CLINICAL STUDY PROTOCOL

IN-SITU CANCER VACCINE: PHASE I/IIB OPEN-LABEL STUDY TO ASSESS THE SAFETY OF ALLOSTIM® IN COMBINATION WITH CRYOABLATION AS THIRD LINE THERAPY FOR METASTATIC COLORECTAL CANCER

PROTOCOL #ITL-019-CORK-CRYVAC

IND Number:	13936
Drug Development Phase:	Phase IIA
Investigational Products:	AlloStim®
Investigational Treatment:	AlloStim® and targeted tumor cryoablation
Indication:	Metastatic colorectal cancer third line therapy
Sponsor:	Immunovative Therapies, Ltd. Malcha Technology Park Building 1, First Floor Jerusalem, Israel 96951
Original Protocol Date:	**December 23, 2014 v.2**
Amendment 1.0 Date:	**September 16, 2015**
Amendment 2.0 Date:	**September 24, 2015**
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Amendment 5.0 Date:	**June 14, 2016**
Amendment 6.0 Date:	**October 25, 2016**
Amendment 7.0 Date:	**May 18, 2017**
Amendment 8.0 Date:	**September 12, 2017**

CONFIDENTIAL INFORMATION:

The information in this document contains commercial information and trade secrets that are privileged or confidential and may not be disclosed unless such disclosure is required by applicable laws and regulations. In any event, persons to whom the information is disclosed must be informed that the information is privileged or confidential and may not be further disclosed by them. These restrictions on disclosure will apply equally to all future information supplied which is indicated as privileged or confidential.

STUDY CONTACTS

This study is to be performed in accordance with Good Clinical Practice (GCP), the ethical principles that have their origin in the Declaration of Helsinki, Title 21 of the Code of Federal Regulations §§ 50, 56, and 312, and the International Conference on Harmonization E6 guidelines.

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1. PROTOCOL APPROVAL SIGNATURE PAGE

SPONSOR: IMMUNOVATIVE THERAPIES, LTD.

I have read and understand the contents of this clinical protocol for study No. ITL-019-CORK-CRYVAC dated September 12, 2017 and I agree to meet all obligations of Immunovative Therapies, Ltd. as detailed in all applicable regulations and guidelines. In addition, I will inform the Principal Investigator and all other Investigators of all relevant information that becomes available during the conduct of this study.

Approved By:

Print Name: DR. MICHAEL HAR-NOY

Signature: _____ Date: _____

Title: Chief Executive Officer

2. PRINCIPAL INVESTIGATOR'S AGREEMENT

I have read and understand the contents of this clinical protocol for study No. ITL-019-CORK-CRYVAC dated September 12, 2017 and will adhere to the study requirements as presented, including all statements regarding confidentiality. In addition, I will conduct the study in accordance with current Good Clinical Practices and applicable US FDA regulatory requirements:

Print Name: _____

Signature: _____ Date: _____

3. PROTOCOL ABSTRACT

Sponsor:	**Investigational Product:**	**Study Phase:**
Immunovative Therapies, Ltd.	AlloStim®	Phase IIA

Study Title:

In-Situ Cancer Vaccine: Phase IIA, Open-Label Study to Assess the Safety of AlloStim® in Combination with Cryoablation as Third Line Therapy for Metastatic Colorectal Cancer

Protocol Number:

ITL-019-CORK-CRYVAC

Study Phase:

Phase IIA

Indication:

Metastatic colorectal cancer third line therapy

CryoVax™:

CryoVax™ is the name provided by the Sponsor to protocols that incorporate an in-situ vaccination step. This protocol includes an in-situ cancer vaccine step, which combines a percutaneous ablation of a single metastatic tumor lesion with intralesional immunotherapy serving as an adjuvant. In this protocol, the ablation step is conducted using a percutaneous cryoablation procedure (CA). The ablation of the selected tumor lesion causes release of tumor-specific antigens into the microenvironment (antigen source) and the intralesional immunotherapy serves as an adjuvant to promote maturation of dendritic cells (DC) that engulf and process released tumor antigens. The mature DC migrate to the draining lymph nodes to elicit a Th1-type tumor-specific immune response. The combination of ablation and intralesional immunotherapy thus creates an in-situ, patient-specific, anti-tumor vaccine.

Some subjects enrolled in this study are scheduled to undergo cryoablation, while other subjects are scheduled to receive IV doses to try to create an in-vivo, patient-specific, anti-tumor in-situ vaccine.

T-Stim™:

T-Stim™ cells are an intermediate product in the process of making the study drug called AlloStim®. T-Stim™ is ex-vivo differentiated and expanded memory CD4 T-cells that uniquely function as both Th1 cells (produce IFN-gamma and not IL-4) and natural killer cells (contain granzyme B and perforin and lyse tumor cells and not normal cells in-vitro). T-Stim™ cells are derived from the blood of normal donors (allogeneic) and are intentionally mismatched to the recipients. The T-Stim™ production process involves first purifying CD4+ T-cells from buffy coat material collected from known normal screened blood donors. The CD4+ cells are cultured in the presence of Dynabeads® *ClinExVivo*™ CD3/CD28 beads for 9 days. After the 9-day culture process, the CD4+ cells expand approximately 50-fold and differentiate into T-Stim™ memory CD4 cells with Th1 and NK-like properties. The T-Stim™ cells are harvested from the culture, washed, the beads are removed and the cells are aliquoted into individual dose vials. The dose vials are stored frozen in liquid nitrogen. Frozen T-Stim™ doses are stable for at least 18 months. T-Stim™ cells are produced in Jerusalem, Israel under cGMP and are certified USP sterile and low endotoxin (validated gel clot method) before release. All blood donors are screened pursuant to 21 CFR 1271 and tested to be free from blood-borne infectious diseases pursuant to US regulations (HIV1,

HIV2, HTLV1, HTLV2, HBV, HCV, syphilis, and additionally screened for CMV. Each batch is also tested to meet pre-determined identity and functional requirements prior to release.

AlloStim®:

AlloStim® is formulated T-Stim™ cells that have been activated by adding CD3/CD28-conjugated microbeads and packaged in a syringe. AlloStim® is prepared by thawing frozen, QC released T-Stim™ cells and incubating the cells for 4 hours with Dynabeads® *ClinExVivo*™ CD3/CD28. The beads are mixed with the cells at a 2:1 bead to cell ratio. After 4 hours, the cells with the beads attached are harvested, washed and suspended in formulation buffer containing PlasmaLyteA® supplemented with 1% human serum albumin. The formulated cells with the beads still attached are then loaded in a syringe for infusion or injection. A sample from the syringe lot is tested for bacterial contamination with a Rapid Microbiological Method (RMM) with results at 24 -48 hours. AlloStim® syringes are packaged in validated temperature-controlled containers and shipped by specialized courier service to the study site at least 48 hours prior to the protocol required treatment day for each enrolled subject. The syringe lots are quarantined at the study site until notification of RMM release. The cells are stable for at least 72 hours in this final formulation. AlloStim® is available in the following dosage forms:

- 1 ml formulation buffer containing 1 x 10^7 cells with 2 x 10^7 beads attached in a 3 ml syringe for intradermal injections (ID) and intratumoral (IT) injection,
- 3 ml formulation buffer containing 3 x 10^7 cells with 6 x 10^7 beads attached in a 10 ml syringe for intravenous injection (IV) and intratumoral (IT) injection,
- 5 ml of formulation buffer containing 5 x 10^7 cells attached to 1 x 10^8 beads in a 10 ml syringe for intravenous infusion (IV).

Note: some cohorts call for a 0.5 ml intradermal dose. For these cohorts, a 1 ml syringe will be provided and the clinic is instructed to administer only 0.5 ml and discard the remaining 0.5 ml.

Study Purpose:

This is a Phase IIa open label, study designed to compare AlloStim+cryoablation vs AlloStim alone

Brief Description of the Protocol:

This is a single center, open label study comparing CryoVax™ personalized anti-tumor vaccine protocol combining the cryoablation of a selected metastatic lesion with intra-lesional immunotherapy with AlloStim® alone. The treatment schedules both have the following steps: (1) priming with intradermal AlloStim®; (2) vaccination combining cryoablation with intratumoral AlloStim® or intravenous AlloStim® dosing in the place of cryoablation; (3) activation with intravenous AlloStim®; and (4) booster with intravenous AlloStim®. All subjects are followed for safety during the dosing period and until 28 days following the last dose (Safety Evaluation Period"). All subjects are continued to be followed for survival after the Safety Evaluation Period.

Dosing Schedule A-1: Treatment With Cryoablation
The priming step: Subjects will receive an ID injection of AlloStim® (0.5 ml) on Days 0, 7, and 14 all in different locations.
The vaccination step: Subjects will receive cryoablation and intratumor (IT) injection of AlloStim® (1 ml) on Day 21.
The activation step: Subjects will receive an IV infusion of AlloStim® (3ml) on Day 28.

The booster step: Subjects will receive two (optional) IV booster infusions of AlloStim® (3ml), one on Day 56, a second on Day 84.

Dosing Schedule A-2: AlloStim® without cryoablation
The priming step: Subjects will receive an ID injection of AlloStim® (1 ml) on Days 0, 3, 7, 10 and 14.
The vaccination step: Subjects will receive an IV injection of AlloStim® (3 ml) of on Day 21.
The activation step: Subjects will receive an IV infusion of AlloStim® (3ml) on Day 28.
The booster step: Subjects will receive three (optional) IV booster infusions of AlloStim® (3ml), one on Day 49, a second on Day 77.

Two types of toxicity are assessed for determination of whether a Dose Limiting Toxicity (DLT) has occurred. An acute dose limiting toxicity (ADLT) is assessed within 48h of a dose administration during the priming, vaccination and activation steps of each protocol. Cumulative dose limiting toxicity (CDLT) is assessed during the complete Safety Evaluation Period including any ADLT that occurs in the booster dosing.

An ADLT is defined as the occurrence of any of the following within 48h of dosing:

- Any ≥ Grade 3 local injection site reaction;
- Any ≥ Grade 3 infusion related symptoms of duration > 2 days;
- Any ≥ Grade 4 infusion reaction of any duration

For any Grade 1-2 acute toxicity, the study drug administration schedule can continue to the next scheduled dose. If an ADLT is observed, the subject will be followed until the toxicity is resolved or the subject is stable. No further dosing for that subject will occur after an ADLT event.

A CDLT event is defined as the occurrence of any of the following within the Safety Evaluation Period:

- Any ≥ Grade 3 graft vs. host disease
- Any toxicity ≥ Grade 3 that is probably or definitely related to the study drug;
- Any toxicity not present at baseline that occurs at ≥ Grade 4
- Any autoimmune toxicity ≥ Grade 3 not present at baseline

All adverse events (AEs) and serious adverse events (SAEs), regardless of attribution to the study drug, will be coded, graded and become part of the final safety report for each cohort. The Data Safety Monitoring Board (DSMB) will review the safety data on each cohort and recommend whether to advance enrollment to the next cohort.

The development of any Grade 4 or greater toxicity in any subject at any time will prompt a review of safety data by the Sponsor, PI and the DSMB prior to accrual of any additional subjects. It is recognized that Grade 4 events and death can occur in this population due to disease progression. Therefore, dosing will continue for currently enrolled subjects at the occurrence of a grade 4 event according to protocol unless the PI determines the Grade 4 or greater event is definitely or probably related to the study drug. If attribution is assigned to the study drug dosing will be suspended pending review by the DSMB.

In accordance with the 3+3 design, three subjects will be accrued into the first dosing schedule. If during the priming, vaccination and activation steps no ADLT are observed in any of the three subjects, accrual into the next cohort is allowed. If an ADLT occurs in two of the three subjects, no further dose frequency escalation is allowed. If ADLT occurs in one of

the three subjects, three additional subjects will be accrued into the same cohort. If no ADLT occurs within the additional three subjects, accrual into the next cohort can begin. If an ADLT is observed in any of the additional three subjects, no further dose escalation is permitted.

The development of any DLT toxicity in ≥ 33% of the accumulated number of subjects in any and all dosing schedules during the Safety Evaluation Period will result in protocol discontinuation.

At the completion of the Safety Evaluation Period for all cohorts, the safety evaluation of Part 1 will be considered completed and all enrollment will be closed. Efficacy evaluation will continue monthly for each subject until death or loss to follow-up.

Objectives:

Primary Objectives

1. To evaluate the safety and anti-tumor effect of AlloStim® with or without cryoablation

Exploratory Objectives

1. To assess the longitudinal changes in tumor burden by RECIST and compare these changes with the histopathological analysis of corresponding biopsies.
2. To assess whether immune response correlates with RECIST or histopathology and Overall Survival (OS).

Study Population:

Adult patients ≥18 years and ≤80 years with adequate performance status (ECOG <2) and adequate organ function with metastatic, histologically confirmed adenocarcinoma of the rectum or colon presenting with metastatic disease and has been previously treated with two lines of active chemotherapy with or without bevacizumab and also present with a liver tumor lesion safely accessible for percutaneous cryoablation.

Total Number of Subjects:

Part 1 will accrue 3 subjects in A-1 and 3 subjects in A-2 and continue with an additional up to 25 subjects in the favored protocol.

Study Duration:

Part 1 is projected to last 30 to 72 weeks. It is projected that approximately one subject will be accrued every two weeks. Part 2 is projected to last an additional 72-104 weeks Subjects, as long as they are available for follow-up, will be followed monthly for 12 months following last dose administration for survival and quarterly thereafter.

Inclusion Criteria:

Each subject must meet the following criteria to be enrolled in this study:

1. Adult males and female subjects aged 18-80 years at screening visit
2. Pathologically confirmed diagnosis of colorectal adenocarcinoma
3. Presenting with metastatic disease:
    - Primary can be intact or previously resected
    - Metastatic lesion(s) in liver must be non-resectable
    - Extrahepatic disease acceptable
4. At least one liver lesion able to be visualized by ultrasound and determined to be

safely assessable for percutaneous cryoablation (if schedule includes cryoablation)

5. Previous treatment failure of two previous lines of active systemic chemotherapy:
   - Previous chemotherapy must have included an oxaliplatin-containing (e.g. FOLFOX) and an irinotecan-containing (e.g. FOLFIRI) regimen
     - With or without bevacizumab
     - Administered in adjuvant setting or for treatment of metastatic disease
     - If KRAS wild type, must have at least one prior anti-EGFR therapy
     - Treatment failure can be due to disease progression or toxicity
     - Disease progression on second line therapy must be documented radiologically and must have occurred during or within 30 days following the last administration of treatment for metastatic disease
6. ECOG performance score: 0-1
7. Adequate hematological function:
   - Absolute granulocyte count $\geq 1{,}200/mm^3$
   - Platelet count $\geq 100{,}000/mm^3$
   - PT/INR ≤ 1.5 or correctable to <1.5 at time of interventional procedures
   - Hemoglobin ≥ 9 g/dL (may be corrected by transfusion)
8. Adequate Organ Function:
   - Creatinine ≤ 1.5 mg/dL
   - Total bilirubin ≤ 1.5 times upper limit of normal (ULN)
   - Alkaline phosphatase ≤ 2.5 times ULN *
   - Aspartate aminotransferase (AST) or (SGOT) ≤ 2.5 times ULN *
   - Alanine aminotransferase (ALT) or (SGPT) ≤ 2.5 times ULN*

     *≤ 5 ULN if liver involvement)
9. EKG without clinically relevant abnormalities
10. Female subjects: Not pregnant or lactating
11. Patients with child bearing potential must agree to use adequate contraception
12. Study specific informed consent in the native language of the subject.

Exclusion Criteria:

Subjects with any of the following will be excluded from the participation in the study:

1. Bowel obstruction or high risk for obstruction
2. Moderate or severe ascites requiring medical intervention
3. Clinical evidence or radiological evidence of brain metastasis or leptomeningeal involvement
4. Symptomatic asthma or COPD
5. Pulmonary lymphangitis or symptomatic pleural effusion (grade ≥ 2) that results in pulmonary dysfunction requiring active treatment or oxygen saturation <92% on room air
6. Bevacizumab (Avastin®) treatment within 6 weeks of scheduled cryoablation procedure (if schedule includes cryoablation)
7. Regorafenib prior to the Study Period
8. Previous treatment with TAS102
9. Any of the following mood disorders: active major depressive episode, history of

suicidal attempt or ideation

10. Taking anticoagulant medication for concomitant medical condition (unless can be safely discontinued for invasive cryoablation, biopsy and intratumoral injection procedures)
11. Prior allogeneic bone marrow/stem cell or solid organ transplant
12. Chronic use (> 2 weeks) of greater than physiologic doses of a corticosteroid agent (dose equivalent to > 5 mg/day of prednisone) within 30 days of the first day of study drug treatment
    - Topical corticosteroids are permitted
13. Prior diagnosis of an active autoimmune disease (e.g., rheumatoid arthritis, multiple sclerosis, autoimmune thyroid disease, uveitis). Well controlled Type I diabetes allowed
14. Prior experimental therapy
15. History of blood transfusion reactions
16. Known allergy to bovine products
17. Progressive viral or bacterial infection
    - All infections must be resolved and the subject must remain afebrile for seven days without antibiotics prior to being placed on study
18. Cardiac disease of symptomatic nature
19. History of HIV positivity or AIDS
20. Concurrent medication known to interfere with platelet function or coagulation (e.g., aspirin, ibuprofen, clopidogrel, or warfarin) unless such medications can be discontinued for an appropriate time period based on the drug half-life and known activity (e.g., aspirin for 7 days) prior to cryoablation and biopsy procedures
21. History of severe hypersensitivity to monoclonal antibody drugs or any contraindication to any of the study drugs
22. Psychiatric or addictive disorders or other condition that, in the opinion of the investigator, would preclude study participation.
23. Subjects that lack ability to provide consent for themselves.

Endpoints:

Primary endpoint: Safety
Exploratory Endpoints: Correlation of immune response with tumor burden by RECIST, histopathology and OS.

Safety Assessments:

The following evaluations will be used to assess the safety of the investigational product:

- Physical examination, vital signs and laboratory evaluations (including a CBC with differential and comprehensive metabolic panel (CMP), coagulation factors, C-reactive protein (CRP), total IgG and IgM, erythrocyte sedimentation rate, serum lipase, and autoimmune blood screens).
- Adverse Events (AEs) recording according to the National Cancer Institute (NCI) Terminology Criteria for Adverse Events (CTCAE) version 4.03.
- Interrogation of subject, caregivers and family regarding any changes in health condition, in person or by telephone.

Radiology Assessment:

All subjects will undergo a CT scan of the head, chest, abdomen and pelvis with and without contrast (contrast can be withheld if medically indicated) at baseline and at Day 91 at same time as biopsy.

As it is difficult to determine if subjects are responding or progressing after immunotherapy by standard radiological methods and RECIST criteria, the analysis of the CT scans is conducted as an experimental end-point. It is known that after immunotherapy that subjects can develop new lesions, experience lymphadenopathy and interval size increases in tumors. These changes would normally be documented as progression. However, in immunotherapy, the changes could represent "pseudo-progression". In some cases, the changes could indicate actual progression, but a response can still occur months after immunotherapy. Other times, immunotherapy can cause a stable disease condition that results in increased survival.

The changes in the images will be analyzed by RECIST and other accepted and novel experimental methods in order to retrospectively determine a method that best correlates with survival outcomes. The CT scans are scheduled on the same day as biopsy procedures in order to correlate the radiological images with pathological response. It is believed that patterns of necrosis, fibrosis and immune cell infiltration patterns with HU numbers on the radiographic images may provide correlates that could distinguish immune response from progression or be predictive of a survival response. Radiographic images can be reviewed by the site radiologists according to established methods. At the discretion of the PI, select subjects may be further evaluated by MRI or PET in order to attempt to distinguish between radiological "pseudo-progression" due to an immune response and actual progression.

Histopathology Assessment:

Subjects will undergo mandatory tumor biopsies of a target lesion at baseline, during intratumoral injections (Schedule A-1) and at Day 91. The intratumoral procedures use a trocar that will have already been inserted into the tumor to conduct the intratumoral injection and thus these biopsies will not add an additional interventional procedure on day 21 Schedule A-1

Additional biopsies will be conducted only if subjects separately consent. Failure to consent for the additional biopsy procedures will not disqualify subjects.

The harvested tissue will be prepared in paraffin blocks and read centrally by a pathologist appointed by the Sponsor. The amount of tumor burden estimated from the pathological evaluation will be compared with the amount of burden shown on the CT images. The amount and type of immune cell infiltration (immunohistochemical analysis), existence of coagulative necrosis, necrotic tumor and fibrosis will also be documented and correlated to the radiological images.

Immunological Monitoring Assessments:

Research blood draws for monitoring of immunological response will be drawn at day 0 prior to study drug injection (baseline). A research blood draw is scheduled prior to study drug administration on Day 0, Day 28, Day 56 and Day 84 in Schedule A-1 and Day 0,21, 49 and 77 in Schedule A-2.

Immunological tests include: engraftment study (HLA), serum cytokines and polyclonal

Th1/Th2 balance. Seroconversion to IL-12 positivity will be evaluated as a possible biomarker that can predict response and extended survival. Development of a biomarker is important, as standard measures such as RECIST and tumor markers don't necessarily correlate with immune response mechanisms.

The Sponsor will prepare and label blood collection tubes for each research blood draw day. The collected research blood will be processed per Sponsor's SOP at or near the site in a laboratory approved by the Sponsor. The laboratory will isolate and store PBMC, serum and plasma for subsequent immunological testing. These samples will be transported in bulk using a dry shipper to the Sponsor's laboratory in Jerusalem for analysis. The research blood collection days are designed to obtain information on the mechanism of action of the drug. It is important that the minimum required amount of blood is collected and processed in accordance with the SOPs. The PI should consider placement of port or indwelling catheter in order to facilitate these blood draws.

Clinical Laboratory Tests:

Changes in laboratory levels are evaluated in order to determine if the study drug is related to, or might cause, any changes in serum chemistry, hematological function, liver or kidney function, or maintenance of immune self-tolerance (autoimmune tests). All subjects will be monitored for changes in laboratory values in CBC, CMP (including liver function test, kidney function tests) lipase, total IgG and IgM, erythrocyte sedimentation rate, and CRP at baseline and weekly during the Safety Evaluation Period. Coagulation studies (PT, PTT, and INR) are conducted at baseline and prior to interventional procedures (biopsy, cryoablation and intratumoral injections). An autoimmune panel will be assessed on day 0 (baseline, prior to drug administration) and on the last day of the Safety Evaluation Period.

