study statistician calculates an updated posterior probability for each dose. The trial will be stopped if the posterior probability that the lowest dose is unacceptably toxic (> 25% of patients) is greater than 80%.

1 Objectives

1.1 Primary Objective

The primary objective of the dose finding component is to determine the maximum tolerated dose (MTD) of HCW9218 as monotherapy in advanced solid tumor cancers except pancreatic cancer and primary brain tumors.

1.2 Secondary Objectives

Secondary objectives of this protocol are:

- Determine safety and feasibility of repeat dosing of HCW9218 as outlined by incidence of adverse events (AEs) based on CTCAE v5.
- Estimate response rate (complete response (CR), partial response (PR) or stable disease (SD)) as a primary efficacy objective.
- Estimate progression free survival (PFS) and overall survival (OS) at 6 months and 1 year from 1st dose of HCW9218.

1.3 Correlative Objectives

Correlative objectives include:

- Characterize the immunogenicity and pharmacokinetic (PK) profiles of HCW9218.
- Evaluate lymphocyte number, phenotype, and function.
- Flow cytometry analysis to include surface markers that define lymphocyte subsets (NK, NKT, B, and T cells, both CD4 and CD8), as well as intracellular markers that define regulatory T cells (Foxp3) and proliferating cells (Ki67).
- Immunohistochemistry analysis and genomic, transcriptomic and proteomic molecular profiling on available tumor biopsies.
- Evaluate effect on quality of life using patient completed FACT-G and skin condition questionnaires.

2 Background and Rationale

2.1 Advanced Solid Tumor Cancer Control after Failure of Initial Treatments

The immunosuppressive tumor microenvironment limits the success of current immunotherapies. The response rates to immune based treatments in some solid tumors has revolutionized the way these cancers are treated. Unfortunately, in most recurrent solid tumors the response to immune based therapy remains inadequate. For example, a cross-sectional study found that the estimated percentage of US patients with cancer who are eligible for checkpoint inhibitor drugs increased from 1.54% in 2011 to 43.63% in 2018 (Hasam 2019). The percentage of patients estimated to respond to checkpoint inhibitor drugs was 0.14% in 2011 and has

increased to 12.46% in 2018 meaning that the estimated percentages of patients who are eligible for and who respond to checkpoint inhibitor drugs remain modest. This data speaks to the critical need for novel immune based therapies in recurrent solid tumors.

2.2 Investigational Product - HCW9218

2.2.1 General Information on HCW9218

HCW9218 is a soluble bifunctional protein complex comprised of dimeric extracellular domains of the human transforming growth factor beta (TGF-β) receptor II (2*TGFβRII) and human interleukin-15 (IL-15) in a complex with the sushi domain of human IL-15 receptor α (IL15RαSu). These domains are linked through a protein scaffold consisting of the truncated extracellular domain of human tissue factor (TF). HCW9218 is produced by recombinant DNA technology in Chinese Hamster Ovary (CHO) cell suspension culture (animal derived component free) and purified through a series of chromatography and viral removal/inactivation steps. Based on the amino acid sequence, HCW9218 has a calculated molecular weight of 108 kDa. Additionally, HCW9218 is a highly glycosylated protein complex (Liu et al, 2021). HCW9218 drug product is a sterile, preservative-free solution in single-use vials. Each 1 mL vial contains 20 mg of HCW9218 and is formulated in potassium phosphate monobasic (KH_2PO_4, 1.36 mg), sodium phosphate dibasic anhydrous (Na_2HPO_4, 1.43 mg), and sodium chloride (NaCl, 8.18 mg), with a pH of 7.4.

2.2.2 Summary of Nonclinical Clinical Findings

Immunotherapeutics, either as immunostimulants or inhibitors of tumor-induced immunosuppression, are promising anti-neoplastic agents and have revolutionized cancer therapy (Robert, 2020; Waldman et al, 2020; Leonard et al, 2019). Additionally, combination approaches with these agents have been demonstrated to additively or synergistically enhance antitumor activities (Wrangle et al, 2018; Fujii et al, 2018). Bifunctional fusion protein approaches, which eliminate the need for complicated multiagent treatment regimens, are also gaining considerable interest (Lan et al, 2018). HCW9218 is a bifunctional protein complex that contains TGF-β antagonist (i.e., dimeric TGFβRII) activity and IL-15/IL-15RαSu immunostimulatory activity. The proposed mechanisms-of-action of HCW9218 are to 1) activate immune effector cells through binding of the IL-15 domain to cell-expressed IL-15βγ receptors and 2) sequester (or trap) soluble immunosuppressive TGF-β isoforms via binding to the dimeric TGFβRII domains (Liu et al, 2021). As detailed in the Investigator's Brochure (IB), in vitro studies verified the binding and functional activities of the IL-15/IL-15RαSu and dimeric TGFβRII domains of HCW9218 using ELISA, surface plasmon resonance (SPR) and cell-based activity assays. Binding studies also revealed that HCW9218

interacts with both latent and active forms of TGF-β, which may represent an efficient pathway for HCW9218 to sequester TGF-β activity and to out compete membrane-associated receptors for TGF-β binding. In contrast to the activities of the IL-15/IL-15RαSu and dimeric TGFβRII domains, the TF scaffold of HCW9218 had little or no procoagulant activity in prothrombin time (PT) assays with soluble or cell-bound HCW9218 (Liu et al, 2021), consistent with previous published reports with truncated TF (Martin et al, 1995). Studies also demonstrated that HCW9218 potently activates mouse and cynomolgus monkey natural killer (NK) cells and $CD8^+$ T cells in vitro and in vivo to enhance cell proliferation, metabolism and antitumor cytotoxic activities. Similarly, human immune cells become activated with increased cytotoxicity (and antibody-dependent cellular cytotoxicity (ADCC)) against tumor cells following incubation with HCW9218. This fusion complex also exhibits TGF-β neutralizing activity in vitro and sequesters plasma TGF-β isoforms in mice and cynomolgus monkeys. Comparative studies with a dimeric TGFβRII fusion protein lacking IL-15 activity (HCW9228) indicated that both TGFβRII and IL-15 domains of HCW9218 act together to enhance stimulation of immune cells in vitro and in vitro (Liu et al, 2021). The antitumor efficacy and mechanism-of-action of HCW9218 was evaluated in C57BL/6 mice with subcutaneous (s.c.) B16F10 melanoma tumors. Subcutaneous treatment of B16F10 tumor-bearing mice with HCW9218 alone was found to significantly reduce tumor growth and prolonged survival when compared to control treatment (Liu et al, 2021). Similar antitumor activity was observed in B16F10 tumor bearing mice treated with HCW9218 in combination with the anti-TYRP-1 antibody TA99 which specifically recognizes B16F10 tumor cells. Immune cell depletion studies in B16F10 tumor-bearing mice indicated that both $CD8^+$ T cells and NK cells play a role in HCW9218-mediated activity against melanoma tumors. These results are supported by studies showing that HCW9218 treatment promoted an increase in NK cell and $CD8^+$ T cell infiltration into B16F10 tumors in vivo (Liu et al, 2021). A series of mouse tumor efficacy studies were conducted to evaluate the effects of HCW9218 in combination with chemotherapy and antibody-based therapy, specifically assessing activity against tumor and normal tissue cells that become senescent following chemotherapy (Chaturvedi et al, 2020). Initial in vitro and in vivo studies demonstrated the murine B16F10 melanoma cells and human SW1990 pancreatic tumor cells could be induced to enter senescence and secrete pro-inflammatory factors, termed the senescence associated secretory phenotype (SASP), in response to docetaxel (DTX) and gemcitabine plus nab-paclitaxel (G+A) chemotherapy, respectively. These therapy-induced senescent (TIS) tumor cells could re-enter the cell cycle with greatly enhanced invasiveness and stemness characteristics and were susceptible to HCW9218-activated NK cell cytotoxicity. HCW9218 treatment (alone or in combination with TA99 antibody)

enhanced the antitumor activity of DTX in B16F10 tumor-bearing C57BL/6 mice and eliminated TIS cells in the tumors and normal tissues. Immune cell depletion and tumor infiltration studies showed that both NK and $CD8^+$ T cells play a role in HCW9218+TA99-mediated effects on tumor growth with NK cells predominately acting to eliminate the TIS tumor cells. HCW9218-mediated immune cell infiltration into DTX treated B16F10 tumors, reduced the immunosuppression of the tumor microenvironment (TME), and enhanced the efficacy of anti-PD-L1 therapy. These findings collectively suggest that combination of HCW9218 with chemotherapy, tumor-targeted antibody, and immune checkpoint blockade therapies could promoted increased immune activity in the TME leading to effective tumor control while reducing TIS-mediated adverse effects on normal tissues. Further studies demonstrated that treatment of B6.Cg-Prkdcscid/SzJ mice (B6 SCID mice) bearing subcutaneous SW1990 human pancreatic tumors with HCW9218 alone and in combination with gemcitabine and nab-paclitaxel (G+A) chemotherapy resulted in reduced tumor burden and, in the case of the combination therapy, prolonged survival when compared to control treatment and chemotherapy alone. Thus, HCW9218 has potential as immunotherapy in combination with chemotherapy for treatment of pancreatic cancer. In vitro dose-response studies with human, cynomolgus monkey and murine immune cells indicated that HCW9218 induced similar levels of cell activation, proliferation and cytokine release for human and cynomolgus monkey cells. HCW9218 mediated responses in immune cells from C57BL/6 mice were also observed but at >2-fold higher EC50 values than human immune cells. However, HCW9218 did not induce a broad-based cytokine release or “cytokine storm” by human, cynomolgus monkey or murine immune cells. This activity is associated with severe pro-inflammatory responses observed with certain other immunotherapies. Together, the results of these studies support the use of C57BL/6 mice and cynomolgus monkeys as relevant animal models to evaluate the toxicity, pharmacodynamic and PK profiles of HCW9218. An initial non-GLP pilot dose-finding toxicity study was conducted in C57BL/6 mice treated with HCW9218 as a single (at 3, 10, 50, 100, 200, and 400 mg/kg) or two (at 3, 10, 50, and 200 mg/kg, separated by 2 weeks) s.c. doses. Mice receiving a single dose of HCW9218 at 200 and 400 mg/kg exhibited body weight loss beginning 3 days after the injection (study day (SD) 0) and reaching a nadir on SD7 before returning to pre-dose levels by SD11 post treatment. Mortality was observed at SD10 in one of 3 mice receiving 200 mg/kg HCW9218. There were no apparent treatment-mediated effects on body weight or other clinical signs in any other dose group or in mice receiving a second dose at up to 200 mg/kg two weeks after the first dose. Compared to the PBS group, mice receiving 200 mg/kg HCW9218 exhibited a 25-fold increase in WBC counts at SD7 post treatment, which remained 5-fold higher 7 days after the second 200 mg/kg HCW9218 dose (SD21). WBC subset analysis

revealed a 16-fold increase in absolute lymphocyte counts and >50-fold increases in neutrophil, monocyte, eosinophil, and basophil counts at SD7 in the 200 mg/kg HCW9218 group. These changes were not observed at lower HCW9218 dose levels but were expected as they were similar to those reported for C57BL/6 mice treated subcutaneously with IL-15/IL15Rα complexes (Liu et al, 2018). HCW9218-mediated effects were greatest 7 days after the first dose and were reduced after the second dose, consistent with previous studies showing decreased immune responses in mice following repeat dosing with IL-15/IL15Rα (Frutoso et al, 2018; Elpek et al, 2010). Based on the lack of test article-related changes on body weight, clinical signs, hematology with complete blood count, and plasma chemistry, one and two s.c. doses of HCW9218 at up to 50 mg/kg were well tolerated by C57BL/6 mice. This dose range is significantly higher than the HCW9218 dose level (3 mg/kg) shown to be immunostimulatory and efficacious in normal and tumor-bearing mice, respectively. Thus, the dose range of repeated s.c. HCW9218 administration between 3 and 100 mg/kg was selected for evaluation in a GLP toxicity study in C57BL/6 mice. In a follow-up GLP toxicity study in C57BL/6 mice, s.c. administration of HCW9218 at 5, 25, and 100 mg/kg in two doses on Day 1 and Day 15 was well tolerated. No early termination/mortality in any animal was noted. Additionally, no test article related changes were noted in clinical signs; injection site observations; ophthalmology; clinical pathology including hematology, coagulation parameters, clinical chemistry and urinalysis; and pathology including organ weights and coefficients and macroscopic and microscopic examinations. The only test article related effects were transient decreases in body weight and food consumption for the 100 mg/kg group during the first week of the dosing period. Therefore, the NOAEL (No Observed Adverse Effect Level) in this study was considered to be 100 mg/kg. Toxicokinetic (TK) analysis of C57BL/6 mice receiving a single s.c. dose of 25 mg/kg HCW9218 demonstrated a maximal serum concentration (Cmax) in males and females of 5.97 µg/mL (55 nM) and 5.63 µg/mL (52 nM) post treatment, respectively. The time to Cmax (Tmax) was 8 h for males and 16 h for females. Assuming a 50 mL/kg serum volume in mice (Davies and Morris, 1993), the average Cmax represents a serum recovery of about 1.2% for the subcutaneously administered 25 mg/kg HCW9218 dose. The terminal half-life (t1/2) of s.c. HCW9218 in mice averaged about 15 hours, the area under the concentration-time curve extrapolated to infinity (AUC0-∞) was approximately 230 h*µg/mL, the apparent clearance (Cl) was about 109 mL/h/kg and the volume of distribution (Vz) averaged about 2,350 mL/kg. No obvious gender differences in the TK parameters of HCW9218 were noted. In a GLP toxicology study in cynomolgus monkeys, s.c. administration of HCW9218 at 1, 3, and 10 mg/kg in two doses on Day 1 and Day 15 was tolerated. One male monkey treated with two doses of HCW9218 at 3 mg/kg was found dead on Day