The laboratory reports should be de-identified and provided to the Sponsor. The Sponsor will provide graphs indicating the changing values of select markers. The graphs will be provided to the PI or designee for review and indication of clinical significant findings.

Statistical Analysis:

Safety analyses will be performed on the safety population, defined as all subjects in the Intent to Treat (ITT) population that received any dose of study medication. The number of AEs reported will be summarized. Subjects will be censored is still alive at data cut-off or if they withdraw consent. The incidence and percentage of subjects with at least one occurrence of a preferred term will be included, according to the most severe NCI-CTCAE Version 4.03 grade.

Causality (relationship to study drug) will be summarized separately. Duration of AE will be determined and included in the listings along with action taken and outcome.

Laboratory results will be classified according to NCI-CTCAE Version 4.03. Incidence of laboratory abnormalities will be summarized; laboratory results not corresponding to an NCI-CTCAE Version 4.03 terms will not be graded. Laboratory toxicity shifts from baseline to worst grade will also be provided.

Any clinically significant results from physical examination and vital signs measurements will be tabulated.

Survival

Subjects will be followed for survival after the Safety Evaluation Period by monthly telephonic contact for first 12 months and quarterly thereafter.

4. GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviations	Explanation
aBMT	Allogeneic Bone Marrow Transplantation
ADLT	Acute Dose Limiting Toxicity
AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMA	Anti-Mitochondrial Antibody
ANA	Anti-Nuclear Antibody
APC	Antigen Presenting Cells
ART	Anthracycline And Taxane Resistant
ASA	American Society of Anesthesiologists
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CA	Cryoablation
CBC	Complete Blood Count
CDLT	Cumulative Dose Limiting Toxicity
CE	Covered Entities
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CM	Clinical Monitor
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
CPT	Cell Preparation Tube
CRC	Colorectal Cancer
CRF	Case Report Form
CRO	Contract Research Organization
CRP	C-Reactive Protein
CT	Computer Tomography
CTCAE	Common Terminology Criteria For Adverse Events
CTL	Cytotoxic T-Lymphocyte
Da	Dalton
DCs	Dendritic Cells
DHHS	Department Of Health And Human Services
dL	Deciliter
DLT	Dose Limiting Toxicity
DPBS	Dulbecco's Phosphate Buffered Saline
DSM	Data Safety Monitoring

Abbreviations	Explanation
DSMB	Data Safety Monitoring Board
DTR	Deep Tendon Reflexes
eCRF	Electronic Case Report Form
ECOG	Eastern Cooperative Oncology Group
EDC	Electronic Data Capture
e.g.	For Example
EGFR	Epidermal Growth Factor Receptor
EKG	Electrocardiogram
EPAR	European Public Assessment Report
FDA	Food and Drug Administration
H&P	History And Physical Examination
µg	microgram
g	gram
GCP	Good Clinical Practice
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GMP	Good Manufacturing Practice
GVHD	Graft Versus Host Disease
GVT	Graft Versus Tumor
h	Hour
H-2	Histamine Blocker
HBV	Hepatitis B Virus
β-HCG	Beta-Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HIPAA	Health Insurance Portability And Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HMW	High Molecular Weight
HRQoL	Health-Related Quality of Life
HSA	Human Serum Albumin
HTLV	Human T-Lymphotropic Virus
HVG	Host Versus Graft
HVT	Host Versus Tumor
IC/EC	Inclusion Criteria /Exclusion Criteria
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ID	Intradermal Injection
IEC	Independent Ethics Committee
IFN-γ	Interferon-Gamma
Ig	Immunoglobulin

Abbreviations	Explanation
IL	Interleukin
INR	International Normalized Ratio
IRB	Institutional Review Board
irRC	Immune-Related Response Criteria
IT	Intratumoral Injection
ITT	Intent To Treat
IU	International Unit
IV	Intravenous Infusion
L	Liter
LFT	Liver Function Test
LKM	Anti-Liver-Kidney Microsome Antibodies
MCRC	Metastatic Colorectal Cancer
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
ml	Milliliter
mm	Millimeter
MoAb	Monoclonal Antibody
N	Number
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NK	Natural Killer Cells
NS	Normal Saline
NSAID	Nonsteroidal Anti-Inflammatory Drugs
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PDGF	Platelet Derived Growth Factor
PERRLA	Pupils Equal Round Reactive To Light And Accommodation
PET	Positron Emission Tomography
PFS	Progression Free Survival
PHI	Protected Health Information
PI	Principal Investigator
PO	Oral Route (Per Os)
PP	Per Protocol
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QC	Quality Control
QoL	Quality of Life
RECIST	Response Evaluation Criteria In Solid Tumors

Abbreviations	Explanation
RMM	Rapid Microbiological Method
RN	Registered Nurse
RPR	Rapid Plasma Reagin
RR	Response Rate
RT	Room Temperature
RTK	Receptor Tyrosine Kinase
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SMA	Anti Smooth Muscle Antibody
SOP	Standard Operating Procedure
TEAE	Treatment Emergent Adverse Events
TGF-β	Transforming Growth Factor Beta
Th1/Th2	T Helper Cells (Type 1 And 2)
TKO	To Keep Open
TNFα	Tumor Necrosis Factor-Alpha
TTP	Time to Progression
ULN	Upper Limit Normal
US	United States
USA	United States Of America
USP	United States Pharmacopoeia
v	Version
VEGF	Vascular Endothelial Growth Factor
VOD	Veno-Occlusive Disease
vs	Versus
w	With
w/o	Without
yo	Year Old
yr	Year

5. TABLE OF CONTENTS

6. INTRODUCTION

6.1. INVESTIGATIONAL PRODUCTS

6.1.1. AlloStim®

The active ingredient in AlloStim® is viable polyclonal CD4+ memory Th1-like T-cells with both cytolytic (granzyme B+, perforin+) and Th1 helper (IFN-γ+, IL-4-) properties. In its final formulation, AlloStim® is a combination biological drug (somatic cell therapy) and medical device (microbeads). Source cells are CD4+ T-cells positively selected from buffy coat material prepared from normal, screened, volunteer blood donors. These CD4+ T-cells are differentiated and expanded ex-vivo in a 9-day culture process in a bioreactor to become Th1-like memory cells ("T-Stim™"). The final formulation includes the T-Stim™ cells attached to non-biodegradable microbeads; the formulated combination is called AlloStim®. The microbeads have covalently bound mouse anti-human CD3 and CD28 mAbs (Dynabeads® ClinExVivo™ CD3/CD28, Dynal/Invitrogen, Oslo, Norway) which activate the somatic cells and serve to maintain this activated state upon infusion or injection.

AlloStim® is produced under GMP in clean rooms in Jerusalem, Israel. The source blood is tested to be free from contamination with HIV1, HIV2, HTLV1, HTLV2, HBV, HCV, RPR, mycoplasma and CMV. Each batch is tested to assure it is USP sterile, low endotoxin and meets pre-defined functional and identity criteria.

6.1.2. Cryoablation

The cryoablation procedure is conducted with U.S. FDA approved medical devices and disposable probes as an out-patient procedure. Local and/or conscious sedation is allowed. These treatments are used to kill tumor cells. Cryoablation is a minimally invasive treatment that involves placing a probe into a known tumor percutaneously under image guidance (e.g. CT scan or Ultrasound). The cryoablation procedure should be conducted by a licensed interventional radiologist approved by the Sponsor. Unlike normal ablation methods that seek to ablate all viable tumor and leave no tumor at the margins, the ablation procedure for this protocol should aim to ablate only a portion of the tumor and leave viable tumor margins. Effort should be made to ablate viable tumor and avoid necrotic areas of the tumor. Thus, the edge of the tumor, rather than the center is a preferred target. Intratumoral injection of study drug is best accomplished by first inserting a guide device with a gauge larger than the cryoprobe. Generally, a 14g guide is suggested. In this manner, biopsy tools and the cryoprobe can be inserted through the guide to reach the tumor. On site pathology or radiological confirmation that the guide is inserted within viable tumor is preferred. The ablation should occur with at least two freeze-thaw cycles each of at least 10 minutes. The study drug should only be injected when the temperature is verified to be above 35^0 C. The study drug is best delivered using a needle that has side ports to assure the drug is delivered within the ablated lesion and not injected through the lesion into the normal tissue. After the ablation, the guide

tract should be sealed with mesh material to prevent bleeding. All subjects should be observed for at least 2 hours following the procedure for any signs of bleeding or other adverse events. Subjects should be monitored continuously for pulse, respiration, blood pressure and oxygen saturation during the procedure. The type of probes used, the location of the tumor, the amount of tumor ablated, the cooling and warming cycle times, vital signs and procedure notes should be documented in source medical records.

6.2. BACKGROUND TO THE DISEASE

6.2.1. Colorectal Cancer

Colorectal cancer (CRC) ranks as the third most common cancer worldwide. Metastasis is the main reason of death in CRC patients. CRC includes cancerous growths in the colon, rectum and appendix. The liver and the lung are common metastatic sites of colorectal cancer. The most common colon cancer cell type is adenocarcinoma, which accounts for 95% of cases. [1]

For metastatic CRC (CRC) patients, the exposure to all active chemotherapeutic drugs, including 5-fluorouracil (5-FU), oxaliplatin, and irinotecan, can increase survival. These chemotherapy drugs are usually given in two lines of drug combinations: FOLFOX (leukovorin, 5-FU and oxaliplatin) and FOLFIRI (leukovorin, 5-FU and irinotecan). Leucovorin is a vitamin that improves the effectiveness of 5-FU. However, chemotherapy options are absent after progression on 5-FU, oxaliplatin, and irinotecan.

Targeted biologic treatments for metastatic colorectal cancer are available to add to the chemotherapy regimens. These fall into three groups: inhibitors of vascular endothelial growth factor (VEGF), including bevacizumab and aflibercept, monoclonal antibodies against epidermal growth factor receptor (EGFR) on the surface of tumor cells, including cetuximab and panitumumab. However, these EGFR targets are not applicable to KRAS mutant disease. The only approved therapies for chemotherapy-refractory KRAS mutant disease are Regorafenib (a small molecule inhibitor of intracellular kinase involved in various signaling cascades) and TAS102 (oral chemotherapy, for both, non-mutated and mutated KRAS).

This study targets subjects with mCRC. Untreated patients with mCRC have a median survival of approximately 6 months [2]. Chemotherapy regimens such as FOLFOX and FOLFIRI can extend survival for up to 20 months. The use of anti-EGFR therapy in patients that progress after two lines of chemotherapy drug mixtures is an additional 6.4 months [3]. However this survival advantage occurs only in patients that express a wild type KRAS gene (KRAS wt). Patients with a mutated KRAS (KRAS mutant) gene do not respond adequately to anti-EGFR therapy (e.g., cetuximab, panitumumab).

Standard therapy for mCRC is currently based on the use of four chemotherapy drugs (5-fluorouracil, irinotecan, oxaliplatin, and capecitabine) and three biologic agents (cetuximab, bevacizumab, and panitumumab). The common combinations of these drugs are shown below:

Continuous-infusion 5-fluourouracil (5-FU)–based combinations with irinotecan

Saltz (IFL): Irinotecan 125 mg/m2 IV over 90 minutes on days 1, 8, 15, and 22 plus leucovorin (LV) 20 mg/m2 via IV bolus of days 1, 8, 15, and 22 plus 5-FU 500 mg/m2 via IV bolus on days 1, 8, 15, and 22; repeat every 6 weeks [4].

Douillard Irinotecan 180 mg/m2 over 2 hours on day 1 plus LV 200 mg/m2 IV over 2 hours prior to 5-FU on days 1 and 2, plus 5-FU 400 mg/m2 via IV bolus, then 600 mg/m2 via continuous infusion over 22 hours on days 1 and 2; repeat every 2 weeks [5].

FOLFIRI Irinotecan 180 mg/m2 over 90 minutes on day 1 + LV 200 mg/m2 over 2-hour infusion during irinotecan plus bolus -5FU 400 mg/m2, then 2.4–3 g/m2 via continuous infusion over 46 hours on days 1 and 2; repeat every 2 weeks [6].

Oral capecitabine-based combinations with irinotecan

XELIRI Irinotecan 250 mg/m2 IV on day 1 plus capecitabine 1,000 mg/m2 PO bid from day 1 PM through day 15 AM; repeat every 3 weeks [7].

CapIRI Capecitabine 1,000 mg/m2 PO bid on days 1–14 plus irinotecan 100 mg/m2 on days 1 and 8; repeat every 22 days [8].

Continuous-infusion 5-FU–based combinations with oxaliplatin

FOLFOX6 Oxaliplatin 100 mg/m2 IV over 2 hours on day 1 plus LV 200 mg/m2 IV over 2 hours on day 1 plus 5-FU 400 mg/m2 via IV bolus, then 2.4–3 g/m2 via continuous infusion over 46 hours; repeat every 2 weeks [9].

FOLFOX4 Oxaliplatin 85 mg/m2 IV over 2 hours on day 1 plus LV 200 mg/m2 IV over 2 hours on days 1 and 2, plus 5-FU 400 mg/m2 via IV bolus, then 600 mg/m2 IV via continuous infusion over 22 hours on days 1 and 2; repeat every 2 weeks [10].

mFOLFOX Oxaliplatin 85 mg/m2 IV over 2 hours on day 1 plus LV 175 mg/m2 IV over 2 hours on day 1 plus 5-FU 400 mg/m2 via IV bolus, then 2.4–3 g/m2 via continuous infusion over 46 hours; repeat every 2 weeks [11].

FUFOX Oxaliplatin 60 mg/m2 IV over 2 hours on days 1, 8, 15, and 22 plus LV 500 mg/m2 IV over 2 hours on days 1, 8, 15, and 22 plus 5-FU 2.6 g/m2 via continuous infusion over 24 hours on days 1, 8, 15, and 22; repeat every 36 days [12].

FOLFOX7 Oxaliplatin 130 mg/m2 IV over 2 hours on day 1 plus LV 400 mg/m2 IV over 2 hours plus 5-FU 2.4 g/m2 via continuous infusion over 46 hours; repeat every 2 weeks for 6 cycles [13].

Bolus 5-FU–based combinations with oxaliplatin

FLOX Oxaliplatin 85 mg/m2 IV over 2 hours on days 1, 15, and 29 plus LV 500 mg/m2 IV over 2 hours on days 1, 8, 15, 22, 29, and 36 plus 5-FU 500 mg/m2 1 hour after start of LV on days 1, 8, 15, 22, 29, and 36; repeat every 8 weeks for 3 cycles [14].

bFOL Oxaliplatin 85 mg/m2 IV over 2 hours every 2 weeks plus LV 20 mg/m2 IV over 10–20 minutes on days 1, 8, and 15 plus 5-FU 500 mg/m2 via IV bolus on days 1, 8, and 15; repeat every 28 days [15].

Oral capecitabine-based combinations with oxaliplatin

XELOX Oxaliplatin 130 mg/m2 IV over 2 hours on day 1 plus capecitabine 1,000 mg/m2 PO bid from day 1 PM through day 15 AM; repeat every 3 weeks [16].

Capecitabine 1,000 mg/m2 PO bid on days 1–14 plus oxaliplatin 130 mg/m2 IV on day 1; repeat every 3 weeks for 8 cycles [17].

CapOx Oxaliplatin 70 mg/m2 IV on days 1 and 8 plus capecitabine 1,000 mg/m2 PO bid on days 1–14; repeat every 22 days [18].

Combinations with bevacizumab

TREE-2 study mFOLFOX6 plus bevacizumab 5 mg/kg IV every 14 days vs bFOL plus bevacizumab 5 mg/kg IV every 14 days vs CapOx [XELOX] plus bevacizumab 5 mg/kg IV every 14 days [19].

Combinations with cetuximab

FOLFOX4 + cetuximab FOLFOX4 + cetuximab 400 mg/m2 IV for week 1, then 250 mg/m2 IV weekly thereafter [20].

CapOx + cetuximab Cetuximab 400 mg/m² on day 1 followed by weekly cetuximab 250 mg/m² plus oxaliplatin 70 mg/m² on days 1 and 8 plus capecitabine 1,000 mg/m² PO bid on days 1–14; repeat every 22 days [21].

6.2.2. First and Second Line Therapy

Subjects in this study are recruited at third line metastatic disease after failure of two lines of chemotherapy with one containing oxaliplatin and another irinotecan (with at least one line containing bevicuzumab).

Combination regimens of infusional 5-FU with either oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) form the basis of current first and second line standards of care for mCRC. The choice of first and second line therapies varies by physician preference and toxicity, as toxicity is increased with the three-drug combinations. The most common first and second line combination therapies are: FOLFIRI, FOLFOX, CapeOx, FOLFOXIRI, and XELOX [22-25] [26].

According to NCCN Clinical Practice Guidelines, mCRC is treated with FOLFOX as first line therapy and FOLFIRI or CapOx as second line therapy. Patients diagnosed with mCRC are often treated for prolonged time periods and experience multiple sequelae from chemotherapy toxicity which adversely impacts quality of life. Neurologic and hematologic toxicities limit treatment with oxaliplatin, and irinotecan therapy is limited by hematologic toxicity and diarrhea [27]. Thus failure of first and second lines of chemotherapy can be due to either disease progression or toxicity.

6.2.3. Biologic Agents

In an attempt to improve first and second line chemotherapy regimens, three biological agents have been recently FDA licensed for the treatment of mCRC. These agents target molecular pathways of mCRC tumorigenesis: Bevacizumab, a human monoclonal antibody (MoAb) that targets vascular endothelial growth factor (VEGF); cetuximab, a chimeric human:mouse MoAb aganst the epidermal growth factor receptor (EGFR); and panitumumab, a fully human MoAb that targets the extracellular domain of EGFR.

6.2.4. Bevacizumab

As of 2004, bevacizumab has been FDA approved for first-line treatment of mCRC in combination with irinotecan- or oxaliplatin-based chemotherapy regimens [28]. Bevacizumab was initially approved after it was found to show a significant survival benefit when added to first-line IFL chemotherapy [29] [30]. Yet in a phase III study of 1401 mCRC patients randomized to first-line FOLFOX4 or XELOX with or without bevacizumab, PFS was extended in the bevacizumab group (9.4mo vs. 8.0mo, p=0.0023), but no significant difference was found in median OS (21.9mo vas 19.9mo, p=0.0769) or RR (47% vs. 49%, p=0.31) [31]. What more, the PFS benefit was limited to the XELOX group only (9.3mo vs. 7.4mo, p=0.003). In terms of toxicity, more patients in the bevacizumab arm discontinued treatment because of adverse events, including thromboembolic events and bowel perforations (30% vs. 21%). Thus, it is unclear whether bevacizumab improves outcome with better chemotherapy regimens in the first-line setting. For patients in the refractory mCRC setting, bevacizumab plus FOLFOX has been found to significantly improve RR (22.6% vs. 8.6%, p<0.001), PFS (7.3mo vs. 4.7mo, p<0.0001), and OS (12.9mo vs 10.8mo, p=0.0011) [32]. Maintenance bevacizumab after initial response to combination treatment is being investigated, yet this drug has little activity when used alone. Therefore, prior use of bevacizumab is optional in this protocol.

6.2.5. Cetuximab and Panitumumab

The fact that approximately 80% of colorectal tumors stain positively for EGFR has led to relatively rapid FDA approval for cetuximab and panitumumab. As of 2004, cetuximab has been FDA approved for use as monotherapy or combination therapy for pretreated chemotherapy-resistant disease. In a head to head comparison of cetuximab versus bevacizumab in first-line treatment of KRAS mutant mCRC, neither strategy demonstrated a clearly superior outcome [33]. However, the association with longer overall survival suggests that FOLFIRI plus cetuximab could be the preferred first-line regimen for patients with KRAS wt mCRC [34]. In the refractory-setting FOLFIRI plus cetuximab improves RR (23% vs. 11%, p=0.007) and TTP (4.1mo vs. 1.5mo, p<0.0001) over FOLFIRI alone [35].

Panitumumab was intially FDA approved in 2007 for use in EGFR-expressing mCRC after failure of fluoropyrimide-, oxaliplatin-, and irinotecan-based chemotherapy regimens, on the basis of a phase III trial that compared panitumumab plus best supportive care (BSC) vs. BSC alone (Chamoto et al. 2003). In this phase III trial, 231 patients were randomized to panitumumab plus BSC, and 232 to BSC alone. After a median follow-up of 35 weeks, OS was similar in both groups, but patients in the panitumumab arm had a significantly greater PFS (8 weeks, 95%CI, 7.9-8.4wks) than the BSC alone arm (7.3 wks, 95% CI, 7.1-7.7wks) p<0.001 (Chamoto et al. 2003). A retrospective analysis of the panitumumab phase III approval trial showed a significantly greater effect on PFS for patients with wild-type KRAS than with mutant KRAS (12.3 vs. 7.4 wks; p<0.0001) [36]. Investigators concluded that KRAS status should be determined when considering panitumumab monotherapy [37]. (see discussion of KRAS status and treatment response below).

6.2.6. KRAS Mutation Status

The oncogene KRAS is the most commonly mutated gene in various human cancers. Being constitutively activated, it can bypass the EGFR-driven signaling cascade and impair the clinical efficacy of EGFR inhibitors such as cetuximab, nimotuzumab, panutinumab and aflibercep. *KRAS* is currently considered a clinically useful biomarker, and it is common practice in the oncology community to test *KRAS* mutational status prior to initiating therapy with either cetuximab or panitumumab. Roughly 40% of patients with mCRC have somatic activating *KRAS* mutations [38]. The mutant status of *KRAS*, a gene encoding the *KRAS* proto-oncogene downstream effector of EGFR, has been associated with poor prognosis and nonresponse to EGFR antagonists [38, 39]. Therefore, these agents are indicated for KRAS wt disease only.

On October 2, 2007, the FDA expanded labeling and granted regular approval for single-agent cetuximab for the treatment of patients with EGFR-expressing mCRC after failure of both irinotecan- and oxaliplatin-based chemotherapy regimens. On July 17, 2009, the FDA ordered changes to the product labels of cetuximab (Erbitux, ImClone Systems) and panitumumab (Vectibix Amgen) due to retrospective subset analyses of trials in patients

with colorectal cancers having KRAS mutations which showed a lack of benefit associated with these monoclonal antibodies.