19, mainly due to lung inflammation that was not noted for other study animals. Therefore, this death may not be test article related. There were no test article related changes in clinical signs, body weight, ophthalmology, ECG, blood pressure, or gross pathology. Test article related non-adverse changes included transient decreases in food consumption, decreases in erythroid cell, neutrophil and eosinophil parameters, albumin and albumin/globin ratio, increases in basophil, monocyte, and lymphocyte parameters and weights of spleen, spleen/body weight ratio and spleen/brain ratio, and fluctuations of platelet, reticulocyte and cytokine parameters. Dose-dependent related increases of MCP-1 and decreases of TGFβ1 and TGFβ2 in the serum were observed, which may relate to pharmacodynamics of the test article. Immunophenotyping indicated that HCW9218 induced dose-dependent increases in the percentage of Ki67 positive cells and absolute cell numbers of CD4, CD8, Treg and $CD16^{+}$ NK cells. Clinical pathological and organ weight changes may be related to inflammatory cell infiltration in adrenal gland, heart, kidney, liver, dosing site, and increased cellularity in the red pulp of the spleen and decreased lymphocytes in the cortex of the thymus. Due to the tendency of these changes to recover and the expected immunostimulatory mechanism-of-action of the test article, these changes were not considered to be adverse. Therefore, the NOAEL in this study was considered to be 10 mg/kg. Companion TK analysis of the cynomolgus monkey toxicology study showed an average Cmax after the first dose of 0.19 µg/mL (Tmax 20 h), 1.2 µg/mL (Tmax 30 h), and 3.4 µg/mL (Tmax 24 h) for 1, 3, and 10 mg/kg HCW9218, respectively. The average Cmax after the second dose was 0.24 µg/mL (Tmax 8 h), 1.8 µg/mL (Tmax 8 h), and 3.2 µg/mL at (Tmax 8 h) for 1, 3, and 10 mg/kg HCW9218, respectively. Similar to the results reported in mice, the averaged Cmax values after the first dose represent maximum serum recoveries of 0.7%, 1.5%, and 1.1% for subcutaneously administered doses of 1, 3 and 10 mg/kg, respectively, assuming a 36 mL/kg serum volume in cynomolgus monkeys (Ageyama et al, 2001). The average t1/2 values after the first dose of 1, 3, and 10 mg/kg s.c. HCW9218 were 21, 11.5, and 11.5 h, respectively. The results confirm that exposures to HCW9218 increased in a dose-dependent manner and that no apparent gender differences in the TK parameters of HCW9218 were observed. Additionally, there was no apparent accumulation of HCW9218 with repeated dosing every 14 days. While the serum Cmax levels were low, the findings were consistent with the results reported in nonclinical monkey studies and clinical trials of other IL-15 and IL-15/IL-15Rα product candidates where the Cmax observed for s.c. administration was ~200-fold less than that for the intravenous (i.v.) route and s.c. bioavailability (based on $AUC_{0\text{-}t}$) was ~3% (Margolin et al, 2018; Romee et al, 2018; Sneller et al, 2011). In these early phase clinical trials, s.c. administration was found to be advantageous in reducing i.v. IL-15-related constitutional adverse

events while maintaining immunostimulatory activity (Margolin et al, 2018; Romee et al, 2018).

2.2.3 Summary of Clinical Findings

HCW9128 has not yet been evaluated in human clinical studies. The published adverse event (AE) profiles of related immunotherapeutics, including recombinant IL-2, recombinant human IL-15 (rhIL-15), IL-15 mutein/IL-15RαSu-Fc complex (ALT-803, nogapendekin alfa inbakicept), TGFβRII-Fc fusion (AVID200), TGFβRII-anti-PD-L1 IgG1 fusion (M7824, bintrafusp alfa) and anti-TGF-β antibody (fresolimumab), are provided in Appendix II. These agents contain protein domains that are similar in structure and/or mechanism-of-action as those of HCW9218. See additional information in the IB.

2.3 Study Rationale

2.3.1 TGF-β and Cancer

TGF-β is a multifunctional cytokine that acts in a cell- and context-dependent manner as a tumor promoter or tumor suppressor. As a tumor promoter, the TGF-β pathway enhances cell proliferation, migratory invasion, metastatic spread within the tumor microenvironment and suppresses immunosurveillance (Moses 2016, Massague 2008). Collectively, the pleiotropic nature of TGF-β signaling contributes to drug resistance, tumor escape and undermines clinical response to therapy. Activation of the TGF-β signaling pathway can elicit either tumor-suppressing or tumor-promoting effects in a cell- and context-dependent manner. In normal tissues, these effects maintain homeostasis and prevent the early stages of tumor formation (Smith 2012). In cancer cells, the TGF-β signaling pathway is deregulated or mutated, and TGF-β no longer controls proliferation. As a result of mutations or epigenetic modifications introduced during cancer progression, tumors can become resistant to the suppressive effects of TGF-β signaling (Ikushima and Miyazono 2010). Based on current human TCGA datasets, esophageal, gastric colorectal (CRC), and pancreatic (PDA) adenocarcinomas contain mutations or deletions in TGF-β receptor genes in 25%–50% of cases. Head and neck, bladder, and endometrial adenocarcinomas and cervical and lung squamous carcinomas harbor such mutations in 10%–20% of cases.

TGF-β has been shown to play a major role in promoting immunosuppressive responses and fibrosis in the TME as well as inducing epithelial–mesenchymal transition (EMT) of tumor cells, which facilitates their migratory and invasive capabilities (Ciardiello et al, 2020; Principe et al, 2016). As a result, elevated serum TGF-β levels have been shown to correlate with a poor prognosis in patients with solid tumors (Huang and Blobe 2016). Early phase clinical studies have provided evidence that strategies to decrease TGF-β levels or signaling can provide clinical benefit, including objective responses and prolonged survival, in certain patients

with advanced/metastatic cancer (Lind et al, 2020; Melisi et al, 2018). Recently, several approaches have also been proposed to combine TGF-β antagonists with immunostimulatory agents, such as cytokines or immune checkpoint inhibitors, to improve infiltration and activation of effector $CD8^+$ T cells and NK cells in the TME (Lind et al, 2020; Ganesh and Massague, 2018; Ng et al, 2016; Alvarez et al, 2014). Additionally, studies in mouse tumor models support evaluation of combination therapies including chemotherapy to enhance antigen presentation by tumors, TGF-β antagonists to reduce TME immunosuppression, and immunostimulatory agents to augment antitumor T cells responses (Principe et al, 2020).

2.3.2 Rational for HCW9218-based Immunotherapy

As described above, the tumor microenvironment of solid tumor cancer patients is immunosuppressive and resistant to chemotherapy due to a variety of mechanisms (Bazzichetto et al, 2020; Binnewies et al, 2018). HCW9218 has been designed to overcome immunosuppression in the immunosuppressive effects of the TME by 1) activating and promoting tumor infiltration of immune effector cells through binding of the IL-15 domain to cell-expressed IL-15βγ receptors and 2) sequestering soluble immunosuppressive TGF-β isoforms via binding to the dimeric TGFβRII domains. As a result, HCW9218 is expected to promote antitumor immune responses in solid tumor cancer patients.

2.3.3 Rationale for the Starting Dose and Dose Range of HCW9218 in Humans

Since HCW9218 comprises immunostimulatory agonist (IL-15/IL-15RαSu domain) and TGF-β antagonist (dimeric TGFβRII domain) components, the activities of both of these components as well as the extracellular TF scaffold domain were evaluated in nonclinical studies to determine the safe starting dose and dose range for subcutaneously administered HCW9218 in the first-in-human clinical study in persons with advanced/metastatic malignancies. In calculating the safe starting dose for an immune activating product in patients with advanced pancreatic cancer, the minimum anticipated biological effect level (MABEL) approach, including considerations of receptor and ligand occupancy, the biological activity of HCW9218 in vitro and in vivo, and the toxicity and pharmacokinetic profiles of HCW9218 in relevant animal models, was used (Saber et al, 2016), and findings compared to starting and recommended phase 2 dose (RP2D) levels of other clinical-stage immunotherapeutics comprising IL-15/IL-15RαSu and TGFβRII domains. Details of this analysis are provided in the IB. Based on these considerations, a s.c. dose of 0.25 mg/kg HCW9218 is expected to be a safe starting dose for patients with advanced solid tumors. This dose is expected to have immunostimulatory activity without causing significant adverse events. The

plan is for every 3-week dosing based on ease of administration of HCW9218 in combination with other therapies which are most commonly administered on an every 3-week basis. De-escalation to 0.1 mg/kg will be carried out if excessive toxicity is observed per Section 6.2.1. HCW9218 will be evaluated in planned dose escalation to 1.2 mg/kg. The tolerability of HCW9218 in patients will be closely monitored and its dose-dependent stimulatory effects on patients' immune system will be evaluated based on correlative studies, as described in this protocol.

2.3.4 Rationale that HCW9218 Plays a Role in Therapy Induced Senescence (TIS)

Senescence is a tumor suppressor mechanism that is activated in response to stress and results in cell cycle arrest and leads to a secretory phenotype that is pro-inflammatory and tissue damaging (Zhu 2015). The accumulation of senescent cells is considered a hallmark of aging and thought to contribute to the aging pathologies. Senolytics are able to delay or attenuate age-related diseases (Baker 2015). TGF-β exerts potent growth inhibitory activities in various cell types, and multiple growth regulatory mechanisms have reportedly been linked to the phenotypes of cellular senescence and stem cell aging in previous studies. There is increasing evidence that there is a multifaceted association between TGF-β signaling and aging-associated disorders, including Alzheimer's disease, muscle atrophy, and obesity. TGF-β reportedly induces reactive oxygen species in the mitochondria in several cell types including hepatocytes and lung epithelial cells (Albright 2003, Yoon 2005). In addition, TGF-β suppresses telomerase activities by downregulating the expression of telomerase reverse transcriptase (TERT) in various cell types including rat fibroblasts and human breast cancer cells (Hu 2006, Li 2006). A recent report showed that TGF-β signaling can compromise DNA damage repair and promote senescence. This pathway contributed to cardiac aging in vivo, and the inhibition of TGF-β signaling restored H4K20me3 (an epigenetic modification to the DNA packaging protein Histone H4) and improved cardiac function in older mice (Lyu 2018). TGF-β induces the expression of inhibitory Smads (I-Smads), Smad6 and Smad7, thus incorporating negative feedback mechanisms. In bronchial epithelial cells, TGF-β causes senescence by inducing p21 (Minagawa 2011). Immune cells appear to senesce with age and are characterized by increased levels of p16INK4a (CDKN2A) and p21CIP1 (CDKN1A) and a senescence-associated secretory phenotype (SASP). A recent study showed that immunosenescence is a phenomenon that is characterized by attrition and senescence of immune cells in mice subjected to increased endogenous DNA damage. Markers of senescence (p16 and p21) were significantly increased in the DNA repair protein knock out (Ercc1) mice in the following immune cells: B cells ($B220^+$ $CD19^+$), T cells ($CD3^+$), natural killer cells ($CD3^-$ $NK1.1^+$) and macrophages ($CD11b^+$ $F4/80^+$) compared to the wild-type mice

(Yousefrzadeh, et al. 2021). Inhibiting senescence in the immune compartment may have a profound effect on the cytotoxic functions of both innate and adaptive immune cells. We propose to examine markers of senescence on immune cells obtained from blood throughout the proposed trial. We hypothesize that

HCW9218 will act as a senolytic by "trapping" excess TGF-β, therefore inhibiting its ability to promote senescence. We believe that this combined with the immune activating effect of IL-15 will be a biologically relevant therapeutic with potential to at least partially reverse therapy induced senescence (TIS) or in the future, perhaps have an effect even on physiologic aging.