Labeling changes have been implemented in the 'indications and usage', 'clinical pharmacology' and 'clinical studies' sections of both cetuximab and panitumumab product labels. See FDA website:
http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm172905.htm

However, while approved for KRAS wt mCRC, not all patients with a KRAS wt genotype respond to EGFR-targeted agents. Recently, activating mutations of BRAF, which encode for a protein acting downstream of KRAS, were also shown to be responsible for resistance to EGFR inhibitors in chemorefractory mCRC. A recent publication confirms, as for KRAS, a high concordance of BRAF mutations in primary CRC and related metastatic sites [40]. Because the KRAS pathway is central to many nodes of receptor tyrosine kinase (RTK) signaling, the same hurdles are to be expected in inhibition of other RTKs.

Accordingly, development of new therapeutic strategies for KRAS mutant as well as BRAF mutant tumors are therefore highly needed.

This study targets the population of mCRC patients that have progressed after two lines of chemotherapy and are not eligible for targeted therapies in order to offer a new category of drug (immunotherapy) to a population of patients with unmet medical needs in an indication requiring more effective therapies.

6.2.7. Regorafenib

Regorafenib (Stivarga) is an oral multi-kinase inhibitor developed by Bayer which targets angiogenic, stromal and oncogenic receptor tyrosine kinase (RTK). Regorafenib shows anti-angiogenic activity due to its dual targeted VEGFR2-TIE2 tyrosine kinase inhibition. Regorafenib demonstrated to increase the overall survival of patients with mCRC and was approved by the US FDA on September 27, 2012 for use in third- or fourth-line therapy.

Regorafenib was approved based on results from the CORRECT pivotal trial which randomized 760 patients; 505 to Regorafenib and 255 to placebo. Regorafenib had a median benefit of 1.4 months compared to placebo (6.4 months vs 5.0 months, HR=0.77, p=0.0052). The subset analysis in the CORRECT trial, indicated that median OS in the KRAS mutant subset was 6.2 months compared to control of 5.1 months (1.1 month difference, HR=0.87 95%CI), whereas the KRAS wt subset had median OS of 7.3 months compared to 5.0 months for placebo control (2.3 months difference, HR=0.65, 95%CI). There were no complete responses and only 1% of patients had a partial response.

Based on the results of studies evaluating Regorafenib in metastatic colorectal cancer, the National Comprehensive Cancer Network (NCCN) has updated their guidelines

regarding the treatment of colon and rectal cancers. Regorafenib is recommended as a treatment option after first, second, or third progression on therapies containing 5-fluorouracil/ leucovorin, irinotecan, oxaliplatin (e.g., FOLFOX and FOLFIRI), bevacizumab, and cetuximab or panitumumab (if KRAS wt.). Patients with KRAS mutant disease are recommended for consideration of regorafenib therapy in the third-line setting after progression on FOLFOX/FOLFIRI (or other oxaliplatin and irinotecan combinations) with bevacizumab.

However, due to the marginal survival benefit and high toxicity of Regorafenib , the NCCN guidelines also state that physicians can consider to enroll KRAS mutant patients who progress on FOLFOX and FOLFIRI with bevacizumab and maintain adequate performance status and end-organ function into clinical trials, including phase I trials [41]. The EMA European Public Assessment Report (EPAR) for Regorafenib states that the drug is indicated in adult patients with metastatic colorectal cancer who have been previously treated with or are not considered candidates for other available therapies, including patients with KRAS mutant tumors, though physicians are recommended to carefully evaluate benefits and risks when prescribing Regorafenib in patients with KRAS mutant tumors.

Although Regorafenib is a recommended therapy for metastatic colorectal cancer after failure of standard therapies, it is important to note that it comes at a starting cost of $9,350 per 28-day cycle. A recent editorial questions the cost-effectiveness of Regorafenib therapy. The authors comment on the small incremental survival benefit, short PFS, and the potentially substantial adverse effects. They suggest that identification of the subset of patients most likely to derive significant clinical benefit from Regorafenib therapy become a high priority [42].

This protocol does not require prior Regorafenib for eligibility. This is based on the subset analysis of the CORRECT trial demonstrating little OS benefit for the KRAS mutant subset of patients and is consistent with NCCN guidelines recommending consideration of clinical trial options for third-line KRAS mutant mCRC. Subjects in this protocol that are progressing or not responding to experimental therapy can add Regorafenib after the Safety Evaluation Period as salvage therapy.

6.2.1. TAS-102

TAS-102 (Lonsurf®) is an oral drug that combines two agents, trifluridine (FTD), a nucleoside metabolic inhibitor, and tipiracil (TPI), a thymidine phosphorylase inhibitor, developed by Taiho Oncology, Inc.

The FDA approved the use of the drug on September 22, 2015 for patients with metastatic colorectal cancer following an international, multicenter, phase III RECOURSE trial. The study enrolled 800 patients with mCRC from Japan, the United States, Europe, and Australia, randomly assigned in a 2:1 ratio to receive TAS-102 or placebo. Most of the patients in the study had disease that no longer responded to fluoropyrimidines. To be eligible for the study, the patients had to undergo tumor testing

to determine whether their cancers harbored a mutant KRAS gene. Patients were also required to have received chemotherapy with each of the following agents: a fluoropyrimidine, oxaliplatin, irinotecan, bevacizumab, and—for patients with non-mutant KRAS tumors—cetuximab or panitumumab. The primary endpoint was overall survival. Secondary end points included progression-free survival, response rate (the proportion of patients whose best response was a complete or partial response), and safety.

TAS-102 showed improved survival (median overall survival was 7.1 months in the TAS-102 group and 5.3 months in the placebo group), including those with both non-mutated and mutated KRAS genes. The overall survival for KRAS-mutant patients was 6.5 months if they received TAS-102, compared to 4.9 months if they did not. The 1-year overall survival rates were 27 percent and 18 percent, respectively. Additional analyses showed that the survival benefit of TAS-102 was seen in essentially all pre-specified subgroups, including KRAS status.

The median progression-free survival was 2.0 months in the TAS-102 group and 1.7 months in the placebo group. 221 patients (44 percent) in the TAS-102 group and 42 patients (16 percent) in the placebo group had achieved disease control (complete response, partial response, or stable disease) measured at least 6 weeks after the patients were randomly assigned to treatment groups. In addition, the time to worsening of performance status was significantly longer in the TAS-102 group than in the placebo group (5.7 versus 4.0 months). Patients in the TAS-102 group experienced few serious adverse events. Overall, adverse events of grade 3 or higher occurred more frequently in the TAS-102 group than in the placebo group (69 percent versus 52 percent). The most common adverse events associated with TAS-102 included neutropenia, which occurred in 38 percent of those treated, and leukopenia, which occurred in 21 percent. Four percent of the patients in the TAS-102 group developed febrile neutropenia, and there was one death related to TAS-102 [43].

Based upon review of data submitted, the NCCN decided (in a Web Conference on July 24, 2015) to add to the NCCN-Guidelines V.1.2016 the regimen of trifluridine + tipiracil as a subsequent therapy option for patients with disease progression after oxaliplatin- and irinotecan-based chemotherapy.

This protocol does not require prior TAS-102 for eligibility. This is based on the minimal efficacy of TAS 102 in the subject population. A reported phase II trial of TAS-102 found an overall survival benefit in Japanese patients with metastatic colorectal cancer refractory to treatment. The RECOURSE study was a global phase III trial conducted in 13 countries. Patients had metastatic colorectal cancer refractory to all standard therapies including with wild-type KRAS tumors. Patients were treated with TAS-102 (534 patients) or placebo (266 patients) and directly compared. The researchers found that TAS-102 prolonged overall survival experiencing a median overall survival of 7.1 months for TAS-102 and 5.3 months for placebo, a 1.8 month increase.

Subjects in this protocol that are progressing or not responding to experimental therapy can add TAS-102 after the Safety Evaluation Period as salvage therapy.

6.3. CANCER VACCINES AND RATIONALE FOR CRYOVAX™

The current drugs used to treat colorectal cancer provide important treatment options for patients, their limitations including drug resistance, poor efficacy and severe side effects has focused efforts on developing safer and more effective therapies for these patients. The rationale for development of cancer vaccines is based on experimental work in animal models, in which it has been demonstrated that specific CD8+ cytotoxic T-cells (CTL) are able to eliminate tumors and develop memory which protects against recurrence. However, despite the promise of cancer vaccination shown in animal models, pooled results of published vaccine trials reveal a very weak clinical response rate of <1% for active specific immunization procedures with an objective response rate of 2.6%, mainly among melanoma patients [44] even though about 50% of these vaccinated patients were shown to have developed specific CD8+ cytotoxic T-cells (CTL) able to specifically recognize and kill tumor cells in-vitro.

The poor clinical results of immunotherapy vaccine approaches to cancer treatment have been attributed to tumor immunoavoidance mechanisms [45]. Tumors employ many escape strategies in order to evade immune attack. These strategies include downregulation of MHC molecules in order to hide from immune recognition [46], expression of inhibitory factors and immunosuppressive cytokines [47] [48] [49], including TGF-β [50, 51], IL-10 [52], and recruitment of regulatory immune cells CD4+CD25+FoxP3+ Tregs [53, 54], Tr1 cells [55], tolerogenic DCs, and myeloid suppressor cells, including immature macrophages, granulocytes, DCs and other myeloid cells at earlier stages of differentiation [56-58] [59]

These immunoavoidance mechanisms employed by tumors render the immune system tolerant and permit tumors to grow unimpeded by immune surveillance. Establishment of this type of self-tolerance is a part of a natural immune regulatory mechanism which prevents autoimmune disease against organ-specific self-antigens. However, this normally beneficial effect may be responsible for tumor immune evasion, as many of the tolerance mechanisms that prevent autoimmunity are the same as employed by tumors to prevent immune destruction [60, 61].

Therefore, the mechanisms of autoimmune disease serve as a model for developing strategies to break immune tolerance to tumors. A key mechanism for breaking self-tolerance and induction of autoimmunity is related to inflammation. Autoimmune attack of self-tissues requires two conditions: activated T-cells and the "conditioning" of the target organ by irradiation or infection [62]. These two conditions are created in the CryoVax™ protocol by first an ablation of a tumor lesion and then intratumoral injection of allogeneic activated immune cells. The combination creates an inflammatory microenvironment around the dead tumor cells.

In order to develop an effective immunotherapy strategy for metastatic cancer, new approaches are required that not only can create and enhance tumor-specific immunity, but also are able to counter-act the ability of the tumor to evade immune destruction. The experimental drug, AlloStim®, is designed to break tolerance to tumor tissues by taking advantage of the known mechanisms of breaking tolerance to self-tissue in autoimmune disease. AlloStim® provides a highly inflammatory environment. Inflammation in the presence of tumor tissue that has died pathologically is a formula that has potential to break tolerance.

Type 1 cytokines including IL-1, TNFα, IFN-γ, IL-12, IL-18, and GM-CSF are generally believed to be required components of therapeutic cancer vaccines. AlloStim® cells are differentiated in culture to produce large amounts of these Type 1 cytokines. Because AlloStim® cells are allogeneic and recognized as foreign by the host immune system, they will be rejected by the host. Since these foreign cells produce large amounts of Type 1 cytokines, they are expected to steer the immune response to the foreign antigens to Th1. In this manner, the rejection process is expected to increase the number of Th1 memory immune cells in the circulation of patients injected with AlloStim®. This is significant because cancer patients are known to have an excess of Th2 immune cells and absence of Th1 cells. This imbalance is thought to be a mechanism of tumor immune evasion.

The design of this *in-situ* anti-tumor vaccine is based upon an important paradigm shift in the field of immunology regarding the regulation of immunity. A new concept has emerged that proposes that the regulation of immunity and tolerance is not only determined by the specificity of immune T-cells as previously thought, but also by the context in which the antigens are presented to the immune system [63] [64].

The antigens are presented to the immune system by a network of specialized cells that are known as professional antigen-presenting cells (APCs) or dendritic cells (DCs). DCs are responsible for inducing immunity to pathogens or tumors by presenting antigens to naive T-cells, resulting in the differentiation of the T-cells into effector and memory T-cells specific for the antigens. Effector T-cells, mainly CD8+ cytolytic T-cells (CTL), are capable of destroying cells which express the antigens. Memory T-cells provide immune protection against recurrence or reinfection. Differentiation of the DCs into potent APCs is triggered by molecular stimuli that are released as a result of the tissue disturbance and a local inflammatory response.

The implications are that if a pathogenic infection occurs in the absence of appropriate inflammatory reactions, pathogen-specific T-cells will not be activated despite the presence of pathogen-specific foreign antigens, and, conversely, autoreactive T-cells, including those that are directed against tumor cells, can be readily activated provided the self (tumor) antigens are presented by DCs during an inflammatory response. Since tumors produce anti-inflammatory cytokines, they are capable of influencing the immune response by preventing an inflammatory response.

Therefore, successful anti-tumor immunity will develop in the situation where DCs are processing tumor antigens in the presence of an inflammatory reaction ("danger signals") which is potent enough to also downregulate tumor-mediated immunosuppressive cytokine production. The magnitude and duration of the immune response will be dependent on the extent and quality of the local inflammatory response and will be contained by a variety of tolerogenic mechanisms.

In summary, previous attempts at developing therapeutic cancer vaccines have demonstrated that it is possible to elicit specific immunity against self-tumor antigens. Recent insights on how immunity and tolerance are regulated indicate that the failure of these vaccines in the clinic may be related to the absence of sufficient danger signals at the time tumor antigens are processed by DCs. AlloStim® is designed to provide the necessary danger signals to elicit anti-tumor immunity in this CryoVax™ protocol.

6.4. ALLOSTIM® RATIONALE IN METASTATIC COLORECTAL CANCER

Anti-cancer vaccines are believed to hold great promise as an anti-cancer treatment method. While the promise of this approach has been demonstrated in animal models, it has been difficult to translate these results to the clinic. Several leading cancer vaccine candidates that achieved promising results in Phase I and/or II studies later failed to obtain statistical significance for critical end points in randomized Phase II or III studies. Such disappointing outcomes have led to a critical evaluation of anti-cancer vaccine concepts. The failures of previous vaccines are thought to be due to differences in the mouse immune system compared to human immune system and the enhanced ability of human tumors to evade immune recognition and attack. These problems are addressed in the current vaccine design concept using the experimental AlloStim® drug.

AlloStim® is based upon a novel approach [65]. Rather than being developed in animals as were previous vaccine concepts, AlloStim® was instead designed to elicit an immune mechanism already proven to be clinically effective in humans. This mechanism is known as the graft vs. tumor (GVT) effect that occurs after allogeneic stem cell/bone marrow transplant procedures.

It is well accepted that solid tumors, including mCRC, can be effectively treated with the GVT effect that occurs after myeloablative allogeneic transplantation techniques [66] [67] [68]. Improved reduced-intensity allogeneic transplantation has also been shown to elicit an effective GVT effect in patients with breast cancer [69].

The GVT effect has been described as the most powerful and most effective anti-tumor mechanism ever described in humans [70]. Unfortunately, these beneficial GVT effects are accompanied by graft-versus-host-disease (GVHD) toxicity. GVHD is associated with significant morbidity and is the leading cause for mortality after allogeneic hematopoietic stem cell transplantation. This toxicity significantly limits the clinical application of the GVT mechanism [71].

By analyzing the known immune-mediated mechanisms of the inter-related GVT and GVHD effects, it was hypothesized that instead of separating the GVT/GVHD effects, it might be possible to maintain the interrelationship of these immune mechanisms by reversing the direction of the effects. In the transplant setting, the immune effects flow from the graft to the host (GVT and GVHD). AlloStim® reverses these interrelated effects so that the effects flow from the host to the graft. Successful reversal of the direction of the effects is hypothesized to result in a beneficial host vs. tumor (HVT) effect coupled to a non-toxic host vs. graft (HVG) rejection effect [64]. Pre-clinical studies validated this approach LaCasse, 2011 [65, 72-75].

Accordingly, this protocol also focuses on the replication of the proven GVT mechanism against metastatic colorectal cancer while eliminating the toxic effects of GVHD. The approach also eliminates the need for a tissue-matched donor. In addition, AlloStim® has demonstrated the unique ability to dysregulate the suppressor circuits which tumors use to evade immune attack [73].

6.5. PRE-CLINICAL STUDIES

In vitro activity studies demonstrated that AlloStim® is capable of eliciting anti-tumor effects in immunocompetent, non-conditioned tumor-bearing hosts, providing extended survival and curative immunity in both low and high tumor burden settings. For more detailed information and references on AlloStim®, please refer to the Investigator's Brochure.

6.6. PREVIOUS HUMAN EXPERIENCE

A Phase I/II study under a US IND was conducted from September 2009 until May 2011. The study was placed on clinical hold by US FDA in May 2010 due to deficiencies found in the conduct of the trial during on-site inspections. Please refer to the Investigator's Brochure and Appendix 5 for additional details. Clinical Toxicity data submitted to the FDA is presented in Appendix 3.

6.6.1. Clinical Toxicity data from the Phase I/II under the US IND

Adverse events reported for the ITL-002-CRYO study are presented in the tables below. Please also refer to Appendix 3 for additional details regarding the Clinical Toxicity data which was submitted to the FDA.

Summary of Treatment-Emergent Adverse Events in Subjects Treated with AlloStim™ ITL-002-CRYO

Adverse Event	Total No. of Subjects	% of Total	Grade 1		Grade 2		Grade 3	
	n	%	n	%	n	%	n	%
Alkaline Phosphatase	3	7.14	2	4.76		0.00	1	2.38
Allergy, Immunology and Other	39	92.86	39	92.86		0.00		0.00
Ecchymosis	3	7.14	3	7.14		0.00		0.00
Edema (Limbs)	1	2.38	1	2.38		0.00		0.00
Flu-like Symptoms	34	80.95	33	78.57	1	2.38		0.00
Insomnia	1	2.38	1	2.38		0.00		0.00
Lymphadenopathy	7	16.67	7	16.67		0.00		0.00
Lymphopenia (Low Abs Lymphs)	2	4.76	2	4.76		0.00		0.00
Nausea	2	4.76	2	4.76		0.00		0.00
Neutropenia (Low ANC)	1	2.38	1	2.38		0.00		0.00
Pain-NOS	37	88.10	34	80.95	3	7.14		0.00
Pruritis/Itching	10	23.81	10	23.81		0.00		0.00
Rash	6	14.29	6	14.29		0.00		0.00
Sweating/Diaphoresis	7	16.67	7	16.67		0.00		0.00

The following table summarizes all AE regardless the causality:

Summary of Adverse Events Regardless the Causality ITL-002-CRYO

Adverse Event (CTCAEv3)	Total # of Subjects		% of Total		Grade 1		Grade 2		Grade 3		Grade 4		Grade 5	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Alkaline phosphatase (High)	25	59.52	15	35.71	9	21.43	1	2.38		0		0		
ALT (High)	16	38.10	11	26.19	3	7.14	2	4.76		0		0		
AST (High)	25	59.52	15	35.71	7	16.67	3	7.14		0		0		
Allergy, Immunology Reaction	41	97.62	40	95.24	1	2.38		0		0		0		
Anorexia/Decreased Appetite	7	16.67	4	9.52	2	4.76		0	1	2.38		0		
Bilirubin (Hyperbilrubinemia)	8	19.05			1	2.38	5	11.90	2	4.76		0		
Calcium (Hypocalcemia)	31	73.81	21	50.00	10	23.81		0		0		0		
Calcium (Hypercalcemia)	2	4.76	2	4.76		0		0		0		0		
Constipation	7	16.67	7	16.67		0		0		0		0		
Creatinine (High)	5	11.90	3	7.14	2	4.76		0		0		0		
Distention/Bloating (Abdominal)	4	9.52			4	9.52		0		0		0		
Diarrhea	8	19.05	7	16.67	1	2.38		0		0		0		
Dizziness/Vertigo	6	14.29	6	14.29		0		0		0		0		
Dyspnea (SOB)	10	23.81	2	4.76	5	11.90	2	4.76	1	2.38		0		
Dysphagia	1	2.38			1	2.38		0		0		0		
Edema (Limbs)	14	33.33	5	11.90	7	16.67	2	4.76		0		0		
Ecchymosis	5	11.90	5	11.90		0		0		0		0		
Fracture	1	2.38				0	1	2.38		0		0		
Flu-like Symptoms	38	90.48	34	80.95	4	9.52		0		0		0		
Heartburn/Dyspepsia	3	7.14	3	7.14		0		0		0		0		
Hemoglobin (Low)	34	80.95	15	35.71	16	38.10	3	7.14		0		0		
Hypertension	1	2.38	1	2.38				0		0		0		
Hypoalbunemia (Low Albumin)	29	69.05	13	30.95	12	28.57	4	9.52		0		0		
Hypotension	2	4.76	2	4.76		0		0		0		0		

Summary of Adverse Events Regardless the Causality ITL-002-CRYO

Adverse Event (CTCAEv3)	Total # of Subjects		% of Total		Grade 1		Grade 2		Grade 3		Grade 4		Grade 5	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Infection/Normal ANC	2	4.76	1	2.38	1	2.38		0		0		0		0
Insomnia	13	30.95	10	23.81	3	7.14		0		0		0		0
Leukopenia (Decreased WBC)	19	45.24	14	33.33	5	11.90		0		0		0		0
Lymphadenopathy	15	35.71	15	35.71		0		0		0		0		0
Lymphopenia (Low Abs. Lymphs)	32	76.19	6	14.29	9	21.43	16	38.10	1	2.38		0		0
Mood Alteration-Agitation	2	4.76	2	4.76		0		0		0		0		0
Mood Alteration-Anxiety	4	9.52	3	7.14	1	2.38		0		0		0		0
Mood Alteration-Depression	1	2.38			1	2.38		0		0		0		0
Nausea	17	40.48	15	35.71	2	4.76		0		0		0		0
Neuropathy/Sensory	7	16.67	3	7.14	4	9.52		0		0		0		0
Neutrophils (Low Abs. Neutrophils)	3	7.14	2	4.76	1	2.38		0		0		0		0
Pain-NOS	39	92.86	23	54.76	15	35.71	1	2.38		0		0		0
Palpitations	1	2.38	1	2.38		0		0		0		0		0
Platelets (Low Platelets)	7	16.67	5	11.90	2	4.76		0		0		0		0
Potassium (Hyperkalemia)	4	9.52	4	9.52		0		0		0		0		0
Potassium (Hypokalemia)	10	23.81	9	21.43		0	1	2.38		0		0		0
Pruritus/Itching	13	30.95	13	30.95		0		0		0		0		0
Rash/Desquamation	7	16.67	7	16.67		0		0		0		0		0
Sinus Bradycardia	1	2.38	1	2.38		0		0		0		0		0
Sinus Tachycardia	3	7.14	2	4.76	1	2.38		0		0		0		0
Sodium (Hyponatremia)	20	47.62	11	26.19		0	9	21.43		0		0		0
Sweating/Diaphoresis	12	28.57	11	26.19	1	2.38		0		0		0		0
Taste Alteration/Dysgeusia	3	7.14	3	7.14		0		0		0		0		0
Urinary Frequency/Urgency	4	9.52	4	9.52		0		0		0		0		0
Vomiting	9	21.43	7	16.67	2	4.76		0		0		0		0

Summary of Adverse Events Regardless the Causality ITL-002-CRYO

Adverse Event (CTCAEv3)	Total # of Subjects		% of Total		Grade 1		Grade 2		Grade 3		Grade 4		Grade 5	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vision (Blurred/Spots)	1	2.38	1	2.38		0		0		0		0		0
Weakness	13	30.95	7	16.67	4	9.52	2	4.76		0		0		0
Weight loss	5	11.90	1	2.38	4	9.52		0		0		0		0

6.7. EXPECTED BENEFITS FROM THE PROPOSED STUDY

Subjects in this study have the potential to benefit from a new approach to cancer vaccination that is designed to provide the same tumor debulking effects of non-myeloablative allogeneic bone marrow transplantation (aBMT) without the graft-versus-host-disease (GVHD) toxicity or need for a matched donor. Patients with mCRC chemotherapy-refractory disease have only the option of Regorafenib. However, this drug has little, if any, efficacy in KRAS and BRAF mutant disease.

The goal of this research is to develop new treatment options for mCRC KRAS mutant and BRAF mutant patients. This approach has the potential to benefit patients that would otherwise either not be treated or would be treated with minimally effective and highly toxic Regorafenib that provides a median of only 1.1 months of survival advantage. AlloStim®, combined with a percutaneous ablation technique of a selected metastatic lesion, creates an in-situ anti-tumor vaccine customized to the patient's own tumor. The ablation causes the release of the internal contents of the tumor cells and exposes tumor-specific antigens to the immune system. AlloStim® is then injected into the ablated lesion as an adjuvant to create the necessary inflammatory environment ("danger signals") to steer the immune response to tumor-specific Th1 immunity. Accordingly, this study will provide patients suffering from a disease with an unmet medical need with access to a new class of treatment (immunotherapy) with a mechanism of action that has potential to debulk tumor and extend survival.

6.8. RATIONALE FOR THE STUDY

This study is evaluating a personalized in-situ anti-cancer vaccine combining AlloStim® and tumor ablation or AlloStim® alone. The protocol is designed to elicit a Th1 anti-tumor immune response in patients with mCRC KRAS mutant and BRAF mutant disease. Such immune response may be capable of debulking metastatic disease. Immune cell infiltration at the margins of primary colorectal disease has been correlated with longer survival in mCRC [76]. Specifically, increased infiltration of CD8+ CTL also correlated with survival [77]. In addition, colorectal cancer patients present with skewed Th1/Th2 ratios [78]and high Th1 expression has correlated with survival [79].

6.8.1. Rationale for the Doses and the Dosing Regimen

In the Part 1 of the trial, the different dosing schedules will be evaluated. Assessment of different schedules should allow for better assessments of treatment safety, tolerability, and efficacy.

There are 4 phases of dosing in this study:

1. Priming phase:
   Since most metastatic patients will present with polarized Th2 immunity (or depleted Th1 immunity) [78, 80, 81], the objective of the "priming" phase of the protocol is to increase the amount of circulating Th1 cells in these patients. To accomplish this objective, patients will be injected with intradermal AlloStim® cells. The allogeneic CD4+ T-cells in AlloStim® are expected to be rejected by the patient and the Th1 type cytokines produced by AlloStim® are expected to steer the rejection response to Th1 immunity, thus creating an increased pool of Th1 memory cells in the circulation

specific for the alloantigens contained within AlloStim®. In other words, the patients are expected to become immune to the investigational product with a Th1 bias.

2. Vaccination phase:

   The objective of the "vaccination" phase of the protocol for cohorts with cryoablation is to create anti-tumor specific immunity through an in-situ vaccination method. Tumor vaccines generally contain a source of tumor antigen combined with an adjuvant which promotes immune recognition and primes for development of a specific immune response against the tumor antigen. In this protocol, a source of tumor antigen is created in-situ by ablating a selected tumor lesion using the minimally-invasive technique of image guided cryoablation.

   The ablation technique is designed to cause pathological death of tumor cells, releasing internal contents to the microenvironment. It is thought that necrotic cell death stimulates active cellular immunity, whereas apoptotic death stimulates immune tolerance.

   The ablation procedure results in release of large amounts of tumor debris into the tumor microenvironment that serves as a source of patient-specific tumor antigens. The ablation procedure causes a pathological type of tumor killing (necrosis) that alerts the immune system and mobilizes the cells that will respond to clean up and repair the debris, including immature dendritic cells (DC) which are responsible for directing an immune response to the engulfed antigens to either Th1 or Th2.

   In order to provide an adjuvant to drive correct DC maturation, AlloStim® will be injected into the necrotic center of the ablated tumor lesion within approximately ten minutes to one hour following the ablation procedure. AlloStim® cells produce large amounts of inflammatory cytokines and express surface molecules (e.g. CD40L) which are known to cause the maturation of dendritic cells and promote development of Th1 anti-tumor immunity. Further, since the patients will be immune to the alloantigens in AlloStim®, intratumoral injection (IT) is expected to elicit a potent memory response of Th1 cells to reject these allogeneic cells. All these factors are expected to serve as an adjuvant by promoting maturation of DC to prime for anti-tumor specific Th1 immunity.

   In the cohort without cryoablation, the IV AlloStim® infusion and subsequent rejection is expected to cause release of inflammatory cytokines that will activate NK cells and memory T-cells and cause them to traffic to metastatic tumor lesions, creating an in-situ vaccination step intended to create anti-tumor specific immunity. Tumor vaccines generally contain a source of tumor antigen combined with an adjuvant which promotes immune recognition and primes for development of a specific immune response against the tumor antigen. In these schedules, a source of tumor antigen is created in-vivo by AlloStim® leading to the release of internal antigens. These factors are expected to serve as an adjuvant by promoting maturation of DC to prime for anti-tumor specific Th1 immunity.

3. Activation phase:
   Intravenous infusions of AlloStim® are designed to create a "cytokine storm" of inflammatory cytokines capable of suppressing the ability of tumors to evade immune responsiveness. In addition, intravenous AlloStim® has been shown to activate host T-

cells and macrophages through CD40L-CD40 interaction and cause these activated cells to extravasate to the tissues. Since priming and vaccination are expected to increase the titer of circulating Type 1 memory cells, extravasation causes an increase in immune cell infiltration at sites of inflammation, including metastatic tumor sites. This provides a mean to maintain and support an active cellular immune response against widely disseminated tumors. However, the inflammation is non-specific such that any inflammatory lesion such as arthritic lesions, inflammatory bowel or sites of previous injury can also become inflamed or the existing inflammation becomes exacerbated.

Since tumors are known to be capable of evading Th1 immune responses, the "activation" phase of the protocol is designed to both activate the previously created immunity and to try to disable these tumor immunoavoidance mechanisms. It is known that a highly inflammatory environment can have the effect of suppressing tumor immune avoidance and breaking tolerance to the tumor antigens in much the same manner as inflammation can break tolerance to self-tissue antigens and promote autoimmunity. In order to create this inflammatory environment, AlloStim® is infused intravenously. The infusion of the foreign cells in AlloStim® is expected to cause a highly inflammatory environment as the primed immune system of the patient activates to reject these cells. In addition, the rejection of AlloStim® is expected to have the secondary effect of activating components of the host innate immune system (such as NK cells and macrophages) and activation of both allo-specific and tumor-specific Th1 cells which should initiate the cascade of immunological events necessary for systemic tumor elimination and suppressing the ability of the tumor to avoid this immune attack.

4. Booster phase:
   Intravenous infusions of AlloStim® are designed to maintain the highly inflammatory environment believed to be necessary in order to maintain an active cellular immune response against the tumor and to suppress the tumor's ability to evade immune attack.

   The final "booster" phase of the protocol is designed to maintain the chronic inflammatory environment to suppress tumor immunoavoidance and to maintain the activation of both innate and adaptive immunity. To meet this objective two-three intravenous infusions of AlloStim®

7. STUDY OBJECTIVES

7.1. PRIMARY OBJECTIVES

1. To determine the safety of AlloStim® combined with cryoablation vs AlloStim® alone.
2. Determine the schedule that provides the greatest efficacy and least adverse events

7.2. EXPLORATORY OBJECTIVES

1. To assess the longitudinal changes in tumor burden by RECIST and compare these changes with the histopathological analysis of corresponding biopsies (Parts 1 and 2).

2. To assess whether immune response correlates with RECIST, histopathology, plasma biomarkers and Overall Survival (OS).

8. STUDY DESIGN

This is a single center, open label study of CryoVax™ personalized anti-tumor vaccine protocol combining the cryoablation of a selected metastatic lesion with intra-lesional immunotherapy compared to AlloStim® alone.

Part 1

3 subjects will be accrued sequentially into schedule A-1 and A-2 (Part 1). After accrual of 6 subjects, up to 25 additional subjects will be accrued into the most favorable schedule (Part 2)

Dosing Schedule A-1: Intradermal and Intravenous Dosing Combined With Cryoablation

The priming step: Subjects will receive an ID injection of AlloStim® (0.5ml) on Days 0, 7, and 14 all in different locations.
The vaccination step: Subjects will receive cryoablation and intratumor (IT) injection of AlloStim® (3 ml) on Day 21.
The activation step: Subjects will receive an IV infusion of AlloStim® (3ml) on Day 28.
The booster step: Subjects will receive two (optional) IV booster infusions of AlloStim® (5ml), one on Day 56 and Day 84.

Dosing Schedule A-2: Intradermal and Intravenous Dosing With AlloStim® alone

The priming step: Subjects will receive an ID injection of AlloStim® (0.5 ml) on Days 0, 3, 7, 10 and 14.
The vaccination step: Subjects will receive IV AlloStim® (3 ml) on Day 21.
The activation step: Subjects will receive an IV infusion of AlloStim® (3ml) on Day 28.
The booster step: Subjects will receive two IV booster infusions of AlloStim® (3ml), one on Day 49 and another on Day 77.

Toxicity Monitoring

Two types of toxicity are assessed for determination of whether a Dose Limiting Toxicity (DLT) has occurred. An acute dose limiting toxicity (ADLT) is assessed within 48h of a dose administration during the priming, vaccination and activation steps of each protocol. Cumulative dose limiting toxicity (CDLT) is assessed during the complete Safety Evaluation Period including any ADLT that occurs in the booster dosing.

An ADLT is defined as the occurrence of any of the following within 48h of dosing:

- Any ≥ Grade 3 local injection site reaction;
- Any ≥ Grade 3 graft vs host disease;
- Any ≥ Grade 3 infusion related symptoms of duration > 2 days;
- Any ≥ Grade 4 infusion reaction of any duration

For any Grade 1-2 acute toxicity, the study drug administration schedule can continue to the next scheduled dose. If an ADLT is observed, the subject will be followed until the toxicity is

resolved or the subject is stable. No further dosing for that subject will occur after an ADLT event.

A CDLT event is defined as the occurrence of any of the following within the Safety Evaluation Period:

- Any ≥ Grade 3 graft vs. host disease
- Any toxicity ≥ Grade 3 that is probably or definitely related to the study drug;
- Any toxicity not present at baseline that occurs at ≥ Grade 4
- Any autoimmune toxicity ≥ Grade 3 not present at baseline

All adverse events (AEs) and serious adverse events (SAEs), regardless of attribution to the study drug, will be coded, graded and become part of the final safety report for each cohort. The DSMB will review the safety data on each cohort and recommend whether to advance enrollment to the next cohort.

The development of any Grade 4 or greater toxicity in any subject at any time will prompt a review of safety data by the Sponsor, PI and the DSMB prior to accrual of any additional subjects. It is recognized that Grade 4 events and death can occur in this population due to disease progression. Therefore, dosing will continue for currently enrolled subjects at the occurrence of a grade 4 event according to protocol unless the PI determines the event is definitely or probably related to the study drug. If attribution is assigned to the study drug dosing will be suspended pending review by DSMB.

In accordance with the 3+3 design, three subjects will be accrued into the first dosing schedule. If during the priming, vaccination and activation steps no ADLT are observed in any of the three subjects, accrual into the next cohort is allowed. If an ADLT occurs in two of the three subjects, no further dosing is allowed. If ADLT occurs in one of the three subjects, three additional subjects will be accrued into the same cohort. If no ADLT occurs within the additional three subjects, accrual into the next cohort can begin. If an ADLT is observed in any of the additional three subjects, no further dose escalation is permitted.

The development of any DLT toxicity in ≥ 33% of the accumulated number of subjects in any and all dosing schedules during the Safety Evaluation Period will result in protocol discontinuation.

At the completion of the Safety Evaluation Period for all cohorts, the safety evaluation of Part 1 will be considered completed and all enrollment will be closed. Efficacy evaluation will continue monthly for each subject until death or loss to follow-up.

Part 2

The safety and efficacy data from Part 1 will be evaluated and the favored schedule advanced to Part 2. In Part 2 of the trial, up to 25 additional subjects may be accrued.

9. STUDY ENDPOINTS

9.1. PRIMARY ENDPOINT

Safety will be the primary endpoint of this study and will be evaluated on the basis of the following parameters:

- Physical examination and vital signs (temperature, blood pressure, pulse rate, respiratory rate, and blood oxygen saturation).

- Clinical laboratory profile (complete blood count (CBC), comprehensive metabolic panel (CMP), C-reactive protein (CRP), coagulation factors and autoimmune parameters.

- Adverse events according to the National Cancer Institute (NCI) Terminology Criteria for Adverse Events (CTCAE) version 4.03 (refer to section 15 for AE/SAE management and reporting):

9.2. EXPLORATORY ENDPOINT

- Correlation of tumor burden by RECIST, pathological response and immune response to Overall Survival (OS).

  - Overall Survival (OS) is evaluated from time of enrollment until death from any cause. Subjects are followed for survival during the trial, as long as they are alive, for 12 months after the last study treatment through follow-up phone calls.

  - Traditionally, oncology investigators have relied upon Response Evaluation Criteria in Solid Tumors (RECIST) to serve as a surrogate to predict clinical benefit. RECIST criteria for clinical outcomes rely upon the assumption that agent activity results in shrinkage of tumor and that enlargement of disease is a result of tumor growth. RECIST is a composite evaluation of response based upon measurements of the changes in tumor size [82]. RECIST criteria have been criticized for not being a reliable method to predict OS benefit for cytotoxic agents [83].

    In order to determine the utility of RECIST in evaluating response of patients to immunotherapy, RECIST 1.1 [82] guidelines will be used to determine objective tumor response. This endpoint will be determined and analyzed for correlation with OS. Malignant tumor masses (target lesions) will be selected on the basis of their size and their suitability for accurate repetitive measurements. All patients must have at least one target lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) greater than or equal to 1.0 cm with spiral CT scan. To assess objective response, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Whenever possible, several measurable lesions, up to a maximum of 2 lesions per organ and 5 total target lesions representative of involved organs, should be identified, measured and recorded at baseline. To allow later retrieval, lesions should be clearly marked on the films or otherwise identified. If the lesion is detected by more than one method at baseline, the investigator should select at baseline the method to be used at subsequent evaluations. All measurements should be performed using a caliper or ruler and should be recorded in metric notation in centimeters. A sum of the longest diameter for all target lesions will be calculated and reported as the baseline sum longest diameter. The baseline sum longest diameters will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease by repeated assessment of the sum of the longest diameters during treatment.

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present" or "absent". All baseline evaluations should be performed as closely as possible to the treatment start and never more than 14 days before the beginning of the treatment.

- RECIST evaluations are particularly problematic for evaluating responses to immunotherapy, as the mechanism of action of immunotherapy drugs may cause swelling or flare of tumors, resulting in the appearance of increased size on CT scans, but may actually represent decreased burden of viable tumor. In addition, immune-mediate responses may manifest after an initial increase in tumor burden or may delay or stop tumor growth, resulting in disease stabilization and increased survival, but will be scored as progressive disease by RECIST criteria. Therefore, RECIST may erroneously predict progression in a subject treated with immunotherapy.

  For this reason, this study proposes:

  1. Longitudinal serial biopsies for comparison and correlation with RECIST data in order assess whether RECIST can be used to predict survival when using this immunotherapy.
  2. Evaluation of subjects according to newly proposed immune-related response criteria (irRC) [85]. The irRC is thought to better address patients' treatment, investigators' patient management and sponsors' analysis needs for targeted immunotherapy developments in oncology.

- Subjects treated on this study will undergo evaluation of immune response to treatment. The results will be used to correlate response to vaccination with subsequent clinical outcome and OS.

10. SELECTION OF SUBJECTS

10.1. NUMBER OF SUBJECTS

Approximately 6 subjects will be enrolled in the dose schedule evaluation Part 1 of the trial. In Part 2 of the trial, up to 25 additional subjects may be accrued in the one of the dose schedules. Recruitment Methods

Efforts will be made to include all eligible subjects in this study. Subjects will be recruited from a population of patients who have been previously evaluated at the participating institution or have been referred from other institutions. The subject's medical record will be reviewed by the investigator to determine his/her eligibility for the study.

Each subject or subject's legally acceptable representative must sign a current Institutional Review Board (IRB) approved Informed Consent Form (ICF) before any study-related procedures are performed.

10.2. INCLUSION CRITERIA

1. Adult males and female subjects aged 18-80 years at screening visit
2. Pathologically confirmed diagnosis of colorectal adenocarcinoma

3. Presenting with metastatic disease:
    - Primary can be intact or previously resected
    - Metastatic lesion(s) in liver must be non-resectable
    - Extrahepatic disease acceptable
4. At least one liver lesion able to be visualized by ultrasound and determined to be safely assessable for percutaneous cryoablation (if schedule includes cryoablation)
5. Previous treatment failure of two previous lines of active systemic chemotherapy:
    - Previous chemotherapy must have included an oxaliplatin-containing (e.g. FOLFOX) and an irinotecan-containing (e.g. FOLFIRI) regimen
        - With or without bevacizumab
        - Administered in adjuvant setting or for treatment of metastatic disease
        - If KRAS wild type, must have at least one prior anti-EGFR therapy
        - Treatment failure can be due to disease progression or toxicity
        - Disease progression on second line therapy must be documented radiologically and must have occurred during or within 30 days following the last administration of treatment for metastatic disease
6. ECOG performance score: 0-1
7. Adequate hematological function:
    - Absolute granulocyte count $\geq$ 1,200/mm^3
    - Platelet count $\geq$ 100,000/mm^3
    - PT/INR $\leq$ 1.5 or correctable to <1.5 at time of interventional procedures
    - Hemoglobin $\geq$ 9 g/dL (may be corrected by transfusion)
8. Adequate Organ Function:
    - Creatinine $\leq$ 1.5 mg/dL
    - Total bilirubin $\leq$ 1.5 times upper limit of normal (ULN)
    - Alkaline phosphatase $\leq$ 2.5 times ULN *
    - Aspartate aminotransferase (AST) or (SGOT) $\leq$ 2.5 times ULN *
    - Alanine aminotransferase (ALT) or (SGPT) $\leq$ 2.5 times ULN *

    *or $\leq$5x ULN if liver involvement
9. EKG without clinically relevant abnormalities
10. Female subjects: Not pregnant or lactating
11. Patients with child bearing potential must agree to use adequate contraception
12. Study specific informed consent in the native language of the subject.

10.3. EXCLUSION CRITERIA

1. Bowel obstruction or high risk for obstruction
2. Moderate or severe ascites requiring medical intervention
3. Clinical evidence or radiological evidence of brain metastasis or leptomeningeal involvement
4. Symptomatic asthma or COPD
5. Pulmonary lymphangitis or symptomatic pleural effusion (grade $\geq$ 2) that results in pulmonary dysfunction requiring active treatment or oxygen saturation <92% on room air

6. Bevacizumab (Avastin®) treatment within 6 weeks of scheduled cryoablation procedure (if schedule includes cryoablation)
7. Regorafenib prior to the Study Period
8. Previous treatment with TAS102
9. Any of the following mood disorders: active major depressive episode, history of suicidal attempt or ideation
10. Taking anticoagulant medication for concomitant medical condition (unless can be safely discontinued for invasive cryoablation, biopsy and intratumoral injection procedures)
11. Prior allogeneic bone marrow/stem cell or solid organ transplant
12. Chronic use (> 2 weeks) of greater than physiologic doses of a corticosteroid agent (dose equivalent to > 5 mg/day of prednisone) within 30 days of the first day of study drug treatment
    - Topical corticosteroids are permitted
13. Prior diagnosis of an active autoimmune disease (e.g., rheumatoid arthritis, multiple sclerosis, autoimmune thyroid disease, uveitis). Well controlled Type I diabetes allowed
14. Prior experimental therapy
15. History of blood transfusion reactions
16. Known allergy to bovine products
17. Progressive viral or bacterial infection
    - All infections must be resolved and the subject must remain afebrile for seven days without antibiotics prior to being placed on study
18. Cardiac disease of symptomatic nature
19. History of HIV positivity or AIDS
20. Concurrent medication known to interfere with platelet function or coagulation (e.g., aspirin, ibuprofen, clopidogrel, or warfarin) unless such medications can be discontinued for an appropriate time period based on the drug half-life and known activity (e.g., aspirin for 7 days) prior to cryoablation and biopsy procedures
21. History of severe hypersensitivity to monoclonal antibody drugs or any contraindication to any of the study drugs
22. Psychiatric or addictive disorders or other condition that, in the opinion of the investigator, would preclude study participation.
23. Subjects that lack ability to provide consent for themselves

11. STUDY PLAN AND PROCEDURES

11.1. OVERALL STUDY PLAN

Subjects will be screened for eligibility for the study. The screening process involves examining medical records to determine eligibility based on the IC/EC criteria. After obtaining informed consent, subjects will undergo physical exam, provide blood for lab analysis (CBC, CMP, total IgG and IgM, lipase, erythrocyte sedimentation rate, and CRP), an ultrasound of the liver, and a CT scan. The results of these baseline tests will be reviewed by the Principal Investigator (PI) and the Sponsor's medical monitoring team to determine if the subject still meets IC/EC. In schedule A-1, the study interventional radiologist will determine

if the subject has a lesion assessable for cryoablation. If the subject meets IC/EC criteria, a biopsy and tumor harvest procedure will be conducted.