3 Study Plan

This is a single center Phase I dose finding study of HCW9218 for the treatment of select advanced solid tumor cancers, including, but not limited to breast, ovarian, prostate and colorectal. Pancreatic cancer is excluded due to a competing HCW9218 protocol. Primary brain tumors are not eligible.

HCW9218 is a novel bi-functional fusion protein complex administered by subcutaneous (SC) injection. It is comprised of a soluble fusion of two human TGFβRII domains, human tissue factor, and human IL-15, and a second soluble fusion of two human TGFβRII domains and a sushi domain of human IL-15Rα. HCW9218 activates IL-15R signaling on effector immune cells and the dimeric TGFβRII functions as a "trap" for all three human TGF-β isoforms.

HCW9218 is administered by subcutaneous injection in the outpatient setting once every 3 weeks for a minimum of two cycles unless medically contraindicated or the participant wishes to discontinue treatment as detailed in Section 6.6. Treatment may continue until disease progression, unacceptable toxicity, patient refusal, or treatment is felt to be no longer of benefit. Patients are followed for 12 months from the 1st dose of HCW9218 to determine progression free survival (PFS) and overall survival (OS).

Dose Level (DL) Cohort	HCW9218 Dose (mg/kg)	Enrollment Plan
-1	0.1	Administer HCW9218 as monotherapy at assigned dose by SC injection once every 3 weeks. All patients are assessed for AEs and dose limiting toxicities (DLT). A minimum of 21 days must separate each dose cohort. Within a 3 patient dose cohort, a minimum of 14 days must separate the 1st and 2nd patient.
1 (start)	0.25	
2	0.5	
3	0.8	
4	1.2	

The maximum sample size will be 24 patients.

4 Patient Selection

Study entry is open to those 18 years of age and older regardless of gender, race, or ethnic background. While there will be every effort to seek out and include minority patients, the patient population is expected to be no different than that of other advanced solid tumor studies at the University of Minnesota.

A potential participant must meet all of the inclusion and none of the exclusion criteria to be considered eligible for study participation.

4.1 Inclusion Criteria

4.1.1 Any histologically or cytologically confirmed advanced/metastatic solid tumor cancer (except pancreatic cancer and primary brain tumor), that has failed at least 2 prior lines of therapy given either in the recurrent or metastatic setting and must be refractory to or intolerant of existing therapy(ies) known to provide clinical benefit for their condition.

4.1.2 Measurable disease per RECIST v 1.1. A single target lesion is considered measurable disease provided the lesion meets the definition of measurability as described in RECIST 1.1 (Schwartz 2016). If a target lesion has been surgically removed or treated with radiotherapy, it is not evaluable for response. Although not a requirement for eligibility, patients with tumor assessable may be asked if willing to undergo an optional research related biopsy prior to treatment start and prior to the 3rd treatment course – interested participants would provide consent through a separate optional biopsy consent form. **Note:** If a biopsy is obtained prior to treatment start (any time after consent) and a patient is later deemed ineligible to receive treatment, the tumor sample will not be used for research purposes.

4.1.3 Resolved acute effects of any prior therapy to baseline or Grade ≤1 NCI CTCAE v5 except for AEs not constituting a safety risk in the opinion of the enrolling Investigator.

4.1.4 Age 18 years or older at the time of consent.

4.1.5 ECOG Performance Status 0 or 1. (Refer to Appendix I).

4.1.6 Evidence of adequate organ function within 14 days prior to treatment on Cycle 1 Day 1 defined as:

- hemoglobin ≥9 g/dL (may be transfused not more than 2 units of pRBCs within previous 2 week to meet this requirement)
- absolute neutrophil count (ANC) ≥1500/ul
- platelets ≥100 x 10^9/L (may be transfused not more than 1 unit of platelets within previous 2 week to meet this requirement)
- estimated glomerular filtration rate (eGFR) >40 mL/min or creatinine: ≤1.5 x upper limit of institutional normal (ULN)
- ALT, AST and ALP ≤ 2.5 x ULN or ≤ 5 x ULN if liver metastases

4.1.7 Adequate pulmonary function with PFTs >50% FEV1 if symptomatic or known impairment.

4.1.8 Sexually active persons of child-bearing potential or persons with partners of childbearing potential must agree to use (or agree to their partners' use of) a highly effective form of contraception for at least 28 days after the last dose of HCW9218. Non-childbearing is defined as >1 year post-menopausal or surgically sterilized.

Examples of highly effective birth control methods include but are not limited to the following:

- Using twice the normal protection of birth control (i.e., double-barrier) by using a condom AND spermicidal jelly or foam, or a diaphragm AND spermicidal jelly or foam. A spermicidal jelly or foam must be used in addition to a barrier method (e.g., condom or diaphragm).
- Oral contraceptive pills
- Depot or injectable birth control
- Intrauterine Device (IUD)
- Transdermal contraceptive patch
- Vaginal contraceptive ring
- Contraceptive implants

Note: Rhythm method or abstinence alone is not considered to be an adequate method of contraception.

4.1.9 Provides voluntary written consent prior to the performance of any research related activity.

4.2 Exclusion Criteria

4.2.1 Pregnant or breastfeeding. Persons of child-bearing potential must have a negative pregnancy test (serum or urine) within 14 days of treatment start.

4.2.2 History of clinically significant vascular disease, including any of the following within 6 months prior to start of study treatment: MI or unstable angina, percutaneous coronary intervention, bypass grafting, ventricular arrhythmia requiring medication, stroke or transient ischemic attack, symptomatic peripheral arterial disease.

4.2.3 Marked baseline prolongation of QT/QTc interval (e.g., demonstration of a QTc interval ≥470 milliseconds by Fridericia correction). If undiagnosed MI(s) appear on ECG, work up as medically warranted and determine eligibility based on findings. Cardiac function should be monitored by clinical examination and assessment of vital signs for hypotension, arrhythmia, angina, and myocardial infarction during the study period per Section 6.1.1.

4.2.4 Known or suspected untreated CNS metastases are excluded. Persons are eligible if CNS metastases are treated and they are neurologically stable for at least 2 weeks prior to enrollment. In addition, must be either off

corticosteroids, or on a stable or decreasing dose of ≤ 10 mg daily prednisone (or equivalent).

4.2.5 Anti-cancer treatment including surgery, radiotherapy, chemotherapy, other immunotherapy, or investigational therapy within 14 days before treatment start.

4.2.6 Prior malignancy except for the following: adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, adequately treated Stage I or II cancer currently in complete remission, or any other cancer from which the patient has been disease-free for 3 years after surgical treatment.

4.2.7 Known hypersensitivity or history of allergic reactions attributed to compounds of similar chemical or biologic composition to the agents used in the study.

4.2.8 Prior therapy with TGF-β antagonist, IL-15 or analogs.

4.2.9 Ongoing use of St. John's Wort and/or other herbal CYP modulators as certain cytokines, including IL-2 and TGF-β, have been found to suppress the activities of cytochrome P450 enzymes. The participant may be considered for the study if they discontinued the herbal CYP modulator at least 7 days before the 1st dose of HCW9218 and agree not resume it until after the End of Treatment visit.

4.2.10 Known autoimmune disease requiring active treatment. Persons with a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of enrollment, with the exception of use for persons with CT contrast allergies. Inhaled or topical steroids, and adrenal replacement steroid doses ≤ 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease.

4.2.11 Active systemic infection requiring parenteral antibiotic therapy. All prior systemic infections must have resolved following optimal therapy.

4.2.12 Prior organ allograft or allogeneic transplantation.

4.2.13 Known HIV-positive or AIDS.

4.2.14 Psychiatric illness/social situations that would limit compliance with study requirements.

4.2.15 Other illness or a medical issue that in the opinion of the Investigator would exclude the subject from participating in this study.

5 Patient Screening and Study Enrollment

Written consent must be obtained prior to the performance of any research related tests or procedures.

5.1 Consent and Study Screening in UMN OnCore

Any patient who has been consented is to be entered in OnCore by the Study Coordinator or designee. If a patient is consented but is not enrolled in the study treatment, the patient's record is updated in OnCore as a screen failure and reason for exclusion recorded.

5.2 Study Enrollment and Treatment Assignment

To be eligible for this study, the patient must sign the treatment consent and meet each inclusion criteria and none of the exclusion criteria listed on the eligibility checklist based on the eligibility assessment documented in the patient's medical record.

If a patient is not starting study treatment within 14 days of baseline eligibility lab work, the blood work in Section 4.1.6 must be repeated within 14 days prior to treatment on Cycle 1 Day 1 to confirm continuing eligibility. If one or more of the lab results do not meet the criteria the patient is taken off study per Section 5.3.

The Study Coordinator or designee updates OnCore with the assigned HCW9218 dose level to complete study enrollment.

5.3 Inability to Begin Study Treatment

If a patient is enrolled to the study and is later found not able to begin study treatment, for whatever reason, the patient will be taken off study and treated at the physician's discretion. The Study Coordinator or designee will update OnCore of the patient's non-treatment status. The patient will be replaced to fulfill enrollment requirements and will not be included in the outcome analysis.

6 Treatment Plan

6.1 Administration of HCW9218

HCW9218 is given as a subcutaneous (SC) injection at the patient assigned dose once every 3 weeks until disease progression, unacceptable toxicity or continuation of treatment is longer of benefit.

HCW9218 dose calculation is based on patient's assigned dose level and actual body weight recorded at Cycle 1 Day 1 (or within 14 days prior).

This dose is used for the duration of the study treatment unless there is a ±10% or greater change on a subsequent cycle's "Day 1" weight from the weight used to calculate the Cycle 1 Day 1 dose. In this situation, the HCW9218 dose is recalculated using the more recent weight. The new dose is used for subsequent HCW9218 injections, unless a ≥10% weight change occurs based on the newer weight. Depending on the following:

- The 10% weight change is because their weight returned to around their Cycle 1 Day 1 weight – dose may be recalculated and the patient continue HCW9218
- The 10% weight change represents a ≥20% weight change from the Cycle 1 Day 1 (representing a Grade 3 weight change based on CTCAE v 5) meeting the criteria for discontinuing treatment per Section 6.6.

If the total subcutaneous (SC) dose is greater than 1.5 mL, the dose will be divided by IDS into 2-3 SC injections as needed. Injections are given in the abdominal area. The injection site should be rotated per institutional guidelines and each injection site separated by at least 1 inch.

HCW9218 can be administered on an outpatient basis, or if the patient is in the hospital for another reason, as inpatient treatment.

6.1.1 Provider's Assessment Prior to Each HCW9218 and Dose Delay

In order to proceed with a planned HCW9218 injection, a provider must assess the patient prior to each HCW9218 dose to rule-out any of the following contraindications for dosing:

- the previous injection site reaction is not showing signs of resolving (improving) based on measurement or intensity
- signs or symptoms of a new infection
- fever > 101°F (38.3 °C)
- cardiac function by clinical exam and assessment of vitals for signs hypotension, arrhythmia, angina and myocardial infarction or symptoms of chest pain, murmurs, gallops, irregular rhythm, or palpitations with further assessment as clinically indicated.

The HCW9218 dose will be delayed for up to 1 week if one or more of the above conditions are present.

In addition, HCW9218 may be delayed for up to 1 week on the day of a planned dose for either of the following situations:

- Any ongoing treatment related toxicity.
- If in the opinion of the treating physician, holding HCW9218 would be of benefit to the patient.

If after a 1 week rest:

- The patient meets the above criteria for treatment, the patient may receive HCW9218. The patient calendar would be adjusted (reset) and the patient returns in 3 weeks for the next course of treatment.

- The patient does not meet the above criteria for treatment or the Investigator feels holding treatment would be of benefit to the patient, the dosing may be skipped. The patient would return for reassessment at the time of next planned dose (i.e. in 3 weeks). A decision is made at that time whether to treat or discontinue HCW9218 permanently. If Cycle 2 cannot be given (is skipped) due to treatment related toxicity, a DLT event must be reported.