When the PI and the Sponsor agree that all IC/EC criteria are met, the subject is accrued/ enrolled in the study and assigned a protocol number. The enrollment date will be documented in CRF/eCRF. Upon accrual, the study site develops an appointment schedule for the subject and sends to the Sponsor for approval. Upon approval, the subject is notified of the schedule. The Sponsor will produce and ship AlloStim® in accordance with the approved schedule.

AlloStim® will be formulated in Israel by the Sponsor, packaged in validated temperature-controlled container and delivered by specialized courier service to the study site at least one day before the established treatment schedule day. AlloStim® is stable in formulation for at least 72 hours. All protocol required CT scans will be conducted at a radiology center. Cryoablation, intratumoral AlloStim® and voluntary tumor biopsies will be conducted at a radiology center.

11.2. STUDY PROCEDURES

11.2.1. Physical Examination

A Comprehensive Physical Examination will be conducted at baseline. The exam will include the following organ systems: Constitutional; Eyes; Ears, Nose, Mouth and Throat; Neck; Respiratory; Cardiovascular; Gastrointestinal (abdomen); Lymphatic; Musculoskeletal; Skin; Neurologic; and Psychiatric. Subjects should be carefully screened at baseline for depression and suicide attempts or ideations. If there are indications or a history of depression is strongly recommended that these patients be closely followed together with behavioral health or psychiatric medical support. Subjects with an established diagnosis of depression or reported previous suicide attempts or suicidal ideations should not be enrolled on this protocol; the risks and benefits of being treated with the study drug should be weighed very carefully in consultation with behavioral health or psychiatry.

A Basic Physical Examination will be performed at each subject visit, the presence of lymphadenopathy, edema, rash or pain will be recorded. The subject's psychological status will be evaluated as part of the physical examination in each study visit. Information on exam will be documented in the medical record and on CRF/eCRF.

Subjects will be evaluated for response by laboratory blood tests, radiological, pathological and immunological tests, as well as by performance status in order to determine if there is evidence of disease progression. Subjects that have clinically deteriorated and do not show evidence of response, in the judgment of the PI, will be discontinued on further experimental treatment and advised of alternative treatment options.

11.2.2. Monitoring Vital Signs

After each ID injection of study drug, the vital signs will be monitored with real-time monitoring equipment for heart rate, respiration rate, blood pressure, temperature, and pulse oximetry. Values will be recorded every 15 minutes for one hour on a provided form. If vital signs are stable, the subject can be discharged after 1 hour. Otherwise, the subject should continue to be observed until stable.

After each IV infusion and IT injection of study drug the vital signs will be monitored with real-time monitoring equipment for heart rate, respiration rate, blood pressure, temperature, and pulse oximetry. Values will be recorded every 15 minutes prior, during and for 2 hours following the infusion. All changes in vital signs from baseline during and after the infusion will be recorded in the treatment notes. If vital signs are stable, the subject can be discharged after 2 hours. Otherwise, the subject should continue to be observed until stable.

11.2.3. Laboratory tests

All scheduled and unscheduled laboratory assessments will be analyzed by the local laboratory.

Hematology Profile – includes a complete blood count (CBC) with differential.

Chemistry Profile – includes sodium, potassium, chloride, calcium, BUN, creatinine, calcium, total protein, albumin, Aspartate transaminase (AST), Alanine transaminase (ALT), alkaline phosphatase, total bilirubin and direct bilirubin.

Inflammation Marker – C-reactive protein (CRP), erythrocyte sedimentation rate, total IgG and IgM, and lipase

Coagulation Profile – includes INR or prothrombin time (PT) if available (whichever is selected should be followed throughout) and partial thromboplastin time (PTT).

Autoimmune Profile – includes complement C3, anti-DNA Ab, anti-nuclear Ab (ANA), anti-mitrochondrial Ab(AMA), anti-smooth muscle Ab (SMA), liver/kidney/microsomal Ab (LKM).

Pregnancy Test – for women of childbearing potential, a pregnancy test (serum or urine) is required pretreatment. Minimum sensitivity 25 IU/L or equivalent units of β-human chorionic gonadotropin (β-HCG).

11.2.4. Tumor Assessment

CT scan of the Head/Chest/Abdomen/Pelvis with and without contrast (if tolerated) will be conducted at baseline and Day 91. All subjects accrued in schedule A-1 will also be evaluated at baseline to confirm they have a liver lesion that can be readily visualized and safely approached percutaneously for cryoablation.

Subjects will undergo mandatory tumor biopsies of at least one selected target lesion (not lesion targeted for ablation) at baseline, during intratumoral injections (day 21 Schedule A1 and on Day 91.

The intratumoral procedures use a trocar that will have already been inserted into the tumor to conduct the intratumoral injection and thus these biopsies will not add an additional invasional procedure.

In order to obtain information on the longitudinal changes in the immune cell patterns of tumors as well changes in tumor size, biopsies will be collected for subjects who consented under a separate inform consent. These additional biopsies will be conducted only if subjects separately consent. Failure to consent for the intermediary biopsy procedures will not disqualify subjects.

The biopsies material should be placed in containers supplied by the Sponsor. The samples should be labeled with protocol number, subject study ID, date of birth, date of sample

collection and sample time point (protocol day). The containers should be kept at room temperature (RT) and delivered to Sponsor's histology lab within 24h of collection to:

Dr. Miriam Bloch, MD (Pathologist):
Immunovative Clinical Research, Inc.,
4824 East Baseline Road
Suite #113, Mesa, AZ 85206, USA

A biopsy is useful because subjects on immunotherapy can present with "flared" lesions that appear to be larger on CT scan, but may not necessarily represent disease progression. Increase in size can be due to immune cell infiltration, edema, coagulative necrosis and/or fibrotic changes rather than tumor growth. In addition, subjects may present with new lesions which may not necessarily represent progressive disease, but may instead represent immune reactivity to microscopic disease previously not visible. The initial appearance of progressive disease may occur during immunologic therapy for at least two reasons. First, immunotherapy may induce lymphocytic tumor infiltration and inflammation of the tumor, and the associated increase in tumor diameter is then scored as progression on radiographic imaging (i.e. 'tumor flare'). Second, the appearance of new lesions or growth of primary lesions may occur after immunotherapy has been administered, since the process of immune activation is complex and delayed. During this period of activation, the tumor may be able to transiently grow even when effective therapy is priming the immune system to eventually respond.

Immunotherapy may induce responses with a variety of kinetic patterns. Some tumors may respond rapidly, meeting criteria for a RECIST response. Other tumors may respond late in the course of treatment, wherein standard 2- and 3-month evaluations of these patients would classify them as non-responders. Some tumors may actually increase in diameter before eventually regressing. Using RECIST criteria, such patients would be classified as having disease progression and generally would be removed from a trial before late response might be documented [84]. Because of the unique patterns of clinical response that arise with immune-modulating therapies, alternative clinical trial design and end points are necessary to properly evaluate these agents.

For these reasons, irRC and longitudinal tumor biopsies are used in this study in order to distinguish whether changes in the radiographic appearance of tumors correlates with progression or tumor flare, while overall survival is used as the primary endpoint due to the unreliability of RECIST as a method to evaluate response to immunotherapy. The experimental endpoint of seroconversion to IL-12 positivity was found in an earlier Phase I/II clinical trial to correlate with survival. Therefore, radiological, pathological and immunological endpoints will be used for assessment of tumor response.

11.2.5. Immunological Research Assays

11.2.6. Cytokines

Subject sera will be collected for cytokines (such as IL-12p70, IFN-γ and TNF-α) detection and analyses. AlloStim® is designed to activate a Th1 immune response. Measuring IL-12, TNF-α and IFN-γ in a subject's sera can provide evidence to support activation and sustainability of a Th1 immune response activated by AlloStim®. In addition, chronic inflammatory adverse events could be attributed to AlloStim® by following these cytokines, especially TNF-α.

When cytokines testing is required, ~5 ml of whole blood is collected in blood tubes provided by the sponsor and incubated for 30 to 60 minutes at room temperature. Tubes will be centrifuged for 15 minutes at ~1000 rcf and the serum will be separated and aliquoted into new pre-labeled tubes. All samples should be labeled with subject study ID, protocol number, protocol day, date and time of collection. Serum samples should be kept at minus 20°C (-20°C) or below until shipped. Samples will be shipped on a dry ice or in a liquid nitrogen dry shipper via overnight courier to:

Immunovative Therapies, Ltd.
Malcha Technology Park
Building 1, First Floor
Jerusalem, Israel 96951
Phone: +972-2-6506288

11.2.7. Th1/Th2 Intracellular Staining

AlloStim® priming is designed to increase the number of Th1 cells in circulation. Since IFN-γ is a prototypic marker of Th1 cells and IL-4 is a prototypic marker of Th2 cells, the Th1/Th2 balance can be detected by activating T-cells and measuring the expression of IFN-γ and IL-4. Th1/Th2 analysis requires ~50 ml of whole blood in blood tubes provided by the sponsor. The PBMC will be isolated from whole blood following the instructions provided by the Sponsor. Briefly, the whole blood tubes should be centrifuged at 1500 rcf for 15 minutes and whitish layer to be collected into new pre-labeled tube. After the cell counting, the separated PBMC will be washed by centrifugation 8 minutes at 402 rcf, and re-suspended in freezing medium to final concentration of 10×10^6/ml. The suspended cells will be aliquoted to 10^7 PBMC/vial. All PBMC samples should be labeled with subject study ID, protocol number, protocol day, date and time of collection. PBMC samples should be frozen and stored at -80°C or below until shipping. All PBMC samples will be shipped on a dry ice or in a liquid nitrogen dry shipper via overnight courier to:

Immunovative Therapies
Malcha Technology Park
Building 1, First Floor
Jerusalem, Israel 96951
Phone: +972-2-6506288

12. DESCRIPTION OF STUDY VISITS

The following tables summarize all planned assessments, tests, and treatments scheduled to occur as part of this study, including pretreatment evaluations, end of therapy assessments, and follow-up. These assessments will be conducted at the PI clinic and a Sponsor-designated radiology center (imaging, cryoablation and biopsy). Some deviations in the schedule of assessments are allowed as indicated in protocol deviation section 12.1.

Table 1 Table of Assessments Schedule A-1

Protocol activity	Baseline	ID# 1	ID# 2	ID# 3	Cryoablation	IV #1	IV booster #1	IV booster #2	CT+biopsy
Protocol day	-14 to-1	0	7	14	21	28	56	84	91
Informed Consent	x								
Demographics	x								
Inclusion/Exclusion Criteria	x								
Medical History	x								
Physical Examination (complete)	x								
Physical Examination (brief)		x	x	x			x		
Vital signs	x	x	x	x	x	x	x	x	
ECOG	x								
EKG	x								
CT Scan (head, chest, abdomen, pelvis)	x								
CT Scan (chest, abdomen, pelvis)									x
Cryoablation under US or CT guidance					x				
Biopsy	x								x
Hematological Profile (CBC)	x		x	x	x	x	x	x	x
Chemistry Profile (CMP)	x		x	x	x	x	x	x	x
Inflammation Biomarkers*	x		x	x	x	x	x	x	x
Pregnancy Test (β-HCG)	x								
Coagulation profile (PT, PTT, INR)	x			x					
Autoimmune Panel**		x							x
HLA engraftment test	x								x
Research blood draw***		x			x		x	x	
Concomitant Medications	x	x	x	x	x	x	x	x	
Adverse Events		x	x	x	x	x	x	x	x

Table 2 Table of Assessments Schedule A-2

Protocol activity	Baseline	ID# 1	ID# 2	ID# 3	ID #4	ID #5	IV #1	IV #2	IV#3	IV #4	CT+biopsy
Protocol day	-14 to-1	0	3	7	10	14	21	28	49	77	91
Informed Consent	x										
Demographics	x										
Inclusion/Exclusion Criteria	x										
Medical History	x										
Physical Examination (complete)	x										
Physical Examination (brief)		x	x	x	x	x	x	x	x	x	
Vital signs	x	x	x	x	x	x	x	x	x	x	x
ECOG	x										
EKG	x										
CT Scan (4 phase) head, chest, abdomen, pelvis)	x										
CT Scan (3 phase) chest, abdomen, pelvis)											
Biopsy	x					x					x
Hematological Profile (CBC)	x		x	x		x		x		x	x
Chemistry Profile (CMP)	x		x	x		x		x		x	x
Inflammation Biomarkers*	x		x	x		x		x		x	x
Pregnancy Test (β-HCG)	x										
Coagulation profile (PT, PTT, INR)	x					x				x	
Autoimmune Panel**		x									
HLA test	x										x
Research blood draw		x		x		x		x	x	x	x
Concomitant Medications	x	x	x	x	x	x	x	x	x	x	x
Adverse Events		x	x	x	x	x	x	x	x	x	x

12.1. SCHEDULE DEVIATIONS

Scheduled protocol activities should be conducted on the specified protocol days. In some cases the scheduling may not be able to be followed precisely. For example, subjects may miss appointments causing deviations due to illness, weather, transportation problems or miscommunication. The clinic may experience deviations in scheduling due to lack of available site personnel, lack of available supplies, mechanical or equipment failures. Deviations may also occur due to unavailability of the study drug, due to QC failures or study drug transport problems. In addition, holidays and weekend scheduling may cause conflicts.

If a scheduled protocol appointment is missed for any reason and the study drug has already been shipped, the protocol procedure can be delayed by one or two days, as long as the study drug is delivered within its 72 hours shelf life time. If the study drug cannot be delivered within the 72 hours shelf-life window, then the protocol procedure should be rescheduled for the next available protocol activity day. When rescheduling protocol treatments, the study drug should not be administered within 3 days of a previous study drug administration. Thus, up to a 7 day delay in protocol procedures is allowed for a missed appoint.

For schedule C, the second intratumoral injection after cryoablation (day 17) must occur 3 days +/- 1 day after the ablation procedure.

Schedules for laboratory and research blood tests and follow-up assessments can deviate +/- 3 days from the scheduled protocol days. Schedules for CT scans can deviate +/- 1 week from the protocol days. All protocol deviations should be documented with the reason and the rescheduling clearly stated on a form provided by the Sponsor for this purpose.

If protocol deviations in treatment days occur more than once or occur outside of the allowed deviation windows, the subject will be censored as of the date of the violating deviation for survival analysis.

12.2. SCREENING ASSESSMENTS FOR ALL SCHEDULES

Any subjects deemed possibly eligible for participation will be recruited and assigned a screening number. Before screening assessments are conducted, the PI or designated representative will explain the protocol procedures, including timing; the expected and unknown risks and possible benefits; and answer all subject's questions and concerns. The informed consent procedure should be documented in the source record and include at least the following: (1) subject screening number; (2) date and time; (3) person performing the consent procedure; (4) persons present during the consent procedure and their relationship to the subject or the study; (5) document any questions asked or concerns expressed by subject or subject's representative(s); (6) confirmation that subject understands that participation is voluntary and can withdraw at any time without penalty, that the protocol is experimental, that no particular outcome can be assured or is known, that there is the possibility of side-effects some of which may be serious, and that the protocol requires commitment of significant time to visit clinic and participate in telephone interviews. Subsequently, the subject must sign and date and receive a copy of an informed consent document that was approved by the Institution Review Board (IRB) or Independent Ethics Committee (IEC) before any study specific procedure is performed. An additional signed copy should be retained and placed in the Study File.

Baseline assessments can be initiated up to two weeks prior to the first scheduled investigational product administration (Day -14) and must be completed at least 4 day prior to the scheduled investigational product administration (Day -4) due to formulation logistics and transits times. Results of tests or examinations performed as standard of care prior to obtaining informed consent and within 14 days prior to protocol Day 0 may be used, rather than repeating required tests. The following evaluations and procedures will be performed during screening:

- Written informed consent(s)
  - *Study informed consent*
- Complete medical history, including disease history (date of diagnosis, prior cancer therapy and surgery, tumor/node/metastasis staging at the time of diagnosis and study entry and pre-existing toxicities from previous therapies)
- Prior (within 30 days) and current concomitant medications
- Complete comprehensive physical examination, including the evaluation of any evidence of major depression and suicidal behavior (subject's previous suicide attempts and any suicidal ideations)
- Demographics
- Vital signs, including heart rate, respiration rate, blood pressure, temperature, pulse oximetry, and weight.
- EKG
- Blood draws for CBC, CMP, Total IgG and IgM, erythrocyte sedimentation rate, lipase, and CRP
- Pregnancy test (if applicable)
- Coagulation test (PTT, PT, INR)
- HLA baseline test
- ECOG performance score
- Baseline CT scan of head, chest, abdomen, and pelvis (w/ and w/o contrast). If brain involvement at baseline, subjects will be excluded. Future scans only require chest, abdomen and pelvis (unless symptoms are present which suggest possible brain metastasis). Subjects with medical conditions which contraindicate the use of contrast will be scanned without contrast. The baseline scan will be reviewed by an interventional radiologist for determination of a target lesion for cryoablation. An ultrasound study will be conducted on the target lesion(s) to determine feasibility of using ultrasound guidance for the procedure. The interventional radiologist must certify that a metastatic lesion exists in a location that can be safely approached percutaneously for image-guided cryoablation. This certification along with a summary report describing the target lesion will be sent to the referring PI and Sponsor.
- Tumor biopsy (mandatory). Place biopsy in the container provided by the Sponsor and send for pathology analysis

12.3. ELIGIBILITY DETERMINATION (SCREENING) FOR ALL SCHEDULES (STUDY SITE)

After all the baseline data are obtained (baseline H&P, laboratory and imaging data), the PI or designee will conduct a final assessment including opinion of the interventional radiologist regarding tumor accessibility for cryoablation and biopsy (subject need not be present) in

order to assure that the subject meets all inclusion/exclusion (IC/EC) criteria prior to the first investigational product administration (on or before Day -1). Subjects failing to meet IC/EC criteria after baseline assessment will be dropped from the study. A baseline assessment form will be filed by the PI with the Sponsor certifying either enrollment or drop status of the subject after baseline assessment.

Accepted subjects will be assigned a subject number by the Sponsor that will be used for all future reference to the subject.

13. DISCONTINUATION CRITERIA

13.1. EARLY DISCONTINUATION OF THE STUDY

The study may be discontinued due to clinical hold or other regulatory action. The Sponsor can discontinue the study at any time for any reason with appropriate notice to the study site and the IRB with oversight responsibility. The Sponsor can terminate the participation of the study site if the site is not conducting the protocol in accordance with the procedures defined in the approved protocol (i.e. protocol deviations, failure to ensure the quality of the data collected, etc.) or due to low rate of recruiting.

The DSMB can recommend termination of the study if they are of the opinion that the benefit/risk ratio has become adverse to the subject. The PI can terminate participation in the study if in the opinion of the investigator, information on the investigational product causes doubt as to the benefit/risk ratio for participation.

13.2. EARLY DISCONTINUATION OF INDIVIDUAL SUBJECTS

Subjects may discontinue participation in the study for the following reasons:

- Protocol violation
- Withdrawal of consent
- DLT event
- Non-compliance
- Changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the Principal Investigator (the reason(s) must be documented)
- Subject becomes pregnant (withdrawal is required)
- Subject is lost to follow-up

After termination of study therapy, the subject will be treated as clinically indicated by the investigator. All subjects will be followed until resolution or stabilization of any study-related toxicity. If a subject is discontinued from all study therapy, the reason(s) for discontinuation should be documented in the subject’s medical record and CRF/eCRF. A follow-up evaluation should be performed approximately 28 days after the last dose of study drug.

14. STUDY TREATMENT

14.1. STUDY DRUG FORMULATIONS

AlloStim® is formulated T-Stim™ cells that have been activated by adding CD3/CD28-conjugated microbeads and packaged in a syringe. AlloStim® is prepared by thawing frozen, QC released T-Stim™ cells and incubating the cells for 4 hours with Dynabeads® *ClinExVivo*™ CD3/CD28. The beads are mixed with the cells at a 2:1 bead to cell ratio. After 4 hours, the cells with the beads attached are harvested, washed and suspended in formulation buffer containing PlasmaLyteA® supplemented with 1% human serum albumin. The formulated cells with the beads still attached are then loaded in a syringe for infusion or injection. A sample from the syringe lot is tested for bacterial contamination with a Rapid Microbiological Method (RMM) with results available in 48 hours. The investigational product AlloStim® is formulated in syringes as follows:

- Intradermal (ID) dose: 1 x 10^7 cells with 2 x 10^7 beads in 1 ml in a 3 ml syringe
- Intravenous (IV) dose: 3 x 10^7 cells with 6 x 10^7 beads in 3 ml in a 10 ml syringe

14.2. DRUG STORAGE AND RELEASE

The AlloStim® syringes are packaged in validated temperature-controlled containers and shipped by specialized courier service to the study site at least 48 hours prior to the protocol required treatment day.

Each shipment is accompanied by a Shipment Receipt Form and a Certificate of Analysis for product safety testing. Upon receipt, at the clinical site, the product is unpacked by a designated person(s) and immediately transferred to 2˚C to 8˚C (36˚F to 46˚F) storage. The syringe lot is quarantined at the study site until notification of Rapid Microbiological Method (RMM) release. The lot must pass RMM test, prior to release of each syringe to the clinic. The release must be documented on provided forms.

The AlloStim® is stable for 72 hours under refrigeration at 2˚C to 8˚C (36˚F to 46˚F). **DO NOT FREEZE.** Do not use AlloStim® after the expiry time and date that are printed on the label. If the AlloStim® is not administered and the expiry time and date have passed, the syringes should be discarded and another dose should be requested for use.

14.3. DRUG PREPARATION PRIOR TO ADMINISTRATION

Prior to use, the AlloStim® is removed from storage and kept at room temperature for 15-45 minutes. The AlloStim® is stable for 90 minutes at room temperature. If the AlloStim® is not administered within 90 minutes window, the syringes should be discarded and another dose should be requested for use.

For each AlloStim® use, the study drug lot number, time and date the cells released to the clinic, incubation time at room temperature, administration date, time, and dose, injection site location (as applicable) should be documented in subject's medical records and provided forms.

14.4. TREATMENT ADMINISTRATION

14.4.1. Intradermal Injection

Each ID injection contains 1 x 10^7 AlloStim® cells formulated in 0.5ml of 1% human serum albumin in PlasmaLyteA. One pre-formulated 3 ml syringe of AlloStim® will be delivered to the study site per subject for the ID injection days.

For schedules A-1 and A-2, 0.5 ml of AlloStim® will be administered, and the remaining 0.5 ml will be discarded.

ID Injections shall be administered into the forearm (abdomen and deltoid areas can also be used) for schedule A-1 and A-3 on days 0, 7, 14, and 21, schedules A-2 and A-4 on days 0, 7, 14 and for schedule B, C, D, E and F on days 0, 3, 7, and 10. The injection on Day 3 must be in the same location as on days 0. The day 7 injection should be in different place than the day 0 and 3 injections and the day 10 injection in the same location as the day 7 injection. If the skin in the selected site is hardened, inflamed or otherwise not suitable for ID injection, select a different site. The site selected for each injection and the barcode sticker indicating the batch of the dose will be recorded on a provided form.

For the ID injection, the needle should be at a shallow angle, approximately 15 degrees (just under the skin). Some resistance should be felt, if not, the needle is likely too deep and should be pulled back until the bevel is barely under the skin. The plunger should be pressed slowly and a wheal or bubble should appear at the injection site. The longest diameter of the wheal will be recorded immediately following injection in the source document.

14.4.2. Intravenous Infusions (IV)

Schedules A-1 and A-2: Each IV push contains 3 x 10^7 AlloStim® cells formulated in 3 ml of 1% human serum albumin in PlasmaLyteA. Pre-formulated 10 ml syringes of AlloStim® will be delivered to the study site on or prior to the IV administration day. AlloStim® is administered as an IV push over approximately 5 minutes.

Subjects may be hydrated with 500 - 2000 ml of NS (or similar) prior to infusion and may be medicated with 25-50 mg diphenhydramine PO/IV, 150 mg ranitidine (Zantac) PO or other H-2 blocker, 25-50 mg PO indomethacin (or equivalent NSAID) during or after the infusion if medically indicated.

After each IV administration of study drug, the vital signs will be monitored manually every 15 minutes or with real-time monitoring equipment for heart rate, respiration rate, blood pressure, temperature, and pulse oximetry. Values will be recorded prior, during and for 2 hours following the infusion. All changes in vital signs from baseline during and after the infusion will be recorded in the treatment notes.

The physician on duty has the authority to stop, delay, or cancel the infusion at any time if medically indicated. Any such actions that deviate from the infusion protocol will be recorded in the treatment notes and appropriate CRF or eCRF.

14.4.3. Intratumoral Injections (IT)

Subjects in schedule A-1 will receive an IT injection of AlloStim® (3 ml) on Day 21 into the same ablated lesion on Day 17.

Each IT injection contains either 1 x 10^7 AlloStim® cells formulated in 1 ml or 3 x 10^7 AlloStim® cells formulated in 3 ml of 1% human serum albumin in PlasmaLyteA. Pre-formulated 3 or 10 ml syringes of AlloStim® will be delivered to the study site on or prior to the IT administration day.

After each IT administration of study drug the vital signs will be monitored with real-time monitoring equipment for heart rate, respiration rate, blood pressure, temperature, and pulse oximetry. Values will be recorded every 15 minutes prior, during and for 2 hours following the administration. All changes in vital signs from baseline during and after the administration will be recorded in the treatment notes.

14.5. DRUG ACCOUNTABILITY

As required by FDA regulations, all drug storage, procurement and usage will be carefully monitored and documented. The Principal Investigator will oversee this process and may delegate duties to others as needed to conduct the trial under Good Clinical Practices. The study site will be responsible for tracking receipt of the study drug and storage conditions of the study drug up until the time of administration. In addition, the study site must track which study drug syringe was administered to each subject, including date and time, location and route with forms and labels provided by the Sponsor.

14.6. CONCOMITANT TREATMENTS PROHIBITED AND RESTRICTED THERAPIES DURING THE STUDY

No chemotherapy or other anti-cancer treatment is allowed within the first 140 days for Schedules A1-4 subjects and 105 days for Schedule B, C, D, E and F subjects ("Study Period"). If the PI determines that a subject requires anti-cancer treatment during the Study Period, the subject data will be dropped from the protocol. The safety data will be reported but will be censored as of the treatment date. An additional subject will be accrued for each censored subject.

Subjects with bone metastases may receive palliative radiation therapy to treat pain or prevent pathological fractures during the Study Period.

Subjects experiencing anemia can be transfused as medically required (e.g., if Hgb < 9.0) at discretion of the PI. Erythropoietin may be used in continuation therapy, if indicated.

Subjects will receive medically appropriate supportive care, including treatment of pain and infection during the Study Period.

Additional IV booster infusions are allowed at the discretion of the investigator on a monthly basis after the completion of the Study Period.

Use of steroids is not allowed unless medically indicated. NSAIDs should be used as first choice and methylprednisone or SoluMedrol as next choice if anti-inflammatory medication

is deemed necessary. Inhaled steroids are allowed on study. If a subject is administered systemic steroids during the Study Period for any reason, the subject will be dropped from the study and the data will be censored from the first day of steroid administration.

Dexamethasone therapy is contraindicated in immunotherapy protocols, as it has been shown to selectively cause apoptosis of activated effector immune cells.

Administration of dexamethasone will necessitate dropping the subject from the protocol and censoring data from the first day of administration.

If evidence of severe immune-related adverse events (e.g., GVHD, VOD, autoimmune disease) at any time during the protocol, the investigator should first attempt to suppress the inflammation using high dose IV SoluMedrol.

If the condition is refractory to steroids, additional immunosuppressive agents should be considered (e.g., Imuran, cyclosporine). Specific agents for cytokine storm related events, such as Remicade can be considered. T-cell inflammation can be treated with Orthoclone OKT3, monocyte inflammation with Etoposide and B-Cell inflammation with Rituximab. If immunosuppressive treatment does not reverse a suspected immune-mediated adverse event, dexamethasone should be administered to eliminate any activated immune cells. Dexamethasone can be administered alone or in combination with Cytoxan as a reversal strategy.

All concurrent medical care must be documented. Any use of immunosuppressive agents will result in censor of the subject for analysis, but the AE/SAE must be reported even after administration and documented.

15. ADVERSE EVENTS

An adverse event (AE) is any untoward medical occurrence in a trial subject that has been administered the study drug; the event does not necessarily have to have a causal relationship with AlloStim® treatment or usage.

All observed or volunteered AEs, regardless of causal relationship to the investigational product, will be recorded as an adverse event on a supplied case report form (CRF) or electronic CRF (eCRF). Events involving adverse drug reactions, illnesses with onset during the study, or exacerbations of preexisting illnesses should also be recorded as AEs.

Exacerbation of pre-existing illness, including the disease under study, is defined as manifestation (sign or symptom) of the illness that indicates a significant increase in the severity of the illness as compared to the severity noted at the start of the trial and the known normal course of disease progression for stage IV colorectal cancer. This may include worsening or increase in severity of signs or symptoms of the illness, increase in frequency of signs and symptoms of an intermittent illness, or the appearance of new manifestations/complications. Exacerbation of a pre-existing illness should be considered when a subject requires new or additional concomitant drug or non-drug therapy for the treatment of that illness during the trial.

Lack of, or insufficient clinical response, benefit, efficacy or therapeutic effect, should not be recorded as an AE. The investigator must make the distinction between exacerbation of pre-existing illness and lack of therapeutic efficacy. The investigator can take into consideration

the results of radiology and pathology exams and immunological monitoring data when making this assessment.

In addition, abnormal objective diagnostic procedures findings (e.g., abnormal laboratory test results) should be recorded as an AE if it is fulfills one or more of the following:

- Results in subject's withdrawal by the Investigator;
- Is associated with an SAE;
- Is associated with clinical signs or symptoms;
- Is considered by the physician to be of clinical significance;
- Requires the subject to have study therapy discontinued, modified, or interrupted;
- Requires the subject to receive specific corrective therapy; and
- Requires diagnostic evaluation to assess the risk to the subject.

Laboratory adverse events should be captured on the AE /SAE CRF/eCRF as appropriate. It is expected that wherever possible, the clinical, rather than the laboratory term, will be used by the reporting investigator.

Clinically significant changes in physical examination findings should also be recorded as AEs.

15.1.1. Clinical Laboratory Adverse Events

All laboratory test values captured as part of the study should be recorded on the appropriate laboratory test results pages of the CRF/eCRF. In addition, to collecting additional information about clinically important laboratory abnormalities, at a minimum, the following laboratory abnormalities should be captured on the AE /SAE CRF/eCRF as appropriate:

- Any laboratory test result that meets the criteria for an AE or SAE;
- Any laboratory abnormality that requires the subject to have study therapy discontinued, modified, or interrupted; and
- Any laboratory abnormality that requires the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical, rather than the laboratory term, will be used by the reporting investigator.

15.1.2. Immune-Related Adverse Events

The FDA approved the drug Yervoy (Ipilimumab), a checkpoint blockade immunotherapy for the treatment of Melanoma. The immune mechanism of this drug is novel and resulted also in novel immune-related adverse events. Since the immune mechanism of Yervoy and other drugs of this class may be similar to the mechanism of the Study Drug, the Investigators should be particularly vigilant in diagnosing and reporting Immune-Related Adverse Events.

The manufacturer of Yervoy has provided literature to assist physicians in identifying immune-related adverse events. This literature might serve as a guide to investigators as to the types of adverse events that might occur with drugs that have an immune mechanism of action.

15.1.3. Expected Adverse Events

Expected AEs are any AEs whose nature and severity have been previously observed and documented (in the Investigator Brochure) for the study drug. Expected AEs should not be reported to the IRB/EC.

The following adverse events are known to be associated with the administration of AlloStim®:

- Allergy, Immunology Reaction
- Flu-like Symptoms
- Pain-NOS

The following adverse events have been observed but have not been attributed to the study drug as they may be related to the underlying disease:

- Hemoglobin (Low)
- Lymphopenia (Low Abs. Lymphs)
- Alkaline phosphatase (High)
- AST (High)
- Calcium (Hypocalcemia)
- Hypoalbunemia (Low Albumin)
- DVT (deep vein thrombosis) or other thrombolic events

In addition, subjects in trials enrolling patients who failed two treatment lines with no available treatment options (such as the subjects in the current study) are expected to die from their disease.

AEs <u>do not</u> include the following:

- Medical surgical procedures are not AEs (e.g., surgery, endoscopy, tooth extraction, transfusion). The condition that leads to the procedure is an AE if the procedure was not planned at screening visit.
- Overdose of concomitant medication without any signs or symptoms unless the subject is hospitalized for observation.
- Hospitalization for elective surgery planned prior to study (situation where an untoward medical occurrence has not occurred).

AEs will be solicited from physical exam, subject and caregiver/family interview in person or by telephone and laboratory test results. The investigator is to report all directly observed adverse events and all adverse events spontaneously reported by the trial subject or caregiver/family member. Each trial subject will be questioned about adverse events at each clinic visit following initiation of treatment and by telephone at least once a month during the follow-up phase. The question asked should be, "Since your last clinic visit have you had any health problems?", or a similar type of open-ended query.

For all AEs, the investigator must pursue and obtain information adequate both to determine the outcome of the AE, and to assess whether it meets the criteria for classification as a serious adverse event (SAE). For all AEs, sufficient information should be obtained by the investigator to determine the causality of the AE (i.e., investigational product or other illness). The investigator is required to assess causality and indicate that assessment on the CRF/eCRF. Follow-up of the AE is required if the AE persists. Follow-up is required until

the event is resolved or stabilized at a level acceptable to the investigator and the Sponsor's medical monitor or his/her designated representative.

AE severity will be recorded and graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 (June 14, 2010) and coded into the database according to the latest version of MedDRA.

Table 7 Severity of Adverse Events According to CTCAE (Version 4.03)

Grade	Description
0	No AE or within normal limits
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
4	Life-threatening consequences; urgent intervention indicated
5	Death related to AE

A semi-colon indicates 'or' within the description of the grade.
A single dash (-) indicates a grade is not available

The following definitions should be used for toxicities/AEs that are not specified in the CTCAE:

- Mild (Grade 1): The AE is noticeable to the subject, it does not require discontinuation or reduction of the dose of the investigational product, but may require additional therapy.
- Moderate (Grade 2): The AE interferes with the subject's daily activities; it does not require discontinuation of the investigational product, but may require additional therapy.
- Severe (Grade 3): The AE is intolerable and necessitates discontinuing or reducing the dose of the investigational product or additional therapy.
- Life-threatening (Grade 4): The patient is at immediate risk of death from the AE.
- Death (Grade 5).

The PI will assess the relationship between the investigational product and the occurrence of each AE or SAE by using his or her best clinical judgment. The PI must document the reasoning for any decision on attribution. Other potential causes of an AE other than the Study Drug, such as the history of the underlying disease, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational product will be considered and investigated. All alternative causation that was considered, investigated and ruled out will be documented. The causality of all AEs will be based on the Principal

Investigator's assessment of the AE using the causality terms and assessment criteria as presented in Table 8 below. The investigator must provide written justification to demonstrate that a particular event met the chosen criteria and did not meet the criteria that was not selected.

Table 8 Causality of Adverse Event

Relationship	Assessment Criteria
Definitely Related	There is evidence of exposure to the study drug. The time sequence of drug intake and event is reasonable The AE is more likely explained by the study drug than by another cause The event corresponds to what is known about the drug A direct cause and effect relationship between the suspected drug and the AE has been demonstrated The event ceases or diminishes upon stopping drug intake or diminishes during the interval before rechallenge (dechallenge) The event resumes upon restarting drug intake (rechallenge)
Probably Related	There is evidence of exposure to the study drug. The time sequence of drug intake and event is reasonable The event corresponds to what is known about the drug The AE is more likely explained by the study drug than by another cause The event ceases or diminishes upon stopping drug intake or diminishes during the interval before rechallenge (dechallenge) The effect of rechallenge is unknown or does not elicit or exacerbate the event
Possibly Related	There is evidence of exposure to the study drug. The time sequence of the drug intake and event is reasonable The event corresponds to what is known about the drug The effect either remains or continues to exacerbate after dechallenge No change occurs after rechallenge The AE could have been due to another equally likely caus
Unlikely	There is evidence of exposure to the study drug. However, it does not follow a reasonable temporal sequence from administration of drug; or The event does not correspond to what is known about the drug; or The effect either remains or continues to exacerbate after dechallenge and no change occurs after rechallenge; or There is another more likely cause of the AE
Not Related	The subject did not receive the study drug; or The temporal sequence of the AE onset relative to administration of the study drug is not reasonable; or There is another obvious cause of the AE

Outcome to Date are classified as follows:

- Recovered/Resolved: The subject has fully recovered from the AE with no residual effects observable
- Recovered/Resolved with sequelae: The subject has recovered from the AE with residual effects observable

- Improved: The subject status improved but has not been fully recovered
- Ongoing: AE is not recovered/not Resolved
- Fatal
- Unknown

AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) AE dictionary.

All AEs, serious and not serious, will be recorded on the AE CRF page, and if relevant, the Concomitant Medications Record in the CRF will be updated. Particular attention should be made to ensure no discrepancies between the AE and the SAE form (i.e. outcome, severity, relationship must be consistent).

15.2. SERIOUS ADVERSE EVENTS

A Serious Adverse Event (SAE) is an untoward medical occurrence, regardless of whether or not it is considered related to the study medication. Seriousness (not severity) serves as a guide for determining if an AE qualifies as a SAE.

The following must be reported as SAE:

- Death (regardless of the cause) while on treatment that occurs during the safety evaluation period, including deaths due to disease progression.
    - Deaths occurring after the Safety Evaluation Period need not be reported as SAE unless they are a result of an event that started while on treatment during the Safety Evaluation Period.
- A life-threatening AE (e.g., the patient was at immediate risk of death from the event as it occurred) should be reported as SAE. This does not include an event that, had it occurred in a more serious form, might have caused death.
- In-patient hospitalization or prolongation of existing hospitalization
    - Excluding hospitalizations for:
        - administration of the investigational product or procedures required by the study protocol or tumor-related diagnostic procedures
        - other planned hospitalization
        - social reasons and respite care in the absence of any deterioration in the subject's general condition
    - Emergency room visits do not automatically qualify as SAE. Treatments in the emergency room for procedures such as hydration that did not require admitting the patient to the hospital are not considered a SAE, but should be reported as AE.
- A Persistent or significant disability/incapacity.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAE drug experiences when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An event need not be reported as SAE if it exclusively represents a relapse or an expected change or progression of the baseline malignant disease. This type of event needs only to be reported as AE.

Pre-planned hospitalizations or procedures for pre-existing conditions that are already recorded in the subject's medical history at the time of study enrollment will not be considered SAEs.

Any newly emergent SAEs, after treatment is discontinued or the subject has completed the study, and is considered to be related to the study drug or study participation, should be recorded and reported immediately to the Sponsor. The post-study period for the purpose of SAE reporting is up to 30 days following last IV booster administration.

15.2.1. Definition of an Unexpected Adverse Event

An **unexpected** adverse drug event is any AE, the specificity or severity of which is not consistent with information in the current Investigator's Brochure (IB) for an unapproved investigational product.

Serious Unexpected Suspected Adverse Reaction (SUSAR) is a SAE assessed as unexpected by the Sponsor and that is judged by either the reporting investigator or the Sponsor to have a reasonable causal relationship to a medical product.

15.2.2. Handling of Serious Adverse Events

For all SAEs, the investigator is obligated to pursue and provide information as requested by the Sponsor's clinical monitor or designated representative in addition to that on the CRF/eCRF. In general, this will include a description of the SAE in sufficient details to allow for a complete medical assessment of the case and independent determination of possible causation. Information on other possible causes of the event, including concomitant medications and illnesses must be provided. The investigator's assessment of causality must also be provided. If causality is unknown, it should be indicated. In the case of a subject death, a summary of available autopsy findings must be submitted as soon as possible to the Sponsor or its designated representative. The investigator should ensure that information reported immediately by telephone or other means and information entered in the CRF/eCRF are accurate and consistent.

Any SAE or death must be reported immediately to the Sponsor, independent of the circumstance or suspected cause, if it occurs or comes to the attention of the investigator at any time during the study through the last follow-up visit required by the protocol. Any SAE occurring at any other time after completion of the study must also be promptly reported if a causal relationship to the investigational product is suspected.

Any AEs classified as "serious" must be recorded on the AE/SAE CRF/eCRF and require expeditious handling and reporting to Sponsor to comply with regulatory requirements, independent whether considered related or unrelated to the study therapy. These SAEs will include the immediate cause of deaths, regardless of their causal relationship to the investigational product. All SAEs must be reported using the AE/SAE Form. To the extent possible, the descriptive terminology and other SAE attributes entered on the form should approximate similar information in the CRF/eCRF. The completed form must be faxed/emailed to the sponsor within 24 hours of the study site personnel's initial notification/awareness of the event, even if only limited information is available. Preliminary or supplemental SAE information captured on the clinic note, concomitant report, and laboratory report, may alternatively be scanned and transmitted to the Sponsor or designee. The authorized study site personnel may sign completed AE/SAE forms. The investigator signs each final SAE report. The original AE/SAE form must be kept on file at the study site.

Collection of complete information concerning SAEs is extremely important. Thus, follow-up information that becomes available as the SAE evolves, as well as supporting documentation (e.g. hospital discharge summaries, additional laboratory and test results, autopsy reports, etc.), should be collected subsequently, if not available at the time of the initial report, and immediately sent to the Sponsor or designee using the same procedure as the initial SAE report.

Follow-up of all SAEs that occur during the study will continue until their satisfactory resolution or stabilization. In outstanding cases, it may be defined as "ongoing without further follow-up" by the Investigator's and Sponsor's joint decision.

A follow-up SAE Report Form must be completed by the site (marked as "follow-up report") and emailed within 24 hours to the sponsor (safety@immunovative.com). A SAE follow-up report is required whether or not there is any additional information to the initial report.

As for the initial SAE report, once faxed/emailed, the follow-up SAE report and accompanying documentation should be placed in the SAE section of the Investigator's site file.

For ease of analysis, worldwide standardization, and regulatory reporting, Sponsor or designee will code each reported SAE or symptom to its corresponding preferred term and system organ class in the Medical Dictionary for Regulatory Activities (MedDRA®). The NCI-CTCAE Version 4.03 will serve as the reference document for determining/grading the severity or toxicity grade of all SAEs and other symptoms.

For SAEs whose toxicity grading is not contained within the NCI-CTCAE Version 4.03 toxicity criteria, the Principal Investigator will be responsible for assessing severity based on the intensity of the event as it presented. Investigators and study site personnel enter the SAE term on the CRF/eCRF and AE/SAE form as accurately as possible, regardless of the NCI-CTCAE Version 4.03 terminology for the event.

15.2.1. Exceptions in the Reporting of Serious Adverse Events

According to EU Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use, ENTR/CT3, 5.1.9, regarding clinical trials in high morbidity or mortality diseases, it is acceptable to define some exceptions in the immediate reporting of specific SAEs.
Metastatic colorectal cancer is a recognized condition with high morbidity and/or high mortality.

Under these circumstances, it seems appropriate that the SAEs, clearly related to the MCRC (i.e. disease-related) could be an exception for an immediate systematic reporting.
These AEs will be thoroughly handled and followed up through the CRF (AE form) and will be reviewed monthly by the sponsor's medical monitor and could be re-qualified for reporting if necessary.

Each event must be carefully analyzed by the Investigator to decide whether the SAE could be considered as an exception or must be immediately reported.

15.3. ADVERSE EVENTS REPORTING

15.3.1. Reporting Requirements

- The adverse events reporting period for this trial begins upon receiving the first dose of investigational product and ends on the last day of the Safety Evaluation Period.
- All adverse events that occur in trial subjects during the adverse events reporting period specified in the protocol must be reported to the Sponsor, whether or not the event is considered study medication related.
- In addition, any known untoward event that occurs subsequent to the adverse events reporting period that the investigator assesses as possibly related to the study medication should also be reported as an AE.
- The investigator also should comply with the IRB/IEC procedures for reporting any other safety information.
- All AEs should be reported to the Sponsor **within 10 calendar days.**
- All SAEs must be reported to Sponsor immediately (**within 24 hours**) upon knowledge.
- The Sponsor shall forward these AE reports to the Data Safety Monitoring Board.
- The Sponsor shall forward all SAE reports to the Data Safety Monitoring Board immediately upon receipt.