Refer to Section 6.3 for additional information regarding DLTs.

6.1.2 Pre Medication

Use of pre-medications is at the discretion of the treating physician on an individual patient basis. Acetaminophen may be given prior to the injection to reduce the intensity of the fever that often occurs a few hours later.

The HCW9218 intervention guidelines described below may be implemented or modified by the Investigator as medically necessary or as appropriate without requiring a protocol amendment or being considered a protocol deviation.

HCW9218 Intervention Guidelines

Condition	Agents	Dose	Route	When
Fever/Chills	Antipyretic*			
	Acetaminophen	up to 650 mg; or 10 to 15 mg/kg recipient weight	Orally	Prior to each dose & repeat 4 hours after dosing prn. Repeat every 4-6 hours if fever present.
	Ibuprofen	up to 400 mg; or 10 mg/kg recipient weight	Orally	Prior to each dose & repeat 4 hours after dosing prn. Repeat every 4-6 hours if fever present
	Naproxen	up to 440 mg	Orally	Prior to each dose & repeat 8 hours after dosing prn. Repeat every 8-12 hours if fever present.

*Antipyretic medication including acetaminophen, ibuprofen, or naproxen may be given per physician discretion following the recommended dosing thresholds:
Acetaminophen: not to exceed 3000 mg (3 grams) in 24 hours
Ibuprofen: not to exceed 2400 mg in 24 hours
Naproxen: not to exceed 1100 mg in 24 hours

H1 and H2 blockers may be given prior to administration of HCW9218 per Investigator's discretion and/or Institution's standard of care related to protein-based therapeutics.

6.1.3 Patient Monitoring Day of HCW9218 Dosing

For Cycle 1 participants are observed for a minimum of 6 hours after HCW9218 dosing for immediate adverse events.

For Cycle 2 participants are observed for a minimum of 3 hours after HCW9218 dosing for immediate adverse events. Vital signs (body temperature, heart rate, respiratory rate, systolic and diastolic blood pressure) are documented prior to each HCW9218 dose and then after dosing at 15 minutes (±5 min), 30 minutes (± 5 min), 60 minutes (±10 min) and 120 minutes (±15 min) minutes then hourly (±15 min) until discharge from the clinic.

In addition, during Cycle 1 only, the targeted toxicity worksheet is completed as detailed in Section 10.2.2.

For subsequent doses (i.e., Cycle 3 and subsequent cycles), if the participant did not experience any severe side effects from the previous doses, they may be discharged from the treatment center 30 minutes after the HCW9218 injection at the discretion of the treating Investigator. Vital signs (body temperature, heart rate, respiratory rate, systolic and diastolic blood pressure) are documented prior to each HCW9218 dose and then after dosing at 15 minutes (±5 min) and at 30 minutes (±5 min).

6.1.4 HCW9218 Dosing Delay and Discontinuation

No dose reductions are permitted (except for HCW9218 dose recalculation for weight change in Section 6.1).

A dose delay is permitted per Section 6.1.1.

All attempts should be made to stay within the allotted timeframes although altering the schedule, if determined to be medically necessary by the Investigator, will not be a protocol deviation.

6.2 HCW9218 Dose Finding Enrollment Plan

Dose limiting Toxicity (DLT) is defined in Section 6.3.

6.2.1 STAGE 1 STEP 1 - Fast-track design (1 patient per dose level)

Start at DL1, enroll 1 patient per DL separated by a minimum of 21 days (DLT period) until the:

- **1st pre-defined adverse event** - Any of the following treatment emergent Grade 2 events lasting >72 hours within 5 days of 1st dose of HCW9218: fatigue, flu-like symptoms or injection site reaction.
- And the patient completes the 21 day DLT period with no DLT- **Activate Stage 1 Step 2 in Section 6.2.2.**

OR

- **1st DLT event** within 21 days after the 1st dose of HCW9218 - **Activate Stage 2 in Section 6.2.3** - Stage 1 Step 2 is not used.

OR

- **9 patients are treated at the DL4** - This completes enrollment - Stage 1 Step 2 and Stage 2 are not used.

6.2.2 STAGE 1 STEP 2 - Expand Current and Future DLs to 3 Patients

The cohort size increases from 1 to 3 patients with 2 additional patients added to the current cohort. A minimum of 21 days must separate each dose cohort. Continue dose escalation until:

- **1st DLT event - Activate Stage 2 in Section 6.2.3.**

OR

- **9 patients are treated at the DL4** - This completes enrollment and Stage 2 is not used.

6.2.3 STAGE 2 Continual Reassessment Method (CRM)

The study design changes to an application of the continual reassessment method (CRM). Enrollment occurs in cohorts of three with a minimum of 14 days between the 1st and 2nd patient. Each new cohort of three patients are sequentially assigned to the most appropriate dose by the study statistician based on the updated toxicity probabilities once the 3rd patient in a cohort reaches Day 21 (DLT period). The goal will be to identify one of the 4 dose level strategies corresponding to the desired maximum toxicity rate of ≤25%. The MTD will be identified by the minimum of the following criteria: (1) the total Stage 2 sample size of 24 is exhausted or (2) 9 consecutive patients are enrolled at the same dose.

6.3 Monitoring for Dose Limiting Toxicities and Excessive Toxicity

Toxicity and adverse events are classified and graded according to NCI's Common Terminology Criteria for Adverse Events V 5.0 (CTCAE) unless otherwise noted in Section 10.

Note during the fast-track dose escalation only (Stage 1, Step 1), patients also are monitored for the pre-defined treatment emergent events defined in Section 6.2.1.

Dose Limiting Toxicity (DLT) is defined as any of the following treatment emergent adverse events using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 within the 21 days after the 1st dose of HCW9218:

DLT event	Exceptions:
Any Grade 4 hematologic event	○ Grade 4 lymphopenia hat resolves to Grade 1 or lower (or to Baseline or lower, if pre-existing Grade 2 or higher) within 7 days ○ Grade 4 leukopenia that resolves to Grade 1 or lower (or to Baseline or lower, if pre-existing Grade 2 or higher) within 7 days
Any Grade 3 or Grade 4 febrile neutropenia	
Any Grade 3 thrombocytopenia with bleeding	
Any Grade 3 or higher non-hematologic adverse event	○ Grade 3 nausea, vomiting and/or diarrhea that last < 72 hours ○ Grade 3 or higher electrolyte abnormality that lasts < 72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical interventions ○ Isolated Grade 3 elevation of ALT and/or AST that resolves to Grade 1 or lower (or to Baseline or lower, if pre-existing Grade 2 or higher) within 7 days ○ Grade 3 amylase or lipase that is not associated with symptoms or clinical manifestations of pancreatitis ○ Grade 3 fatigue, not due to disease progression, that lasts < 1 week
Hy's Law case (i.e., alanine aminotransferase/ aspartate aminotransferase (ALT/AST) >3 x ULN in combination with total bilirubin level (TBL) > 2 x ULN in the absence of cholestatic injury (ALP < 2 x ULN (Ricart, 2017; FDA, 2009)) and no other cause identified) after excluding biliary tract obstruction and liver metastasis	
Inability to receive Cycle 2 dosing due to treatment related toxicity	

The Dose Limiting Toxicity (DLT) Observation Period is defined as 21 days after the first dose of HCW9218. The DLT period does not end until the patient is assessed on Cycle 2 Day 1 (+3 days) and the results of lab work drawn on Cycle 2 Day 1 (+3 days) confirms no new DLTs since the previous assessment.

Monitoring for Excessive DLT's (Stage 2 patients only):
A stopping rule is in place to stop the trial in case there are excessive DLTs based on Monitoring Guidelines in place for excessive toxicity. At the end of the 21-day DLT period for each cohort of patients, the study statistician or designee updates posterior probability that the lowest dose is unacceptably toxic. Refer to Section 13.4 for complete details.

6.4 Supportive Care and Concomitant Medications

Supportive care will be provided per institutional guidelines. Guidelines may be updated based on current data/drugs without requiring a protocol amendment or be considered a protocol deviation.

If a rash occurs and the rash area surrounding the HCW9218 injection site is > 6 cm and symptomatic (painful and/or itchy), it may be treated (at the discretion of the treating physician) with topical 0.05% clobetasol propionate (i.e., 0.05% Cormax) or 0.1% triamcinolone (i.e., Kenalog) cream.

Diphenhydramine may be administered pre- (25-50 mg TID orally) and post-dosing (25-50 mg TID orally x 2 days) of HCW9218 at the discretion of the treating physician. Diphenhydramine should be eliminated if not tolerated.

Red blood cell and/or platelet transfusions can be administered as clinically indicated.

Palliative “standard of care” radiation therapy is permitted to non-target tumor(s) such as bony lesions at the discretion of the treating investigator.

6.5 Prohibited Concomitant Therapy

Unless the patient discontinues the study treatment, no investigational or commercial anti-cancer agents or anti-cancer therapies other than HCW9218 and supportive care therapies (i.e., topical steroids) may be administered. Palliative radiation therapy is permitted as described in Section 6.4.

The use of systemic corticosteroids medications may result in loss of therapeutic effects of the investigational product and should be avoided; however, in the event of a life-threatening inflammatory reaction to HCW9218, the administration of dexamethasone or other corticosteroids-based medication is warranted. Prednisone should be avoided, with the exception of use in patients with CT contrast allergies.

Certain cytokines, including IL-2 and TGF-β, have been found to suppress the activities of cytochrome P450 enzymes. This may potentially indirectly affect the metabolism of other medications resulting in significant toxicities in some cases. Since the effects of HCW9218 on human cytochrome P450 enzymes are not yet known, use of cyclosporine, statins, or warfarin (Coumadin) should be avoided in participants receiving HCW9218.

6.6 Duration of Study Treatment

A minimum of two treatment cycles is planned and HCW9218 may continue until disease progression unless any one of the following occurs sooner:

- Patient requests to discontinue treatment early – if in agreement, follow-up would continue as in Section 6.7 unless the patient also withdraws consent.
- In the judgement of the treating Investigator and documented in the medical record, study treatment is no longer in the best interest of the participant.

An End of Treatment (EOT) visit occurs 3 weeks (±1 week) after the last dose of HCW9218 to confirm no ongoing side effects/toxicity. If ongoing toxicity at the EOT visit, it should be managed as medically appropriate and documented, it if meets the criteria for documenting as an AE be followed until resolution or stabilization.

In the situation where a patient's EOT visit falls outside of the visit window due to a prolonged period before the decision to discontinue treatment is made, the EOT related activities may be retrospectively abstracted from the medical record from around the time the EOT would have occurred and/or delayed until the next standard of care visit. Missing items / research samples will not be considered a deviation.

6.7 Follow-Up for Disease Response and Survival (Duration of Study Participation)

After the End of Treatment visit follow up continues for 12 months from the 1st dose of HCW9218 to fulfill the secondary endpoints of disease status at 3, 6 and 12 months and progression free survival / overall survival at 6 and 12 months.

If a patient discontinued treatment for a reason other than disease progression, disease status is documented per standard of care reassessments until progression, and then for survival only until the end of the 12 month follow-up period.

Follow-up assessments may be in person, by medical record review, or local medical doctor if patient is no longer seen at the treating institution. In person follow-up visits solely for this research study are not required and may be linked with a standard of care visit. Refer to Section 8 for allowable time related windows around each follow-up time points.

Follow-up may end early if any of the following apply:

- The patient is discharged to hospice
- The patient withdraws consent
- The patient is lost to follow-up (defined as 3 missed appointments/attempts to contact)

If a patient dies before the end of the study defined follow-up, record the date of death as the off-study date and reason off study as death and add the date of

death. Refer to Section 10.3 regarding reporting of deaths while a patient is on study.

If a person ends follow-up before the 12 month study period (i.e. withdraws consent, lost to follow-up), public records will be searched for survival information for the endpoints of survival at 6 and 12 months.

7 HCW9218 Potential Toxicities and Management

7.1 Clinical Profile

The adverse event (AE) profile of HCW9218 has not yet been evaluated in human subjects.