15.3.2. IND Safety Reports

The sponsor will notify FDA and all participating study investigators of an IND safety report that are Serious and Unexpected Suspected Adverse Reaction (21 CFR 312.32(c)(1)(i)) within 7 or 15 day report, but no later than 15 calendar days as follows:

- For any Unexpected Fatal or Life-threatening event associated with the use of the study drug, the Sponsor notifies the FDA of the SAE by telephone or fax as soon as possible, but no later than **seven (7) calendar days** after initial receipt of the SAE. Then Sponsor will follow with the written report (via MedWatch Form 3500A) no later than **15 days since the occurrence.**
- For any Serious and Unexpected, Non-fatal event associated with the use of the study drug, the Sponsor notifies the FDA as soon as possible, but no later than **15 days** after initial receipt of the event.

All other safety information will be submitted to FDA at the end of Part 1 and in the annual report.

15.3.3. Reporting Adverse Events in the (e)CRFs

The investigator is to report all directly observed adverse events and all adverse events spontaneously reported by the trial subject. In addition, each trial subject will be questioned

about adverse events at each clinic visit following initiation of treatment (toxicity assessment).

Data on all adverse events (serious and non-serious, related or not related) will be collected in the CRFs/eCRF. Minimum requirements of adverse event data to be recorded are type of event, start and stop dates, highest severity grade, seriousness, action taken, outcome, and relatedness to either study medication or tumor.

15.3.4. SAE Contact Information

All AEs should be reported to the Sponsor every 10 days by email to safety@immunovative.com. The Sponsor shall forward these AE reports to the Data Safety Monitoring Board.

All SAEs must be reported to Sponsor immediately (within 24 hours) upon knowledge and sent by email to safety@immunovative.com.

15.4. ALLOSTIM® INFUSION-RELATED ALLERGIC OR AUTOIMMUNE TOXICITY

In this study, subjects will continue to be closely monitored for infusion reactions, any allergic or autoimmune toxicity Grade 3 and higher and other study drug related Grade 3 and higher toxicities. Physicians should always remain vigilant for signs and symptoms of severe hypersensitivity reactions.

Symptoms occurring during or following infusion of the investigational treatment may also be defined according to adverse event categories, such as allergic reaction, anaphylaxis, autoimmune disorder or cytokine release syndrome (Immune system disorders). The NCI-CTCAE version 4.03 definition of Immune system disorders is provided in Table 9.

In the setting of symptoms occurring during or following infusion of the investigational treatment, investigators are encouraged to use the adverse event term listed below and any additional terms (including those not listed here) that best describe the event. Those events described above should be graded as follows:

Table 9 NCI-CTCAE v 4.03 Infusion-related Allergic or Autoimmune Toxicity

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Allergic reaction	Transient flushing or rash, drug fever <38 degrees C (<100.4 degrees F); intervention not indicated	Intervention or infusion interruption indicated; responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics); prophylactic medications indicated for ≤ 24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening consequences; urgent intervention indicated	Death
Autoim	Asymptomatic;	Evidence of	Autoimmune reactions	Life-	Death

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
mune disorder	serologic or other evidence of autoimmune reaction, with normal organ function; intervention not indicated	autoimmune reaction involving a nonessential organ or function (e.g., hypothyroidism)	involving major organ (e.g., colitis, anemia, myocarditis, kidney)	threatening consequences; urgent intervention indicated	
Cytokine release syndrome	Mild reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, I.V. fluids); prophylactic medications indicated for ≤ 24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening consequences; pressor or ventilator support indicated	Death

The following are treatment guidelines for AlloStim® infusion reactions:

Grade 1

- Monitor the subject for worsening of condition.
- For subsequent infusions or IV push, premedicate with 25-50mg diphenhydramine PO/IV, 150mg ranitidine (Zantac) PO or other H-2 blocker, 25-50mg PO indomethacin (or equivalent NSAID). During or after the infusion if showed signs of infusion reactions in prior intravenous dosing (rash, itching, shortness of breath) at the investigator's discretion.

Grade 2

- Monitor for worsening of condition.
- Administer diphenhydramine hydrochloride 50 mg IV (or equivalent), acetaminophen 650 mg orally for fever, and oxygen.
- For subsequent infusions or IV push, premedicate with 25-50mg diphenhydramine PO/IV, 150mg ranitidine (Zantac) PO or other H-2 blocker, 25-50mg PO indomethacin (or equivalent NSAID). During or after the infusion if showed signs of infusion reactions in prior intravenous dosing (rash, itching, shortness of breath) at the investigator's discretion.
- If steroid therapy is medically indicated, methylprednisone or SoluMedrol should be considered as first choice. Administration of dexamethasone will necessitate dropping the subject from the protocol and censoring data from the first day of administration. **Dexamethasone therapy is contraindicated in immunotherapy protocols, as it has been shown to selectively cause apoptosis of activated effector immune cells.**
- If a subject should have an infusion reaction to AlloStim®, all attempts should be made to obtain a cytokine blood sample as close to the onset of the event as possible,

at the resolution of the event, and 30 days following the event. The procedure for sample collection and handling is described in a separate procedural manual.

Grade 3

- Stop the infusion or IV Push if not completed.
- Administer methylprednisone or SoluMedrol (or equivalent) medications/treatment as medically indicated.
- Subjects who have a Grade 3 infusion reaction will not receive further AlloStim® treatment, but will continue to be followed on the protocol.
- If a subject should have an infusion reaction to AlloStim®, all attempts should be made to obtain a cytokine blood sample as close to the onset of the event as possible, at the resolution of the event, and 30 days following the event. The procedure for sample collection and handling is described in a separate procedural manual.

Grade 4

- Stop the infusion or IV push if not completed.
- Administer methylprednisone or SoluMedrol (or equivalent) medications/treatment as medically indicated.
- Hospital admission for observation may be indicated.
- Subjects who have a Grade 4 infusion reaction will not receive further AlloStim® treatment, but will continue to be followed on the protocol.
- If a subject should have an infusion reaction to AlloStim®, all attempts should be made to obtain a cytokine blood sample as close to the onset of the event as possible, at the resolution of the event, and 30 days following the event. The procedure for sample collection and handling is described in a separate procedural manual.

15.5. PRINCIPAL INVESTIGATOR/SPONSOR SAFETY REVIEW

The investigator will ensure that the protocol and consent form are reviewed and approved by the appropriate Institutional Review Board/Independent Ethics Committee (IRB/IEC) prior to the start of any study procedures. The IRB/IEC will be appropriately constituted and will perform its functions in accordance with US Food and Drug Administration (FDA) regulations, International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines, and local requirements as applicable.

The investigator(s) undertake(s) to perform the study in accordance with this protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements. The Investigator is required to ensure compliance with the investigational product administration schedule, visits schedule and procedures required by the protocol. The investigator agrees to provide all information requested in the CRF in an accurate and legible manner according to the instructions and to provide the Sponsor representatives direct access to source documents.

The Principal Investigator will be responsible for monitoring the safety and efficacy of the trial, executing the Data Safety Monitoring (DSM) plan, and complying with all reporting requirements to the Sponsor, local and federal authorities.

15.6. DATA SAFETY MONITORING BOARD

A Data Safety Monitoring Board (DSMB) will be established with a primary function to protect the safety and welfare of study subjects. The DSMB will meet to examine

comprehensive safety data, which will be provided to the DSMB at regular intervals (quarterly) as defined by their charter, but may meet with greater frequency at their discretion. DSMB members will receive automatic electronic notification as soon as an SAE is reported at the study site. The DSMB members will have access to needed subject clinical data for complete documentation of all SAEs. All pertinent safety data including information on deaths, tumor progressions, adverse events and laboratory safety data will be reviewed. Subsequent to each meeting of the DSMB, the members will recommend in writing to the Sponsor that either the study continue in its current form, undergo protocol modification to deal with a critical emerging issue, or be stopped because of safety concerns. DSMB meetings will consist of an open session, which the Sponsor's representatives can attend, and a confidential closed session, which the Sponsor's representatives cannot attend. Minutes will be kept for both, the open and closed sessions of the DSMB meetings. The minutes of the closed sessions will not be revealed to the Sponsor until the study has been completed and the database has been locked.

15.7. STOPPING RULES

An external DSMB will oversee this study. The investigators and representatives of the Sponsor and DSMB will review and discuss subject data for all subjects enrolled by teleconference. The DSMB could recommend stopping the clinical trial for safety purposes.

The development of dose limiting toxicity (DLT) in any subject will prompt a review of safety data by DSMB. The development of any DLT toxicity in ≥ 33% of the accumulated number of subjects at any dosing during the assessment period (28 days after last booster infusion) will result in protocol discontinuation. However, if DTLs only occur in the Activation or Booster phase, then a decision by DSMB may be made to continue enrolling subjects to receive only priming and in-situ vaccination phases.

16. STATISTICAL METHODS

16.1. GENERAL CONSIDERATIONS

The Phase I/IIb study is primarily designed to assess the safety and tolerability of the study drug dosing schedule and the related immune response. Any evidence of clinical objective anti-tumor activity and/or symptomatic changes will be recorded.

Safety analyses will be performed on all subjects who receive any dose of study medication. All summary data will be presented by dosing schedule (Schedule A-1 vs Schedule A-2 vs. Schedule A-3 vs. Schedule A-4 vs. Schedule B vs. Schedule C vs. Schedule D vs. Schedule E vs. Schedule F). Any evidence of clinical objective anti-tumor activity and/or symptomatic changes will be recorded.

Descriptive statistics and/or subjects' listings will be provided for demographic data, incidence of adverse events (overall, by intensity, and by relationship to study medication), incidence of clinically significant laboratory abnormalities, vital signs, medical history and physical exam, and tumor assessments.

If subjects are administered steroids, the subject data will be censored at the date of steroid administration.

16.1.1. Demographics

Demographic data will be summarized with descriptive statistics for gender, age, date of diagnosis of disease, prior treatments and ECOG status (e.g. N, mean, median).

16.1.2. Concomitant Medications or Treatments

Concomitant medications will be presented in data listings.

16.1.3. Primary Endpoint

Safety is the primary endpoint. AEs and SAEs will be coded, graded and summarized by attribution. Safety analysis will be performed on all subjects who receive any dose of study medication. AEs that occur after the Safety Evaluation Period will not be included in safety evaluations.

16.1.4. Adverse Events

All reported terms (investigator descriptions) for AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of AEs will be summarized for those events that occur on or after the date of the first treatment (TEAE: Treatment-Emergent Adverse Events). All SAEs and TEAEs leading to withdrawal, and or other significant TEAEs will be listed separately. The data will be summarized as follow:

- all TEAEs,
- TEAEs related to Study drug,
- Serious TEAEs,
- Serious TEAEs related to Study drug,
- TEAEs resulting in death,
- TEAEs leading to withdrawal.

The incidence of TEAEs will be summarized by system organ classification and preferred term by severity and relationship to study medication. All AEs will be included in the data listings.

Laboratory results will be classified according to NCI-CTCAE Version 4.03. Incidence of laboratory abnormalities will be summarized; laboratory results not corresponding to an NCI-CTCAE Version 4.03 term will not be graded. Laboratory toxicity shifts from baseline to worst grade will also be provided.

The results from medical history, physical examination, and vital signs measurements will be tabulated.

16.1.5. Secondary Endpoint (Part 2 only)

Health-Related Quality of Life will be measured at baseline, during the treatment (for subjects enrolled to schedules A1-4 at day 35 and for subjects in schedules B-F at day 28) and after completion of treatment (28 days following the last booster dose). Analysis will be performed to compare quality of life differences over the time.

16.1.6. Exploratory Endpoints

All data will be summarized using descriptive statistics by dosing schedule. All subjects who received any study medication will be included in the analysis.

If the subject is alive at the end of the follow-up period or is lost to follow-up, OS will be censored on the last date the subject is known to be alive.

16.1.7. Missing and Spurious Data

Every effort will be made to obtain required data at each scheduled evaluation from all subjects who have been enrolled. There will be no imputation for missing data.

16.1.8. Determination of Sample Size

The planned sample size of at least 3 subjects at each dose schedule was considered sufficient to assess and to detect major safety and tolerability problems.

17. REGULATORY, ETHICAL AND LEGAL OBLIGATIONS

17.1. DECLARATION OF HELSINKI

The study will be conducted in accordance with the Declaration of Helsinki and GCP according to International Conference on Harmonisation (ICH) guidelines. Specifically, the study will be conducted under a protocol reviewed by an IRB or IEC; the study will be conducted by scientifically and medically qualified persons; the PI and Sponsor believe the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; and all the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject will give his or her written, informed consent in their native language before any protocol-driven tests or evaluations are performed. Consent to serial biopsies will be asked for separately from the consent to participate in the study. This consent must also be written and in the native language of the subject. A subject can consent to the study and not consent to the serial biopsies and will not be excluded for not consenting to the biopsies.

17.2. INSTITUTIONAL REVIEW BOARDS/ETHICS COMMITTEES

The Investigator will submit this protocol to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) and will maintain a record of IEC/IRB review/approval at the site. During the clinical trial, any amendment or modification to the protocol will be sent to the IEC/IRB. The IEC/IRB will also be informed of any event likely to affect the safety of subjects or the continued conduct of the study, in particular any change in safety and all updates to the Investigator's Brochure will be sent to the IEC/IRB.

The IEC/IRB will approve all protocol amendments, written informed consent documents and document updates, subject recruitment procedures, written information to be provided to the subjects, available safety information, information about payment and compensation available to subjects, the investigator's curriculum vitae and/or other evidence of qualifications, and any other documents requested by the IEC/IRB and regulatory authority, as applicable.

17.3. ETHICAL CONDUCT OF THE STUDY

The investigator is responsible for ensuring that the clinical study is conducted in accordance with the protocol and to Standard Operating Procedures (SOPs), Food and Drug Administration (FDA) Good Clinical Practice (GCP) guidelines, FDA Financial Disclosure regulations, as well as the ICH guidelines and any other regional/national requirements for clinical trials, as applicable.

17.4. PROTOCOL AMENDMENTS

Any amendment to the protocol requires written approval/favorable opinion by the IEC/IRB prior to its implementation, unless there are overriding safety reasons.

In some instances, an amendment may require a change to the Informed Consent Form. The Investigator will obtain an IEC/IRB approval/favorable opinion concerning the revised Informed Consent Form prior to implementation of the change. The investigator understands that subjects must be consented using the most current IEC/IRB approved version of the Informed Consent Form. If the Informed Consent Form is updated, subjects who are still receiving therapy will be re-consented, or at the direction of the IEC/IRB.

17.5. COMPLIANCE WITH THE PROTOCOL AND PROTOCOL REVISIONS

The investigator must comply with all requirements of the protocol. When a situation occurs that requires a temporary departure from the protocol, the Investigator or other physician in attendance should contact the sponsor or designee as soon as possible in order to discuss the situation and agree on an appropriate course of action. The investigator will describe the departure from the protocol and the circumstances requiring it on the CRF/eCRF and will notify the IRB/IEC as appropriate.

17.6. INFORMED CONSENT

The investigator or his/her designee will inform the subject of all aspects pertaining to their participation in the study. The process for obtaining subject informed consent will be in accordance with all applicable regulatory requirements (e.g., CFR Part 50 and ICH E6 Section 4.8).

Prior to the beginning of the study, the investigator must have the IRB/IEC's written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects.

The subject must have been given a copy of the informed consent form (ICF) at least 24 hours prior to being asked to sign the document. The investigator or his/her designee and the subject must both sign and date the ICF before the subject can participate in the study. The subject will receive a copy of the signed and dated form, and the original will be retained in the site's study records. The decision to participate in the study that is made by the subject must be entirely voluntary. The investigator or his/her designee must emphasize to the subject that consent for study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

The informed consent and any other information provided to subjects or the subject's legally acceptable representative should be revised whenever important new information becomes available that is relevant to the subject's consent and should receive IRB/IEC approval/favorable opinion prior to use. The investigator, or a person designated by the investigator, should fully inform the subject or the subject's legally acceptable representative of all pertinent aspects of the study and of any new information relevant to the subject's willingness to continue participation in the study. This communication should be documented. During a subject's participation in the trial, any updates to the consent form and any updates to the written information will be provided to the subject.

17.7. CONFIDENTIALITY AND DISCLOSURE

Confidentiality will be maintained within legal limits in the review of the medical records and consent forms, which may contain the identity of the subject. All subjects' identities will be restricted to their initials and a unique subject number.

Regulatory agencies or ethics committees may review medical records, and other study documentation for auditing or inspection purposes. Investigators and their affiliated institutions that are considered covered entities (CE) under the Health Insurance Portability and Accountability Act (HIPAA) will request that the subject sign a fully informed Authorization that will accompany the consent form. The Authorization will include the Authorization Core Elements (see Privacy Rule, 45 CFR §164.508(c)(1)) and the Authorization Required Statements (see Privacy Rule, 45 CFR §164.508(c)(2)). These activities are intended to ensure that subjects understand how their Protected Health Information (PHI) arising from this research study is to be handled. The Privacy Rule is an additional but similar obligation to those that have been required under the Department of Health and Human Services (DHHS) or the Food and Drug Administration (FDA) Protection of Human Subjects Regulations (e.g., 45 CFR part 46 or 21 CFR parts 50 and 56, respectively) to take measures to protect such PHI from inappropriate use or disclosure.

The investigator and other study site personnel will keep confidential any information (including this protocol) related to this study and all data and records generated in the course of conducting the study, and will not use the information, data, or records for any purpose other than conducting the study. These restrictions do not apply to (1) Information that becomes publicly available through no fault of the investigator or study site personnel; (2) information that it is necessary to disclose in confidence to an IRB/IEC solely for the evaluation of the study; (3) information that it is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results that may be published as described under the Publication Section.

17.8. MONITORING AND AUDITING STUDY DOCUMENTATION

The Sponsor (or designee) of this study is responsible to Health Authorities for taking all reasonable steps to ensure the proper conduct of the study in regards to ethics, protocol compliance, and integrity and validity of the data recorded on the CRFs. The Sponsor is also responsible for monitoring data quality and subject safety. Thus, the main duty of the Sponsor is to help the investigator to maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the study.

For the purposes of ensuring compliance with the protocol, GCP, and applicable regulatory requirements, the investigator will permit monitoring and auditing by the Sponsor or designee and inspection by applicable regulatory authorities.

The investigator agrees to allow the auditors/inspectors to have direct access to his/her study records for review, being understood that these personnel are bound by professional secrecy, and as such will not disclose any personal identity or personal medical information.

The confidentiality of the data verified and the anonymity of the subjects should be respected during these inspections.

17.9. STUDY REPORT, PUBLICATION POLICY AND ARCHIVING OF STUDY DOCUMENTATION

All data and records generated during the study and all inventions discovered in the course of conducting the study are the property of Immunovative Therapies, Ltd.

Immunovative Therapies, Ltd. will retain ownership of all data. Data derived from the trial are the exclusive property of Immunovative. All proposed publications based on this study will be subject to the Sponsor's approval requirements. Any publication or presentation related to the trial must be reviewed and approved by the Sponsor before submission of the manuscript. If the Sponsor publishes a manuscript, each PI and sub-investigator (as appropriate) will be named as an author and will have an opportunity to review and approve the submission of the manuscript.

The investigator (or designee) will be responsible for preparing the Clinical Study Report for the site. An integrated clinical study report for all sites will be prepared by the Sponsor in accordance with ICH E3 guidance.

17.10. DATA CAPTURE

17.10.1. Case Report Forms

The CRF is an integral part of the study and subsequent reports. The CRF must be kept current to reflect subject status during the course of the study. Only the subject number and an alpha code will be used to identify the subject.

After obtaining written source document information from each subject at each visit, the site will enter the data onto a paper CRF or directly into an internet –based data collection system (as described under section 11.50.2; Electronic Data Capture).

Source documents are where subject data are recorded and documented for the first time. They include, but are not limited to hospital records, clinical and office charts, laboratory notes, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and records kept at the pharmacy, laboratories, and other departments involved in a clinical trial.

Source documents that are required to verify the validity and completeness of data transcribed on the CRFs must never be obliterated or destroyed.

The investigator will maintain a Delegation of Authority Page to document all persons authorized to make entries and/or corrections on CRFs. The CRF should be reviewed, signed, and dated by investigator.

In case of paper CRF, all CRFs should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced CRF copies. When making changes or corrections, original entries will be crossed out with a single line, and the changes will be initialed and dated. DO NOT ERASE, OVERWRITE OR USE CORRECTION FLUID ON THE ORIGINAL CRF ENTRY.

According to the GCP guidelines, the investigator (or designee) will check the CRF entries against the source documents, except for the pre-identified source data directly recorded in the CRF.

The following table identifies what are source vs. CRF data in this study:

Table 10 Source vs. CRF Data

Type of Data	Source/CRF	Comment
Informed consent	Source documents	Signature date is captured on CRF
Demographics	Source documents and CRF	
Inclusion/Exclusion Criteria	Source documents and CRF	
Medical History (incl. Disease History)	Source documents and CRF	
Physical Examination	Source documents	Performance date and clinically significant results are documented on CRF
Vital Signs	Source documents	Performance date and clinically significant results are documented on CRF
ECOG	Source documents and CRF	
EKG	Source documents	Source documents/CRF and clinically significant results are documented on CRF
CT Scan	Source documents (CD and printed radiology reports)	Performance date is captured on CRF
Ultrasound	Source documents	Performance date is captured on CRF
Cryoablation	Source documents	Performance date is captured on CRF
Biopsy	Source documents (Pathology reports)	Performance date is captured on CRF
Clinical Laboratory Reports (CBC, CMP, CRP, coagulation)	Source documents (Printed report forms)	Performance date and clinically significant results are documented on CRF
Pregnancy test	Source documents	Performance date is captured on CRF
Autoimmune profile	Source documents	Performance date and clinically

Type of Data	Source/CRF	Comment
	(Printed report forms)	significant results are documented on CRF
Research blood (Cytokines and TH1/TH2 balance)	Source documents (Printed report forms)	Performance date and clinically significant results are documented on CRF
HLA Test	Source documents and CRF	
Date & time of blood sampling	Source documents (Printed report forms)	Dates are documented on CRF
Study drug administration details	Source Documents (Drug administration Form) and CRF	
Drug accountability	Folder in Pharmacy/ Investigator's Site File	
HRQoL Questionnaires	CRF	
Adverse events	Source documents and CRF	
Concomitant Medication	Source documents and CRF	
Protocol Deviations	Source documents	
Trial Termination details	CRF	

The ICF will include a statement by which the subject allows the Sponsor, or designee, the Ethics Committee (IRC/IEC), and the regulatory authorities to have direct access to source data which supports the data on the CRFs (e.g. subject's medical file, appointment books, original laboratory records, etc.).