The HCW9218 protein complex comprises an IL-15 domain, which is a common γ chain cytokine that may have similar immunostimulatory properties as IL-2. HCW9218 treatment, particularly at high-dose levels, could potentially cause similar side effects, including severe cytokine release syndrome or capillary leak syndrome, as commercially available IL-2, Proleukin® (Clinigen, Inc. Yardley, PA) that is approved for use at high doses in advanced melanoma and advanced renal cell carcinoma. Therefore, in general, the Investigator may refer to the approved product package insert for Proleukin® (aldesleukin) for Injection and other published literature describing the management of IL-2-related AEs (Dutcher et al, 2014). Details of these AEs are provided in Appendix II.

Additionally, expected AEs based on the published clinical experience of monotherapy with the related product candidates, including recombinant human IL-15 (rhIL-15) (Conlon et al, 2019; Miller et al, 2018; Conlon et al, 2015), IL-15 mutein/IL-15RαSu-Fc complex (ALT-803, nogapendekin alfa inbakicept) (Margolin et al, 2018; Romee et al, 2018), TGFβRII-Fc fusion (AVID200) (Lafyatis et al, 2020; Mascarenhas et al, 2020; Yap et al, 2020) and TGFβRII-anti-PD-L1 IgG1 fusion (M7824, bintrafusp alfa) (Tan et al, 2021; Khasraw et al, 2021; Kang et al, 2020; Strauss et al, 2020), are provided in Appendix II. These agents contain protein domains that are similar to those of HCW9218. Information on the AEs reported in clinical studies of anti-TGF-β antibody therapy (Lacouture et al, 2015; Morris et al, 2014), assumed to have a similar mechanism-of-action as the HCW9218 TGFβRII domain, also is included in Appendix II.

In general, treatment with ALT-803 monotherapy at up to 0.02 mg/kg was well-tolerated and no DLTs were reported. Dose-dependent transient flu-like constitutional AEs were observed following i.v. administration whereas s.c. delivery resulted in self-limited injection site rashes infiltrated with lymphocytes without acute constitutional symptoms. Treatment with immunotherapies

containing the TGFβRII domain were also well-tolerated at i.v. dose levels up to 15 mg/kg with reported treatment-emergent skin lesions and mucosal bleeding being associated with TGF-β inhibition. The combination of IL-15 immunostimulatory and TGF-β antagonist activities in HCW9218 may result in additional and/or more frequent or severe AEs.

7.2 Most Common and Most Serious Side Effects Seen In Drugs Similar To HCW9218

HCW9128 has not yet been evaluated in human clinical studies. Thus, the expected AEs are based on the published clinical experience with other related immunotherapies, including recombinant human IL-2, recombinant human IL-15, TGFβRII-Fc fusion and anti-TGF-β antibody.

All adverse events listed in Appendix II are defined as "Expected" for the purpose of event documentation and reporting requirements.

The tables below show the most common and most serious side effects seen in drugs similar to HCW9218. This language matches the treatment consent form.

COMMON, SOME MAY BE SERIOUS In 100 people receiving a drug similar to HCW9218, more than 20 and up to 100 may have:	OCCASSIONAL, SOME MAY BE SERIOUS In 100 people receiving a drug similar to HCW9218, from 4 – 20 people may have:	RARE, AND SERIOUS In 100 people receiving a drug similar to HCW9218, 3 or fewer may have:
• redness, itching and pain or swelling at the injection site • infection, especially when white blood cell count is low • anemia, which may require a blood transfusion • bruising, bleeding • nose-bleed • fever, chills, pain • flu like symptoms • swelling in arms and legs • pain in belly • pain in joints • loss of appetite • diarrhea • dizziness • shortness of breath	• chest pain • skin rash • abnormal eye movement • constipation • bloating • cough • headache • sores in mouth • depression • anxiety/ worry • extreme sleepiness • weight gain • abnormal heartbeat • blurred vision	• skin lesions • allergic reaction • fluid in the organs • heart attack • internal bleeding • severe blood infection • damage to multiple organs, heart, kidney, liver, others • swelling, tenderness and/or redness of the veins • stroke

COMMON, SOME MAY BE SERIOUS In 100 people receiving a drug similar to HCW9218, more than 20 and up to 100 may have:	OCCASSIONAL, SOME MAY BE SERIOUS In 100 people receiving a drug similar to HCW9218, from 4 – 20 people may have:	RARE, AND SERIOUS In 100 people receiving a drug similar to HCW9218, 3 or fewer may have:
• fatigue/ tiredness • high blood pressure which may cause headaches • increase/ decrease in urination • drop in blood pressure • itching • dry skin • nausea, vomiting • abnormal lab results • blood clot • confusion		

8 Schedule of Patient Activities

Virtual visits may replace in person clinic visits as relevant. Lab work and disease reassessments must be performed at the enrolling site, unless other arrangements are permitted as discussed with the study PI.

For flexibility “windows” are built into the study calendar to allow for scheduling issues (i.e. holidays, inclement weather, patient request) and may be used as needed. Each time point and its associated window is an independent event and does not affect future time points or the study calendar. This contrasts with treatment delays for toxicity written into the study plan – Refer to Section 6.1.1

Permitted window for scheduling issues:

- A ±1 day window is permitted for visits after Day 1 for Cycles 1 and 2.
- Safety labs may be performed up to 48 hours prior to administration of HCW9218 except for Cycle 2 Day 1 (end of DLT period).
- Beginning with Cycle 3, a ±3 day window is permitted around Day 1 as long as a minimum of 2 weeks separate each HCW9218 in event of a dose delay per Section 6.1.4.
- Imaging studies and tumor markers are performed per standard of care (every 4 cycles (12 weeks) or per SOC if more frequent) – windows do not apply.

- End of Treatment (EOT) visit ± 1 week (refer to Section 6.6 for options if the decision to stop treatment is made > 4 weeks after the last dose of HCW9218).
- Follow-up assessments ± 1 month

In addition, a treatment day may be adjusted by the treating Investigator on an individual patient basis if the change is in the best interest of the patient. This may require reporting the change as a study deviation. Contact the study's Regulatory Specialist or Program Manager for guidance. Refer to Section 14.1 for the definition of protocol deviations.

8.1 Standard of Care Activities

Standard of care assessments and procedures are part of good clinical care and are charged to the patient and their health insurance.

	Baseline / screening Within 28 days of Cycle 1 Day 1 unless o/w noted[1]	Cycle 1						Cycle 2					Cycle 3 and subsequent cycles	Every 4 cycles (12 weeks) or per SOC if more frequent until PD	End of treatment (EOT) visit 3 weeks after the last dose of HCW9218	After EOT visit: Follow-up for response until PD, then survival only every 3 mon for 12 months from C1D1
Study day	-28 to -1 (no Day 0)	1	2	3	5	8	15	1	2	5	8	15	1			
window	Not applicable	±1 day using Day 1 as the anchor						+3 days for Day 1 then ±1 day					±3 days	± 14 days	± 1 week	± 1 month
Written consent	X[1]															
Assess for eligibility	X															
Medical history including prior cancer treatment	X															
Concomitant medications	X	X				X	X	X			X	X	X		X	
AE assessment		X	X	X	X	X	X	X	X	X	X	X	X		X	
Physical exam[6]	X	X				X		X					X		X	
Provider assessment	X	X			X	X	X	X					X		X	
ECOG Performance Status	X	X				X		X							X	
Vitals signs	X	X[3]	X	X	X	X	X	X[3]	X	X	X	X	X[4]		X	
Weight[5] plus height at baseline only	X	X[5]				X		X				X	X		X	
CBC with differential	X[1]	X[2]	X	X	X	X	X	X	X	X	X	X	X[2]		X	
Complete metabolic panel[7]	X[1]	X[2]				X		X					X[2]		X	
CRP, ferritin, amylase, lipase, LDH, mg, phos	X[8]					X[8]										
Diagnosis related biomarker (e.g., CA125, PSA)	X													X		X
Pulmonary function testing only if clinically indicated	X															
Tumor assessment by disease appropriate methods	X[1]													X		X
Disease response based on RECIST 1.1 (until disease progression)	X[1]													X		X
Survival/follow-up																X

1. Baseline/Screening – within 14 days of study treatment start for labs required for eligibility; prior disease assessments may be used if they were performed within 42 days of the 1st HCW9218 dose in the absence of intervening anti-cancer therapy. Consent is exempt from 28 day limit and may be performed at any time prior to starting study related activities. If more than 14 days pass between lab work for eligibility (found in Section 4.1.6) and Cycle 1 Day 1, the pre Cycle 1 lab results must continue to meet the criteria in Section 4.1.6.
2. Safety labs can be collected up to 72 hours prior to administration of HCW9218 except for C2 D1 where labs cannot be collected sooner than Cycle 1 Day 22 / Cycle 2 Day 1 (the end of DLT period).
3. C1D1 and C2D1: Vital signs (body temperature, heart rate, respiratory rate, systolic and diastolic blood pressure) are documented before each HCW9218 dose and then after dosing at 15 min (± 5 min), 30 min (± 5 min), 60 min (± 10 min) and 120 min (± 15 min) then hourly (± 15 min) until discharge from the clinic or at completion of dose monitoring.
4. For C3D1 and subsequent doses: Vital signs (body temperature, heart rate, respiratory rate, systolic / diastolic blood pressure) are documented before each HCW9218 dose and then after dosing at 15 min (± 5 min) and 30 min (± 5 min).

5. HCW9218 dose calculation is based on patient's dose level and actual body weight recorded at Day 1 (or within 14 days prior). Dose adjustments occur only if weight changes by ±10% or greater from weight used to calculate original dose per Section 4.1.
6. Cardiac function should be monitored by clinical examination and assessment of vital signs for hypotension, arrhythmia, angina and myocardial infarction. During the study, participants with signs or symptoms of chest pain, murmurs, gallops, irregular rhythm, or palpitations must be further assessed if clinically indicated, including the need for hospitalization
7. Complete metabolic panel includes: albumin, alkaline phosphatase, ALT, Anion gap, AST, bilirubin (total), calcium, chloride, CO2, creatinine, glucose, potassium, protein (total), sodium, urea nitrogen
8. Baseline and Day 8 measures, repeat only if clinically indicated.

8.2 Research Related Activities

Activities directly related to participation in the research study are paid for by study funding and not charged to the patient or their health plan/health insurance. Research related activities are linked to a standard of care (SOC) visit and may be adjusted to stay with the SOC visit unless otherwise noted.

Tests and procedures	Baseline/ Screening	Cycle 1						Cycle 2					Cycle 3 and all subsequent cycles	Every 4 Cycles (every 12 weeks)	At End of Treatment (EOT visit)
Study day		1	2	3	5	8	15	1	2	5	8	15	Day 1		
Coagulation studies (INR/PTT)[1]	X	X	X	X	X	X	X	X	X	X	X	X			
ECG and cardiac monitoring[2]	X														
Pregnancy testing (serum or urine) for females of child-bearing potential[3]	X	X[3]													
HCW9218 administration (refer to Section 6.1 for dose adjustment based on weight change)		X						X					X		
Targeted toxicity worksheet (Appendix IV) in addition to AE assessment per Section 10.2.2		X[4]	X[4]	X[4]	X[4]										
Blood sample for pharmacokinetics (PK)[9]		Pre and post [5]	X[5]	X[5]	X[5]	X[5]		Pre and post[7]	X[7]	X[7]	X[7]				X
Blood sample for correlative marker (CM) analysis[9]		Pre and post [6]	X[6]	X[6]	X[6]	X[6]	X[6]	Pre and post[8]	X[8]	X[8]	X[8]	X[8]			
Blood sample for immunogenicity (IMG)[9]		X (prior to dose)						X (prior to dose)					X (prior to dose)		X
Blood sample for immune cell analysis and TIS biomarkers (p16 and p21) (at least 10 mL collected in Heparin tube)		X (prior to dose)				X	X	x (prior to dose)					X (prior to dose)		
Blood sample for UMN research related testing (at least 30 mL collected in 3 Heparin tubes)		X (prior to dose)	X	X	X	X	X	x (prior to dose)		X	X	X	X (prior to dose)		
FACT-G QOL questionnaire (Appendix V)	X													X	X
Skin condition questionnaire (Appendix VI)								X					X		X
Tumor biopsy (optional refer to Section 4.1.2)	X[10]												X (prior to cycle 3 dose only)		

1- Coagulation studies are performed in FV labs as usual but charge to research, collect prior to day's dose if applicable
2- An ECG as usual will be performed at baseline/screening but charged to research.
3- A negative serum or urine pregnancy test is required within 14 days prior to enrollment to confirm eligibility for women with childbearing potential (non-childbearing is defined as > 1 year postmenopausal or surgically sterilized). The pregnant test must be repeated (and remain negative) if the baseline testing occurred more than 14 days before Cycle 1 Day 1.

4- Targeted toxicity worksheet is completed at the following timepoints per Section 10.2.2: Prior HCW9218 injection on Cycle1 Day 1 (within 1 hour prior to HCW9218), at 3 hours (±15 minutes) and at 6 hours (±15 minutes) post injection, Cycle 1 Day 2, Cycle 1 Day 3, and Cycle 1 Day 5. The completion of the targeted toxicity worksheet aligns with in person visits and may be shifted to align with the actual day a visit occurs or skipped if a visit is not occurred.
5- **Cycle 1 Blood samples for PK analysis** will be collected in association with the **first dose of HCW9218:** Baseline sample collected within 24 hours prior to HCW9218 dosing, at 30 minutes ± 5 min and 3 hours ± 30 minutes (C1D1), 24 hours ± 6 hours (C1D2), 48 hours ± 6 hours (C1D3), 96 hours ± 24 hours (C1D5) and C1D8 ± 24 hours.
6- **Cycle 1 Blood samples for CM analysis** will be collected in association with the **first dose of HCW9218:** Baseline sample collected within 24 hours prior to HCW9218 dosing, at 30 minutes ± 5 min and 3 hours ± 30 minutes (C1D1), 24 hours ± 6 hours (C1D2), 48 hours ± 6 hours (C1D3), 96 hours ± 24 hours (C1D5), then on C1D8 ± 24 hours and Day 15 ± 24 hours.
7- **Cycle 2 Blood samples for PK analysis of HCW9218** will be collected in association with the **second dose of HCW9218**: Baseline sample collected within 24 hours prior to dose 2 of HCW9218 dosing and at 3 hours ± 30 minutes, 24 hours ± 6 hours (C2D2), 96 hours ± 24 hours (C2D5) and C2D8 ± 24 hours.
8- **Cycle 2 Blood samples for CM analysis** will be collected in association with the **second dose of HCW9218**: Baseline sample collected within 24 hours prior to dose 2 of HCW9218 dosing and at 3 hours ± 30 minutes, 24 hours ± 6 hours (C2D2), 96 hours ± 24 hours (C2D5) then on C1D8 ± 24 hours and Day 15 ± 24 hours.
9- Refer to the HCW Biologics Investigator Initiated Trial (IIT) Manual for sample collection, processing, and shipping details.
10- Patients with accessible tumor and who sign the separate consent for a fresh tumor biopsy collected prior to the 1st HCW9218 dose (any time after consent) and before the 3rd dose of HCW9218. Biopsy collection is exempt from the 28 day limit and may be performed at any time after consent and prior to HCW9218 dose. If a biopsy is obtained prior to the 1st HCW9218 dose and a patient is later deemed ineligible to receive treatment, the tumor sample will not be used for research purposes.

Research related sample collection and activities are linked to a standard of care (SOC) visit and may be adjusted to stay with the SOC visit unless otherwise noted.

Up to 3 additional research samples for each test type may be collected at other time points (i.e., at time of AE, disease progression or response) during the study period.

All research samples go to the Masonic Cancer Center Translational Therapy Lab (TTL) except fresh biopsy or surgical samples are handled as described in the contract with BioNet.

Residual samples (and study data) may be used by HCW Biologics Inc. and shared with University of Minnesota investigators for research studies of additional biomarkers and correlative assessments. Results will not influence a patient's treatment nor will the results be provided to the patient or placed in their medical record.

The following testing is performed at HCW Biologics or their designee lab.

Pharmacokinetic (PK) analysis – Detection of HCW9218 levels over time. If appropriate, PK may also be performed using the same samples as immunogenicity and correlative marker analysis.

Immunogenicity (IMG) - Detection of Anti-HCW9218 Antibodies will be performed at HCW Biologics Inc.

Correlative Marker (CM) Assessments - These tests will be performed at HCW Biologics as research studies using de-identified samples. Serum samples will be collected to evaluate the levels of serum cytokines, chemokines and inflammatory markers, typically but not limited to TGF-β isoforms, IL1α, IL-1β, IL-6, IFN-γ, TNF-α, MCP-1, resistin and CRP.

Residual samples (and study data) may be used by HCW Biologics Inc. and shared with University of Minnesota investigators for research studies of additional biomarkers and correlative assessments. Results will not influence a patient's treatment nor will the results be provided to the patient or placed in their medical record.

The following testing is performed at the University of Minnesota.

Blood Immune Cell Analyses - Flow and/or mass cytometry analysis of blood cells may be conducted to detect surface markers that define immune cell and lymphocyte subsets (such as NK, NKT, B, and T cells, both CD4 and CD8), as well as intracellular markers that define additional parameters (such as regulatory T

cells (Foxp3) and proliferating cells (Ki67)). RNA sequence analysis may also be conducted to assess the gene expression profile in immune cells.

Quality of Life Assessments Exploratory Research Assessments will be performed on various quality of life parameters. Assessments are completed by the participant or, through oral response to a member of the research team administering the assessment. The Functional Assessment of Cancer Therapy - General (FACT-G) is a validated 27-item questionnaire designed to measure four domains of health related quality of life in cancer patients: physical, social, emotional, and functional well-being using a 5 point Likert-type score based on patient recall of the last 7 days.

The Skin Condition Questionnaire is a 3 question binary (Y/N) survey created by HCW Biologics asking about skin changes at the injection site and overall changes in the skin noted by the participant. For yes responses, the participant is asked to describe the changes.

Tumor Biopsies (Optional) Patients will be approached based on safety and feasibility of accessible tumor tissue. A separate research biopsy consent is required. Biopsy samples will be stored for future research studies. Immunohistochemistry analysis and genomic, transcriptomic and proteomic molecular profiling may be performed on fresh/frozen and/or formalin-fixed, paraffin embedded (FFPE) tumor tissue. All such analyses will be retrospective and exploratory. Biopsies will be performed according to the institution's standard procedures. Tumors used for biopsy should not be lesions used as RECIST target lesions unless there are no other lesions suitable for biopsy.

9 HCW9218 Description, Supply and Potential Toxicities

9.1 Formulation

The inactive ingredients of HCW9218 drug product are the formulation buffer (PBS) comprised of 1.36 g/L of potassium phosphate monobasic (KH_2PO_4), 1.43 g/L of sodium phosphate dibasic anhydrous (Na_2HPO_4), and 8.18 g/L of sodium chloride (NaCl), pH 7.4.

9.2 How Supplied

HCW9218 is an investigational drug product supplied to Investigators by HCW Biologics Inc. at Miramar, Florida. Sufficient HCW9218 drug product will be available for this protocol to treat all of the projected enrolled subjects.

The clinical lot of HCW9218 drug product for use in the study will be provided in 2 mL vials containing 1.0 mL of HCW9218 at a concentration of approximately 20 mg/mL (20 mg HCW9218 in 1 mL phosphate buffered solution). These vials will

be packaged in appropriately sized cartons. HCW9218 drug product vials will be labeled, in accordance with 21 CFR §312.6.

HCW9218 drug product will be packed in a temperature-controlled box and shipped to the study site maintained at a temperature between 2°C to 8°C.

9.3 Study Site Storage and Stability

HCW9218 will be received by, stored in, and dispensed by the University of Minnesota, Fairview Investigational Drug Services (IDS) Pharmacy, a secure, limited access facility. Refer to the HCW Biologics Investigator Initiated Trial (IIT) Manual for receipt of the investigational product.

HCW9218 drug product vials will be maintained at a temperature between 2°C and 8°C, protected from excessive light and heat.

Refer to the HCW Biologics Investigator Initiated Trial (IIT) Manual for the reporting of product excursions including storage temperature outside of the recommended temperature range (2ºC – 8ºC) and/or broken or missing vials.

Note: The Investigator or designee(s) must report excursions by email to HCW by the end of the following business day following the identification and followed by event documentation using the Drug Product Excursion Form (DPEF).

9.4 HCW9218 Preparation for Injection

There are no particular hazards associated with HCW9218 and no specific considerations are necessary when handling the vialed product other than handling with gloves.

HCW9218 dose calculation is based on the patient's assigned dose level and actual body weight done on Cycle 1 Day 1 or within the 14 days prior. Dose adjustments occur only if a weight changes by ±10% or greater from the weight used to calculate the Cycle 1 dosing. See Section 6.1.

The calculated amount of HCW9218 will be drawn into a syringe for subcutaneous (SC) injection. The stock concentration is 20 mg/mL, doses will be drawn directly into the syringe for injection.

If the total SC dose is greater than 1.5 mL, the dose will be divided into 2-3 SC injections as needed. Injection sites should be rotated per institutional guidelines and each injection site separated by at least 1 inch.

9.5 Investigational Product Accountability and Reordering

IDS, on behalf the study Investigator, will maintain the records of receipt and disposition of the HCW9218 drug product including patient related dates, dose, and quantities administered. IDS tracks the HCW9218 inventory, including expiration dates, and facilitates reordering.

All partially used and unused HCW9218 drug product vials will be destroyed at the IDS per their standard procedures or returned to the HCW according to the established procedures.

Refer to the HCW Biologics Investigator Initiated Trial (IIT) Manual for instructions on reordering the drug product and the return of unused vials at the end of the study.

9.6 Possible Interactions with Other Drugs

Potential drug-drug interactions with HCW9218 have not yet been defined.

9.7 Potential Side Effects

HCW9128 has not yet been evaluated in human clinical studies.

Thus, the expected AEs are based on the published clinical experience with other related immunotherapies, including recombinant human IL-2, recombinant human IL-15, TGFβRII-Fc fusion and anti-TGF-β antibody.

All adverse events listed in Appendix II are defined as "Expected" for the purpose of event documentation and reporting requirements.

Section 7.2 presents the most common and most serious side effects seen in drugs similar to HCW9218 in table format organized by frequency (common, occasional, and rare) as presented in the treatment consent form.

For additional information refer to the Investigator Brochure.

10 Event Monitoring, Documentation, and Reporting

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events version 5 (CTCAE v5). A copy of the CTCAE can be downloaded from the CTEP home page at

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

The investigational product in this study is the HCW9218.

Event monitoring and documentation begins at the time of the 1st HCW9218 dose and continues through the End of Treatment visit. Active monitoring for adverse events ends

with the End of Treatment visit unless there are ongoing study related toxicity. After the End of Treatment visit through 12 months after the 1st dose of HCW9218, events are documented upon knowledge. Refer to the table in Section 10.2.

10.1 Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Life-Threatening Adverse Event or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious Adverse Event or Serious Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- An important medical event

Unexpected Adverse Event or Unexpected Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

Note: An IB for HCW9128 is provided by HCW Biologics.

Expedited (Rapid) Reporting: Certain events may require rapid notification to entities providing patient safety oversight (e.g. IRB). Refer to Section 10.3.

10.2 Event Monitoring and Documentation

Event monitoring and documentation begins at the time of the 1st HCW9218 dose and continues through the End of Treatment visit. Active monitoring for adverse events ends with the End of Treatment visit unless there are ongoing study related toxicity. After the EOT through 12 months from Cycle 1 Day 1 (follow-up for response and survival), events are documented upon knowledge. Refer to the table below:

Event	Active Treatment Period		During follow-up for 12 months from Cycle 1 Day 1 (events are documented upon knowledge)
	Cycle 1 Day 1 through the end of DLT observation period and the DLT assessment is completed	Cycle 2 Day 1 through though End of Treatment (EOT) visit	
Any event meeting the definition of serious regardless of attribution or expectedness	X	X	X[1]
Any death (date and cause)	X	X	X
1st incidence of a pre-defined adverse event during Fast-track enrollment per Section 6.2.1	X[2]		
Any event meeting the definition of a Dose Limiting Toxicity (DLT)	X[3]		
All ≥Grade 2 AEs regardless of attribution (see exceptions in Section 10.2.1)	X	X	
Targeted Toxicity Worksheet (Appendix IV) – record all AE grades	X (refer to schedule in Section 10.2.2)		

1. Events are documented upon knowledge if they are unexpected and at least possible related to HCW9218.
2. Used only once to trigger change from 1 patient cohort to 3 patient cohort and move to Stage 1 Step 2 or Stage 2 (if a DLT also occurs).
3. Stage 1: Any event meeting the definition of DLT is in association with the 1st treatment cycle (through the end of the DLT period) is also be reported as a DLT event contributing to subsequent dose cohort decisions per Section 12. Stage 2 (CRM component) event Section 13.4.