It is the responsibility of the investigator to maintain adequate and accurate CRFs to record all observations and other data pertinent to the clinical investigation.

The Clinical Monitor (CM) is responsible for performing on-site monitoring at regular intervals throughout the study to verify adherence to the protocol; verify adherence to local regulations on the conduct of clinical research; and ensure completeness, accuracy, and consistency of the data entered in the CRF.

Data review may generate requests for clarifications (Queries) from the Sponsor to which the investigator is obliged to respond by confirming or modifying the data questioned.

17.10.2. Electronic Data Capture

If Electronic Data Capture (EDC) will be used for electronic data acquisition and storage, electronic case report forms (eCRFs) will be provided for transfer of all research data by site personnel from data source documentation to a computer database. Each responsible person at a site will have user access with a unique username and password, with permissions providing each person their needed access. Some personnel will have data entry, data review, and query resolution permissions, while others may only have data read permissions, based on their individual trial roles.

CMs will verify all computerized data against its source. Monitoring will be enhanced by computer assisted data management, identifying missing or possibly erroneous data as soon as data are computerized. This approach will allow initial remote monitoring, and communication between CMs and site personnel before and between site visits, and will expedite data review and cleaning. As each subject's data computerization is completed and fully monitored with all queries resolved, that subject's data set will be locked by the CM to only allow read access for the remainder of the trial.

All trial data are to be housed in a secure computing environment. The EDC system will include a complete audit trail of all data entry, monitoring, and query activity that is compliant with HIPAA (and/or equivalents at each participating country) and meets all requirements for 21 CFR Part 11.

Trial data as required by CMs, other authorized representatives of the Sponsor, and appropriate regulatory authority inspectors will be available through the EDC.

All required trial information must be entered into the EDC system. eCRFs are considered complete when all data fields are complete and acknowledged as correct by the CM. In addition, the investigator, as the person ultimately responsible for the accuracy of all CRF data, must sign the investigator's statement at trial completion for each subject.

Final monitored and/or audited eCRFs will be provided by the Sponsor to the sites at the end of the study in the format of PDF file.

17.11. STUDY DOCUMENTS

17.11.1. Curricula Vitae

An updated copy of the curriculum vitae limited to the experience, qualification and training for the investigator and sub-investigators (and completed FDA 1572 form and Financial Disclosure Forms) will be maintained with the site regulatory documents.

17.11.2. Financial Disclosure

Financial disclosure information must be provided by all investigators and sub-investigators and will be collected by the Sponsor prior to the start of the study.

17.11.3. Archiving of Documents

ICF, source documents, drug accountability and retention records, and other study related documents will be retained in the permanent archives of the study site. These will be available for inspection at any time by the FDA per 21 CFR 312. Records retention for this study is required for a period of 2 years following the date on which this study agent is approved by the FDA for the marketing purposes; or, if no application is to be filed or if the application is not approved for such indication, until two years following the date on which the entire study is completed, terminated, or discontinued, and the FDA is notified.

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19. APPENDICES

APPENDIX 1 BIOPSY, CRYOABLATION SURGICAL PROCEDURE

Subjects will be referred to a sponsor dedicated radiology center for the cryoablation and voluntary biopsy procedures and intratumoral injections.

Preferably, the lesion selected for biopsy will not be the same lesion as is targeted for ablation. However, if a second lesion is not available then the lesion for ablation will be biopsied prior to ablation. The cryoablation and biopsy procedures are conducted under CT and/or ultrasound guidance. Visual documentation of probe placement for the cryoprobe probe will be provided and placed in the source documents. In addition, visual documentation of the kill zone after the ablation will also be provided for cryoablation. The tumor biopsy specimen will be placed in formalin in a pre-labeled specimen container. The tumor biopsy specimen will be sent to the Sponsor for pathological analysis.

Subjects will have a target tumor lesion cryoablated under local or conscious sedation followed by an intratumoral AlloStim® injection at a dose of 1 x 10^7 cells formulated in 1 ml of Plasma-LyteA/1% HAS or 3 x 10^7 cells formulated in 3 ml of Plasma-LyteA/1% HSA. The intent of the ablation procedure is not to completely ablate the target lesion, but only to cause an area of necrosis within the tumor. Therefore, it is not necessary to have perfect margins. The intratumoral injection will occur after the ablation area has warmed to 37^0C.

It is preferred that an interventional radiologist, CT technician, and physician trained in anesthesia or emergency medicine be present during the procedure. A Registered Nurse (RN), medical assistant and a cryoablation technician should also be present to support these procedures (same individual may function in two of these roles). The Sponsor may also have a medical/scientific consultant present. If the ablation target is in or near the lungs, it is recommended that the procedure be conducted in a hospital or near a hospital with the capability to diagnose and treat pneumothorax or related complications.

Cryoablation will be performed by a licensed interventional radiologist or other experienced physician using a CryoCare-28 Percutaneous Probe System (Endocare, CA, USA) or the SeedNet™ System (Galil Medical, Israel). Generally, the Galil system is preferred for approach of lesions in the lungs due to the smaller 17g probe size having less of a chance of pneumothorax complications. These systems use the Joule-Thomson effect to cool the end of a cryoprobe in a closed system. In accordance with the gas coefficient and the dimension of the nozzle, different gaseous elements generate different thermal exchange events at the area close to the nozzle. Argon gas is used for cooling (-187^0C), and helium is used for heating (67^0C).

Subjects will be observed for 1-4h prior to release.

Pre-Procedure Assessments

Subjects may be hydrated prior to the procedure with normal saline or equivalent. All subjects requiring IV conscious sedation will have a pre-procedure assessment which will be documented in the medical records including:

- A medical evaluation (history and physical based) by the interventional radiologist (this should include documentation of current medications and a history of any adverse or allergic drug reactions, including anesthesia or sedation, other allergies, NPO status, and an ASA assessment).

- Assessment of the subject's ability to tolerate conscious sedation
- Review of laboratory studies including coagulation studies. PT/PTT within normal range and INR must be ≤ 1.5. Review with subject the previously signed informed consent for the procedure and plan for sedation.
- Age appropriate assessment, including baseline set of vital signs and a pre-procedure SPO2.
- Note that subjects exhibiting hemodynamic instability, oxygen desaturation, or respiratory depression/failure are not appropriate candidates for IV conscious sedation.

Any of the following drugs (or combination) can be used for conscious sedation at the discretion of the treating physician and administered intravenously (IV) or intramuscularly (IM):

- Diazepam (Valium) adult dose: 2-10 mg.; >60yo: 2-5 mg
- Lorazepam adult dose: 0.5 mg/kg; >60yo: .02-0.05 mg/kg
- Midazolam (Versed) adult dose: .07 - 0.08 mg/kg; >60yo: .02-0.05 mg/kg
- Morphine adult dose: 0.025-0.2 mg/kg; >60yo: 0.05-0.2 mg/kg (Max. of 15 mg)
- Meperidine (Demerol) adult dose: 1-1.5 mg/kg; >60yo: 1-1.5 mg/kg
- Fentanyl (Sublimaze) adult dose: 1-2 mcg/kg; >60yo: 1 mcg/kg

Care must be taken to assure that the total dose does not obtund protective pharyngeal reflexes or render the subject unresponsive to verbal stimuli.

For some procedures, where subject breath movements may affect the ability to accurately place the cryoprobe, DIPRIVAN® (propofol) or similar sedation medication is allowed during the interval of probe placement.

The following equipment/emergency drugs shall be immediately available (Code Cart) prior to IV conscious sedation being administered:

- Oxygen source
- Ambu bag
- Laryngoscope
- Endotracheal Tubes (i.e. sizes 5.0 mm, 6.0 mm, 7.0 mm, 8.0 mm)
- Oral and nasal airways
- Emergency drugs (Narcan, Romazicon)
- Cardiac monitor/defibrillator and suction equipment
- Pulse Oximeter

Immediately Prior to Initiation of Conscious Sedation

- Place the subject on monitoring device for heart rate, pulse oximetry and blood pressure.
- Prior to the initiation of conscious sedation, the following assessments are determined, monitored, and documented:
    - Level of consciousness and mental status
    - Vital signs: heart rate, blood pressure, respiratory rate, temperature and oxygen saturation
    - NPO Status
    - Baseline Aldrete scale

- Peri-procedure monitoring, subject care, and treatment including medications administered will be documented on the Sedation Record Form and included in the medical records.

Intra-Procedure Monitoring and Care

- The physician will order the sedation medication and administer the initial dose. The RN may administer subsequent medications.
- Cardiac monitoring and oxygen saturation are to be monitored continuously and documented every 5 minutes.
- Blood pressure, pulse, respiration and heart rate/rhythm will be taken and documented at least every five minutes, or more frequently depending upon changes in the subject's condition.
- The subject will also be monitored for potential adverse reactions to the medications being administered. Any signs or symptoms of adverse reactions are to be reported immediately to the attending physician and documented.
- Level of consciousness will be monitored and documented.
- Supplemental oxygen will be administered if oxygen saturation is less than 95%.
- Administered drugs should be recorded, including route, time and dosage.
- If subject demonstrates persistent oxygen desaturation (SaO_2 less than 90%) despite the use of supplemental oxygen, or requires airway support, the case should be terminated.

Biopsy and Cryoablation

- Once the percutaneous approach is determined, a sterile field will be created and local anesthesia administered to the planned probe insertion site.
- A guide probe will be inserted percutaneously and verified by CT to be within the target tumor lesion. A needle biopsy system will be used to obtain a sample of tumor to verify ablation of live tumor. Preferably, this determination will be made "real-time" by an on-site pathologist. If a pathologist is not available, the tumor sample will be placed in formalin in a pre-labeled container and sent for retrospective pathological analysis.
- If the subject is undergoing cryoablation, a cryoprobe will be inserted through the guide tube into the tumor. One or two freeze-thaw cycles will be performed. A single probe of 2- or 5-mm will be used according to the size of the target tumor. The time of freezing will be approximately 5-20 minutes dependent on the achievement of an "ice-ball", visible on CT or ultrasound. In the case of a Galil probe, a thermosensor may also be placed in or near the tumor lesion. Thawing will be achieved by input of helium during a period equivalent to the freezing time before the second freezing process will be initiated. The procedure requires ablation of a sample of the tumor lesion and does not require complete tumor ablation with tumor-free margins.
- The lesion will be allowed to cool for approximately 10 min to 1 hour following the second freezing or heating cycle before injection of AlloStim®. AlloStim® is formulated in Plasma-LyteA in a syringe with a locked needle. The needle will be inserted through the guide tube and into the ablated tumor. AlloStim® will be administered intratumorally by injection. The syringe will be flushed into the tumor with additional Plasma-LyteA or saline after injection.
- Following injection, the guide tube will be removed. Hemostasis of the insertion hole will be obtained by Spongel application to the tract of the guide tube and by suture of the insertion site (if necessary).

Immediate Post Procedure Management

- Subjects will go to an appropriate post-anesthesia care observation area, transported by a registered nurse.
- Vital signs (which include: heart rate, blood pressure, respiratory rate, level of consciousness, and oxygen saturation) will be taken every five to fifteen minutes until the subject reaches the defined discharge criteria. Heart rate/rhythm will be monitored by a cardiac monitor. Post sedation assessment shall also include subject's orientation.
- Significant variations in physiologic parameters are to be reported to the physician immediately. These shall include, but not be limited to:
    - A variation of plus or minus 20% in vital signs
    - Serious arrhythmia
    - Oxygen saturation greater than 5% below baseline
    - Dyspnea, apnea, or hypoventilation
    - Diaphoresis, inability to arouse subject , or the need to maintain the subject's airway
    - Other untoward or unexpected subject responses
- Blood is drawn for post-ablation blood tests: CBC, CMP, and inflammation markers. Blood must be drawn within 2 hours after ablation treatment to identify any acute changes.

Discharge Criteria

The RN or medical assistant managing the care of the subject should provide continuous monitoring until a judgment is made by a physician that the subject is ready to be discharged.

Any subject receiving a reversal agent must be observed for 2 hours after the last dose of the reversal agent.

The decision for discharge will be guided by the following criteria which will be documented in the medical records and the discharge order will be written by a physician when the subject reaches a score of seven (7) on the Aldrete scale:

- Subject is able to be aroused
- Blood pressure, heart rate and respiration rate are stable for at least two sets (q 15 min.) and have returned to pre-procedure status
- Oxygen saturation is back to or near baseline
- Subject is oriented to person, place, time and situation and ambulates without difficulty (if these were skills prior to procedure)
- Subject is able to verbalize appropriately; stands and dresses self (if these were skills prior to procedure)
- Subject has minimal/tolerable pain level
- Subject has no nausea, vomiting, or bleeding
- Subject must have a responsible adult to accompany home. In no case should the subject be allowed to drive himself/herself home. For the purpose of this section, a cab or bus driver is not considered a responsible adult
- Subject has received written discharge instructions related to procedure, limitations, etc. and prescription for pain medication

The interventional radiologist will provide the referring PI with a written report of the cryoablation and biopsy procedures within 48h.

APPENDIX 2 CLINICAL HOLD

The FDA placed a clinical hold on IND 13936 in May 2010. The clinical hold was placed after the Sponsor notified FDA that an employee had made allegations regarding the conduct of the trial to the IRB that had oversight and that the IRB had initiated an investigation of the allegations. The IRB subsequently filed a report with FDA finding most allegations without merit. This report is available upon request. The FDA subsequently conducted their own on-site investigation which resulted in a Form 483 being issued to the PI and the Sponsor.

The major issue with respect to the PI related to "planned protocol deviations". This was a process whereby the Investigator would notify the IRB of a planned change in dosing frequency for a small number of subjects. The protocol was sub-divided into sections including intradermal dosing, intraturmoral dosing and intravenous dosing. After intradermal dosing an increased in allo-specific Th1 was expected. After intraturmoral dosing, an increase in tumor-specific Th1 cells was expected and after intravenous dosing, a cytokine storm was expected. When the expected immunological response was not detected, the PI petitioned the IRB to continue the dosing section until the response was noted before advancing to the next part of the protocol. The PI conducted these planned protocol deviations upon obtaining IRB clearance. This resulted in some subjects receiving more doses than others. During the inspection, FDA maintained that the protocol deviations were safety issues that required FDA protocol amendments. The IRB disagreed and argued that they had the authority to authorize this change in a few subjects to determine if a protocol amendment should be submitted.

Numerous other issues were raised and were finally resolved as follows:

1. Sponsor agreed that no protocol deviations would be made without an approved protocol amendment
2. Sponsor agreed to form a DSMB to monitor the safety using an FDA-approved charter
3. Sponsor agreed to have an independent clinical monitor conduct inspections of clinical sites under a FDA-approved charter. The independent monitor will report to FDA monthly on compliance with GCP.

The major issue with the Sponsor was related to the QC release testing of the final formulated lots of study drug. The intermediate of the drug was stored frozen and underwent USP sterility and rapid test for endotoxin. When needed in the clinic, released intermediate was thawed and formulated in a clean room located in the clinic. Since the study drug at the time had only a 4-6h shelf life and USP sterility testing required 14 days, it was not possible to know if a formulated dose was sterile before it was infused. This put increased emphasis on release testing for safety. The release test of greatest concern was a rapid test for endotoxin using an FDA-approved device. The device had internal quality tests to determine that a sample met pre-determined criteria before presenting a result. Because the device used optics and the study drug contained microbeads, occasionally the beads interfered with the device optics requiring re-testing. The FDA expressed that the repeat testing was a safety concern.

This issue was addressed in multiple ways. First, the rapid test assay underwent a rigorous validation protocol with the validation experimental design pre-approved by FDA. This was successfully completed. Second, the endotoxin testing of the intermediate product was changed to a validated gel clot method conducted in a licensed lab. Lastly, a rapid

microbiological test method capable of detecting all organisms in the USP 71 sterility test within 48h was validated and incorporated in the release criteria.

The GMP issues were finally resolved by:

1. Donors for the source blood will be screened and tested in accordance with 21 CFR 1271 tissue regulations which are more stringent than the blood regulations.
2. Sponsor agreed to an independent GMP monitor that will report monthly to FDA. The monitoring will be conducted in accordance with an FDA-approved charter.

APPENDIX 3 CLINICAL TOXICITY DATA

1. Introduction

This appendix presents clinical data for all studies conducted under IND 13,936 (cleared by FDA on August 13, 2009) from September 2009 to May 2010 with follow-up to May 2011. The following table presents an overview of the studies under the IND:

Protocol Number	Objective	Study Status
ITL-001-HM	To evaluate the safety, toxicity, and anti-tumor effect of administration, of AlloStim® in hematological malignancies	Withdrawn
ITL-002-CRYO	To determine the safety and feasibility of creating an individualized *in-situ* anti-tumor vaccine in subjects with refractory or metastatic cancer	Completed

2. Study Summary ITL-001-HM

Title of Study:	A Phase I/II Study of Polyclonally Activated, Intentionally Mis-Matched, Allogeneic Th1 Memory Cells (AlloStim®) in Subjects with Relapsed or Refractory Hematological Malignancy without Prior Conditioning
Protocol Number:	ITL-001-HM
Study Design:	Subjects meeting eligibility criteria receive an initial intravenous priming infusion of 1 x 10^8 AlloStim® on day 0 and booster infusions of 1 x 10^8 AlloStim® cells on days 7, 14, and 21 (+/- 3 days) as tolerated.
Study Purpose:	To evaluate the safety of administration, toxicity and anti-tumor effect of intravenous AlloStim® infusions.
Study Population:	The study population was to consist of up to 50 Subjects with hematological malignancy refractory to standard therapy, or that recurred after standard therapy, or hematological malignancy Subjects referred for allogeneic stem cell transplantation that were unable to locate a suitable donor.
Study Duration:	Subjects were to be evaluated at 30 days, 60 days and 90 days post last infusion for tumor response. Subjects with complete response were to be followed for an additional 180 days and re-evaluated to determine durability of the response. Subjects with stable disease or partial response will be offered an additional cycle of 1 x 10^8 AlloStim® infusions weekly for up to 3 consecutive weeks and then will be re-evaluated for tumor response at 30 days following the 3rd infusion. Subjects showing disease progression at day 90 were to be taken off protocol. The protocol was planned to accrue subjects over a period of approximately 2 years.
Study Status:	Withdrawn (2 subjects completed the treatment)

3. Study Summary ITL-002-CRYO

Title of Study:	A Phase I/II study of an experimental therapeutic cancer vaccine created in-situ in subjects with progressive metastatic cancer refractory to standard therapy.
Protocol Number:	ITL-002-CRYO
Study Design:	This is a single-arm, open label, single institution Phase I/II study to determine the safety and feasibility of creating an individualized in-situ anti-tumor vaccine. The study protocol has three separate steps: (1) priming step; (2) in-situ vaccination step; and (3) immune stimulation step. Subjects meeting eligibility criteria were primed with at least three and up to nine intradermal AlloStim® injections at a frequency of every 2-8 days at doses between 1 x 10^7 to 4 x 10^7 cells. After priming, a selected tumor lesion was cryoablated using a percutaneous, image-guided technique followed by an intratumoral injection of 1-6 x 10^7 AlloStim®. Subjects with palpable tumors also were eligible to receive at least one and up to three alcohol ablation procedures to palpable lesions followed by intratumoral AlloStim®. This was in addition to the cryoablation procedure or in replacement of the cryoablation procedure. At the same time as the intratumoral injections, and/or up to eight days following, the subjects were administered intravenous AlloStim® at doses between 1 x 10^7 to 1 x 10^9 cells. Some subjects with ascites and/or carcinomatosis received intraperitoneal AlloStim® at a dose of 0.5 to 5 x 10^8. Subjects also received intravenous AlloStim® at 1 x 10^7 to 1 x 10^9 cells at the same time or up to 8 days following.
Study Purpose:	This Phase I/II clinical study was designed to investigate the safety and anti-effect of a personalized anti-tumor vaccine created within the body of subjects with advanced cancers.
Study Population:	The study population was to consist of up to 50 subjects, 18 years or older, with progressive metastatic cancer refractory to all standard therapy with at least one lesion accessible for percutaneous cryoablation.
Study Duration:	Total duration of the treatment period for each subject was 90 days. Subjects were evaluated at 30 days after the last AlloStim® treatment for tumor response and again at 60 days and at 90 days.
Study Status:	Completed (42 subjects completed the treatment)

Study ITL-002-CRYO – Tumor Locations:

Tumor Location	No. of Subjects	%
Colorectal	8	19.0
Liver	22	52.4
Esophagus	2	4.8
Soft tissue	7	16.7
Lymph node	20	47.6
Lung	22	52.4
Pancreas	5	11.9
Ovary	3	7.1
Spleen	3	7.1
Bone	24	57.1
Breast	16	38.1
Adrenal	3	7.1
Brain	2	4.8
Omentum/mesentery	5	11.9
Prostate	1	2.4
Gallbladder	1	2.4
Skin	3	7.1
Uterus	1	2.4
Vagina	2	4.8

4. Toxicity Results

4.1. Study ITL-001-HM

No dose limiting toxicity, no adverse events > grade 2 and no adverse events requiring adjustment of dose or medical intervention.

4.1.1. Subject 001:

27 year old male diagnosed with stage IV Hodgkin's disease refractory to chemotherapy. Progressed after myeloablative autologous stem cell transplant performed at UCSD in June 2009. Subject was accrued in study March 2010. Presented at baseline with large mediastinal/pleural mass. No acute toxicity >grade 2 noted. Subject deceased August 20, 2010 due to complications related to surgical procedure. Autopsy revealed significant immune-mediated tumor lysis and fibrosis.

4.1.2. Subject B002:

66 year old male diagnosed with stage IV mantle cell non-hodgkin's lymphoma in May 2009. Underwent 6 cycles of R-hyper CVAD chemotherapy. Disease recurrence in October 2009 in head, chest, abdomen, pelvis and bone marrow. Underwent 2 cycles of R-ICE chemotherapy and did not respond. Started salvage Velcade and acyclovir but discontinued due to HSV encephalitis and severe pancytopenia. Deceased July 15, 2010 from disease complications.