10.2.1 Exceptions to AE Documentation in OnCore

Laboratory abnormalities (e.g., clinical chemistry, hematology, and urinalysis) without clinical significance are not recorded as AEs or SAEs with the exception of abnormalities that meet the definition of DLT per Section 6.3.

Other abnormal assessments (e.g., vital signs, ECG, X-rays) that are not associated with signs or are considered of no clinical significance do not need to be recorded unless they are a targeted toxicity.

Clinical significance is defined as requiring medical or surgical intervention or leading to a study treatment delay or discontinuation. Any laboratory or other assessments meeting the definition of clinically significant must be recorded as an AE, as well as an SAE, if applicable.

10.2.2 Targeted Toxicity Worksheet

The targeted toxicity worksheet is done in addition to AE documentation as described in the table in Section 10.2. It is a focused assessment during the DLT period. The highest grade of toxicity since the last assessment is recorded (which for outpatients may be patient reported). The targeted toxicity worksheet is completed at each of the following timepoints based on an in person assessment at that timepoint:

- Cycle 1 Day 1
  - Prior HCW9218 injection (within 1 hour prior)
  - At 3 hours (±15 minutes) post injection
  - At 6 hours (±15 minutes) post injection
- Cycle 1 Day 2
- Cycle 1 Day 3
- Cycle 1 Day 5

The completion of the targeted toxicity worksheet aligns with in person visits and may be shifted to align with the actual day a visit occurs or skipped if a visit is not occurred.

10.3 Required Reporting: IRB, HCW Biologics and FDA

Certain events or situations require rapid (expedited) reporting as summarized in the table below as well as reporting to the FDA as a part of the IND annual report.

Agency reporting to	Criteria for reporting	Timeframe	Form to Use	Submit to
U of MN IRB	Unanticipated death of a locally enrolled subject(s); New or increased risk; Any adverse event that requires a change to the protocol or consent form – refer to the IRB website for complete details	5 Business Days	RNI	ETHOS
	Deviations that occur at UMN as defined in Section 14.1	Per current IRB requirements	OnCore Deviation Form and IRB Report Form	
FDA	Unexpected and fatal or unexpected and life threatening suspected adverse reaction	No later than 7 Calendar Days	University of Minnesota SAE Report	Submit to FDA as an amendment to IND with a copy to HCW Safety Reporting <safetyreporting@hcwbiologics.com>
	1) Serious, unexpected, and suspected adverse reaction or 2) Increased occurrence of serious suspected adverse reactions over that listed in the investigator brochure or 3) Findings from other sources (other studies, animal or in vitro testing) – not applicable for this study	No later than 15 Calendar Days		
	All other events per CFR 312.32	At time of annual report	FDA Annual report	Submit to FDA as an amendment to IND
HCW Biologics	Any event meeting the definition of an SAE	Within 24 hours of study team knowledge	University of Minnesota SAE Report	Submit to: HCW Safety Reporting <safetyreporting@hcwbiologics.com>

11 Study Data Collection and Monitoring

11.1 Data Management

This study will collect regulatory and clinical data using University of Minnesota CTSI's instance of OnCore® (Online Enterprise Research Management Environment). The OnCore database resides on dedicated secure and PHI compliant hardware. Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore.

Additional immune monitoring data about correlative laboratory samples generated by the Masonic Cancer Center Translational Therapy Laboratory (TTL) from the protocol-directed correlative research samples is stored in their Laboratory Information Management System (LIMS). The LIMS database application is also stored on a production server located in the UMN datacenter (WBOB) and is managed by the Academic Health Center

11.2 Case Report Forms

Participant data will be collected using protocol specific electronic case report forms (e-CRF) developed within the University of Minnesota OnCore based on its library of standardized forms. The e-CRFs will be approved by the Principal Investigator and the Biostatistician prior to release for use. The Study Coordinator or designee will be responsible for registering the patient into OnCore at time of study entry, completing e-CRF based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

11.3 Data and Safety Monitoring Plan

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at https://z.umn.edu/dsmp.

For the purposes of data and safety monitoring, this study is classified as high risk (Phase I under a locally sponsored IND). Therefore the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will review the study's progress quarterly with the understanding the Cancer Protocol Review Committee (CPRC).
- The Masonic Cancer Center PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.

11.4 Regular Team Meetings to Review Safety

Regular meetings will be held to facilitate communication regarding the study's progress in terms of individual patient safety including events meeting the definition of dose limiting toxicity, excessive toxicity (early study stopping rule for CRM only), serious adverse events (SAEs) and overall impact on future enrollment.

At a minimum each patient is reviewed at the end the 1st 21 days to document the DLT status and to confirm the timing and dose level for the next patient cohort enrolled.

The outcome of these discussion will be documented in the meeting minutes and the statistician or designee will send out a confirmatory email summarizing the discussion which will become part of the regulatory record.

Other issues for discussion may include pending patients, patient updates, summary of safety reports, and case report form completion.

The CRC or CRC-RN or designee is responsible for arranging these meetings and preparing the agenda. Meetings will occur every 2 weeks; however, these may be scheduled more or less frequently at the discretion of the PI and/or study staff. The

safety discussion may be part of the regular PI meeting but must be documented as above (in study minutes and post-meeting email from the statistician or designee).

Expected participation includes the Principal Investigator, the study statistician or designee and relevant CTO staff (CRC-RN, CRC, Regulatory Specialist, and Program Manager) or appropriate designee.

In the situation of a patient completing the DLT period between scheduled meetings, communication via email may be used in lieu of a meeting provided the patient was discussed at the prior meeting and will be included on the next meeting's agenda.

11.5 Record Retention

The Investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility until notified by the Masonic Cancer Center Clinical Trials Office that the study records may be destroyed.

12 Study Endpoints

12.1 Primary Endpoint

The primary endpoint is identification of the maximum tolerated dose (MTD) for HCW9218.

12.2 Secondary Endpoints

The safety of repeat dosing of HCW9218 is adverse events (AEs) based on CTCAE v5.

Response and progression endpoints are based on the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1.

- Response rate (CR, PR or SD) at 3 months, 6 months and 12 months
- Progression-free survival (PFS) by 6 month and 1 year is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first
- Overall survival (OS) at 6 month and 1 year from 1st dose of HCW9218 is defined as patients alive at 1 year

13 Statistical Considerations

13.1 Study Design, Objectives and Endpoints

The purpose of this Phase I open-label study will be to identify the maximum tolerated dose (MTD) in a combined study group of all solid tumor cancers (except pancreatic cancer) and provide preliminary estimates of the response for future studies.

The primary endpoint is to provide the MTD of HCW9218 in advanced solid tumor cancers. The primary analysis will be intent-to-treat from the point of 1st injection of HCW9218 both for toxicity and efficacy. Patients who discontinue therapy prior to injection #1 will be replaced. We expect that the number of patients coming off therapy prior to injection #1 will be minimal/none.

There are 5 potential dose levels defined for Phase I. The trial will be conducted with no intra-patient escalation. The starting dose will be 0.25 mg/kg HCW9218 with a safety dose of 0.1 mg/kg HCW9218. The subsequent planned cohorts will be 0.5 mg/kg HCW9218, 0.8 mg/kg HCW9218 and 1.2 mg/kg HCW9218 (Table 1). Given that little to no toxicity is expected, the MTD will be determined using an adaptation of the continual reassessment method (CRM) (O'Quigley, 1996) starting with 1 patient cohorts.

Table 1.

Phase I HCW9218 Dose Levels	
Dose Level	HCW9218 dose
-1	0.1 mg/kg
1 (start)	0.25 mg/kg
2	0.5 mg/kg
3	0.8 mg/kg
4	1.2 mg/kg

This Phase I study will be conducted in two consecutive stages (non-CRM and CRM). A minimum of 21 days must separate each cohort. All patients are assessed for Dose Limiting Toxicity (DLT) per Section 6.3 as defined in the schema below:

Stage 1 Step 1 uses a fast-track design (1 patient per cohort) with a minimum of 21 days between each patient until one of the following occurs:

The 1st occurrence of a **pre-defined treatment emergent adverse event** within 21 days of the 1st HCW injection (Section 6.2.1) at which point the study moves to **Step 2** and the cohort size increases from 1 to 3 patients with 2 additional patients added to the current cohort. A minimum of 21 days must separate each 3 patient cohort

- Escalation in Step 2 continues until the 1st **DLT event** at which point **Stage 2** (CRM) is activated. If Cohort 4 is completed without a DLT, Stage 2 (CRM) is not used.
- The 1st occurrence of a DLT at which point the study moves directly to **Stage 2** (CRM) as detailed below and Step 2 is not used.
- Cohort 4 is completed without a pre-defined AE or a DLT – neither Step 2 nor Stage 2 is used if a total of 9 patients are enrolled in Cohort 4 to complete the study.

Stage 2 is initiated at the 1st DLT. The study design changes to an application of the continual reassessment method (CRM) with an informative skeleton based on constrained maximum likelihood estimation (Iasonos & O'Quigley, 2012). Enrollment occurs in cohorts of three patients. Each new cohort of three patients are sequentially assigned to the most appropriate dose by the study statistician based on the updated toxicity **probabilities once the 3rd patient in a cohort reaches Day 21 (DLT period).** The goal will be to identify one of the 5 dose level strategies corresponding to the desired maximum toxicity rate of ≤25%. The MTD will be identified by the minimum of the following criteria: (1) the total Stage 2 sample size of 24 is exhausted or (2) 9 consecutive patients are enrolled at the same dose. The function 'crm' from the R package 'dfcrm' will calculate posterior means of toxicity probabilities. Dose escalation of more than one level is not permitted with this design.

13.2 Statistical Analysis

The primary analysis study population will be intent-to-treat in that all patients receiving the 1st injection of HCW9218 will be evaluable for toxicity and efficacy.

Primary Endpoint

The MTD will be determined by design.

Secondary Endpoints

If there are sufficient numbers, the secondary endpoints of overall survival and progression-free survival by 6 months and 1 year will be estimated with Kaplan-Meier curves. The primary efficacy endpoint of response rate will be estimated by a simple proportion with 95% confidence limits if sufficient numbers exist. Safety will be estimated by the proportion of adverse events based on CTCAE v5. Further descriptive statistics and plots such as boxplots, spaghetti plots and swimmer plots will be used to assess kinetics and other markers of efficacy such as immune based monitoring and toxicity.

13.3 Sample Size

A maximum of 24 patients will be enrolled with a target DLT rate of ≤25%. Based on the simulations from Table 2, this should be sufficient and safe to define the MTD. Although complete skeleton estimates will not be determined until Stage 2, Table 2 is calculated based on hypothesized values.

Table 2. Operating characteristics for Adaptive-CRM

HCW9218 Dose Levels	Expected DLT, Expected SAE[1,2]			Excessive DLT, Excessive SAE		
	True Probability	Prob. of dose[3]	N[4]	True Probability	Probability of dose	N
0.1 mg/kg (-1)	0.5%, 0.5%	0%	0	5%, 18%	3%	3
0.25 mg/kg (1, start)	0.75%, 1.5%	0%	1	10%, 29%	18%	6
0.5 mg/kg (2)	1.5%, 3%	0%	1	25%, 50%	44%	9
0.8 mg/kg (3)	3%, 5%	0%	1	40%, 66%	34%	5
1.2 mg/kg (4)	7%, 7.5%	100%	9	55%, 77%	1%	1

1. SAE's without a DLT trigger step 2 in stage 1, DLT's regardless of SAE's trigger stage 2
2. Expected/Excessive values are the hypothesized true values under the simulation
3. Probability of dose chosen as the MTD using the Adaptive-CRM design
4. The hypothesized number of patients enrolled at each dose during the trial under the assumed hypothesized "true" probabilities

13.4 Monitoring Guidelines for Stage 2 (Early Study Stopping Rules)

A stopping rule is in place to stop the trial in case there are excessive DLTs (Stage 2) as defined by updated posterior probabilities throughout the trial. At the end of the 21-day evaluation period after each cohort of patients is enrolled, new posterior probabilities will be calculated for each dose. The trial will be stopped if the posterior probability that the lowest dose is unacceptably toxic (> 25% of patients) is greater than 80%. (Zohar & Chevret, 2001).

14 Conduct of the Study

14.1 Protocol Deviations

The following definitions are from the Masonic Cancer Center's Standard Operating Procedure (SOP) Deviation Reporting:

Major Deviation: A deviation or violation that impacts the risks and benefits of the research; may impact subject safety, affect the integrity of research data and/or affect a subject's willingness to participate in the research. Deviations that place a subject at risk, but do not result in harm are considered to be major deviations.

Minor Deviation: A deviation or violation that does not impact subject safety, compromise the integrity of research data and/or affect a subject's willingness to participate in the research.

Deviations on University of Minnesota participants must be reported to the IRB as detailed in Section 10.3. One exception to the reporting of protocol deviations is

noncompliance with timeframes that are subject to clinical judgement or do not impact subject safety or data integrity. Timeframes indicated throughout this protocol are intended to be targeted timeframes unless “must” is specified.

14.2 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

14.3 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the Investigator.

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved consent to review. The Investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

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Appendix I – ECOG Performance Status

Developed by the Eastern Cooperative Oncology Group, Robert L. Comis, MD, Group Chair.*

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

*Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol.* 1982;5:649-655.

Appendix II – Expected Adverse Events for HCW9218

HCW9128 has not yet been evaluated in human clinical studies. Thus, the expected AEs are based on the published clinical experience with other related immunotherapies, including recombinant human IL-2 (Dutcher et al, 2014), recombinant human IL-15 (rhIL-15) (Conlon et al, 2019; Miller et al, 2018; Conlon et al, 2015), IL-15 mutein/IL15RαSu-Fc complex (ALT-803, nogapendekin alfa inbakicept) (Lafyatis et al, 2020; Mascarenhas et al, 2020; Yap et al, 2020), TGFβRII-Fc fusion (AVID200) (Lafyatis et al, 2020; Mascarenhas et al, 2020; Yap et al, 2020), TGFβRII-anti-PD-L1 IgG1 fusion (M7824, bintrafusp alfa) (Tan et al, 2021; Khasraw et al, 2021; Kang et al, 2020; Strauss et al, 2020), and anti-TGF-β antibody (fresolimumab) (Lacouture et al, 2015; Morris et al, 2014).

All Adverse Events listed in the Table below are defined as "Expected" for the purpose of Expedited Reporting. These AEs should be reported according to Section 10.

CTCAE v5.0 System Organ Class (SOC)	**CTCAE Term**	**Reported in Subject Receiving:**		
		IL-2	**IL-15**	**TGF-β antagonist**
Blood and lymphatic system disorders	Anemia	X	X	X
	Disseminated intravascular coagulation	X		
	Eosinophilia	X		
	Febrile neutropenia	X		
	Leukocytosis	X		
Cardiac Disorders	Atrial fibrillation	X		
	Atrial flutter	X		
	Cardiac arrest	X		
	Chest pain - cardiac	X	X	
	Conduction disorder	X		
	Mobitz type I	X		
	Mobitz (type) II atrioventricular block	X		
	Myocardial infarction	X		
	Myocarditis	X		
	Palpitations			X
	Pericardial effusion	X		
	Pericardial tamponade	X		
	Pericarditis	X		
	Restrictive cardiomyopathy	X		
	Supraventricular tachycardia	X	X	
	Sinus bradycardia	X		
	Ventricular arrhythmia	X		
	Ventricular tachycardia	X	X	
Endocrine disorders Eye disorders	Hypothyroidism	X		
	Blurred vision		X	
	Extraocular muscle paresis			X
	Optic nerve disorder	X		
	Other, mydriasis	X		
	Other, pupillary disorder	X		
	Papilledema		X	
	Uveitis		X	
Gastrointestinal disorders	Abdominal distension	X		

CTCAE v5.0 System Organ Class (SOC)	CTCAE Term	Reported in Subject Receiving:		
		IL-2	IL-15	TGF-β antagonist
	Abdominal pain	X	X	X
	Colitis	X		
	Colonic perforation	X		
	Constipation			X
	Diarrhea	X	X	X
	Duodenal hemorrhage	X		
	Duodenal ulcer	X	X	
	Esophageal fistula	X		
	Gastritis	X	X	
	Lower gastrointestinal hemorrhage	X		
	Nausea	X	X	X
	Mucositis oral	X	X	
	Obstruction gastric	X		
	Oral hemorrhage			X
	Pancreatitis	X	X	
	Rectal perforation	X		
	Retroperitoneal hemorrhage	X		
	Small intestinal perforation	X		
	Intra-abdominal hemorrhage	X		
	Upper gastrointestinal hemorrhage	X		
	Visceral arterial ischemia	X	X	
	Vomiting	X	X	X
General disorders and administration site conditions	Chills	X	X	
	Edema limbs	X	X	
	Fatigue	X	X	X
	Fever	X	X	X
	Flu like symptoms	X	X	X
	Hypothermia	X		
	Localized edema	X		
	Malaise	X		
	Multi-organ failure	X		
	Injection site reaction	X	X	X
	Pain	X	X	
Hepatobiliary disorders	Cholecystitis	X		
	Hepatic failure	X		
Immune system disorders	Allergic reaction	X		
	Anaphylaxis	X		
	Cytokine release syndrome	X	X	
Infections and infestations	Bacteremia	X	X	
	Endocarditis infective	X		
	Hepatic infection	X		
	Lung infection	X	X	
	Meningitis	X		
	Shingles			X
	Skin infection	X		
	Sepsis	X	X	

CTCAE v5.0 System Organ Class (SOC)	CTCAE Term	Reported in Subject Receiving:		
		IL-2	IL-15	TGF-β antagonist
Investigations	Alanine aminotransferase increased	X	X	
	Alkaline phosphatase increased	X	X	
	Aspartate aminotransferase increased	X	X	
	Blood bilirubin increased	X	X	
	Cardiac troponin I increased		X	
	CPK increased			X
	Creatinine increased	X	X	
	Lymphocyte count decreased		X	
	Neutrophil count decreased	X	X	
	Platelet count decreased	X	X	X
	Urine output decreased	X	X	
	Weight gain	X		
	White blood cell decreased	X	X	
Metabolism and nutrition disorders	Acidosis	X		
	Anorexia	X	X	
	Hyperglycemia	X		
	Hyperuricemia	X		X
	Hypoalbuminemia		X	
	Hypocalcemia	X	X	
	Hypomagnesemia	X		
	Hypophosphatemia		X	
Musculoskeletal and connective tissue disorders	Arthralgia		X	
	Arthritis	X		
	Myositis	X		
	Rhabdomyolysis	X		
Neoplasms benign, malignant	Tumor hemorrhage			X
Nervous system disorders	Cognitive disturbance	X		
	Dizziness	X		X
	Edema cerebral	X		
	Encephalopathy	X		
	Headache		X	X
	Intracranial hemorrhage	X		X
	Neuralgia	X		
	Peripheral motor neuropathy	X		
	Seizure	X		
	Stroke	X		
	Syncope	X		
	Transient ischemic attacks	X		
Psychiatric disorders	Agitation	X		
	Anxiety	X		
	Confusion	X	X	
	Delirium	X		
	Depressed level of consciousness	X		
	Depression	X		
	Insomnia	X		
	Psychosis	X		

CTCAE v5.0 System Organ Class (SOC)	CTCAE Term	Reported in Subject Receiving:		
		IL-2	IL-15	TGF-β antagonist
	Suicidal ideation	X		
Renal and urinary disorders	Acute kidney injury	X		
	Chronic kidney disease	X		
Respiratory, thoracic and mediastinal disorders	Adult respiratory distress syndrome	X		
	Allergic rhinitis	X	X	
	Apnea	X		
	Cough	X		
	Dyspnea	X	X	
	Epistaxis			X
	Laryngeal hemorrhage	X		X
	Pleural effusion		X	
	Pneumothorax	X		
	Pulmonary edema	X		
	Respiratory failure	X		
	Pharyngeal mucositis	X		
	Tracheal fistula	X		
Skin and subcutaneous tissue disorders	Dry skin		X	
	Erythroderma	X		
	Hyperkeratosis			X
	Pruritus	X	X	X
	Other, actinic keratosis			X
	Other, basal cell carcinoma			X
	Other, keratoacanthoma			X
	Other, lichenoid keratosis			X
	Other, squamous cell carcinoma			X
	Rash acneiform	X		
	Rash maculo-papular	X	X	X
	Stevens-Johnson syndrome	X		
	Urticaria	X		
Vascular Disorders	Arterial thromboembolism	X		
	Capillary leak syndrome	X		
	Hypertension		X	
	Hypotension	X	X	
	Other, mucosal bleeding		X	X
	Phlebitis	X		
	Thromboembolic event	X		
	Vasculitis	X		

Appendix III – Response Evaluation Criteria in Solid Tumors (RECIST) Quick Reference (CTEP)

Eligibility

- Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint.

  Measurable disease - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

  Measurable lesions - lesions that can be accurately measured in at least one dimension with longest diameter ≥20 mm using conventional techniques or ≥10 mm with spiral CT scan.

  Non-measurable lesions - all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques; and.

- All measurements should be taken and recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.
- The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.
- Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Methods of Measurement –

- CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis. Head and neck tumors and those of extremities usually require specific protocols.
- Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.
- The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.
- Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response when all lesions have disappeared.
- Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

Baseline documentation of "Target" and "Non-Target" lesions

- All measurable lesions up to a maximum of five lesions per organ and 10 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 clinically).
- A sum of the longest diameter (LD) for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.
- 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
* Partial Response (PR):	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
* 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 and normalization of tumor marker level
* Incomplete Response/ Stable Disease (SD):	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
* Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions (1)

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

Evaluation of best overall response

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

Target lesions	Non-Target lesions	New Lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/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

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration”. Every effort should be made to document the objective progression even after discontinuation of treatment.
- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Confirmation

- The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.
- To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.
- In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol

Duration of overall response

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

Duration of stable disease

- SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.
- The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

Response review

- For trials where the response rate is the primary endpoint it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

Reporting of results

- All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).
- All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.
- All conclusions should be based on all eligible patients.
- Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.
- The 95% confidence intervals should be provided.

Appendix IV – Targeted Toxicity Worksheet

See Section 10.2 for time points based on CTCAE v5

Patient Initials or ID: _____ **Date of Assessment:** _____ **Assessment Time Point:** _____

ADL = activities of daily living

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Injection site reaction	None	Tenderness with or without associated symptoms (e.g., warmth, erythema, itching)	Pain; lipodystrophy; edema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated
Flu-like symptoms*	None	Mild flu-like symptoms present	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting instrumental ADL	……
*includes fever, chills, body aches, malaise, loss of appetite and dry cough					
Fatigue	None	Fatigue relieved by rest	Fatigue not relieved by rest; limiting instrumental ADL	Fatigue not relieved by rest, limiting self care ADL	……
Fever	None	38.0 - 39.0◦ C (100.4 - 102.2◦ F)	> 39.0 - 40.0◦C (102.3 - 104.0◦F)	> 40.0 ◦ C (>104.0◦ F) for ≤ 24 hrs	> 40.0 ◦ C (>104.0◦ F) for > 24 hrs
Headache	None	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	……
Palpitations	None	Mild symptoms; intervention not indicated	Intervention indicated	……	……
Nausea	None	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated	……
Vomiting	None	Intervention not indicated	Outpatient IV hydration; medical intervention indicated	Tube feeding, TPN, or hospitalization indicated	Life-threatening consequences; urgent intervention indicated
Diarrhea	None	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline; limiting instrumental ADL	Increase of >=7 stools per day over baseline; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Constipation	None	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas; limiting instrumental ADL	Obstipation with manual evacuation indicated; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Other Skin/ Subcutaneous – Specify _____	None	Per CTCAE v5 criteria	Per CTCAE v5 criteria	Per CTCAE v5 criteria	Per CTCAE v5 criteria

Person Completing Form: _____

Signature of Person Completing This Form: _____

Appendix V – FACT-G Quality of Life Questionnaire

FACT-G (Version 4)

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

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

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-G (Version 4)

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

	EMOTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

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

Appendix VI – Skin Condition Questionnaire

The below is the content of the questionnaire – the actual questionnaire will be administered through REDCap.

Patients may have changes in the condition of their skin following treatment with the study medication.

Please indicate the extent to which you have experienced these changes during the past 4 weeks. Please describe any changes you have experienced.

During the past 4 weeks:

1. Have you experienced any changes in the condition of your skin at the site of study medication injection? Yes☐ No☐

If Yes, please describe the changes:

2. Aside from the injection site, have you experienced any new changes in the condition of your skin following treatment with the study medication? Yes☐ No☐

If Yes, please describe the changes:

3. Have you experienced any changes in pre-existing skin conditions following treatment with the study medication? Yes☐ No☐

If Yes, please describe the changes: