PROTOCOL TITLE:

Phase 2A Study Evaluating a Chemokine-Modulatory Regimen in Patients with Colorectal Cancer Metastatic to the Liver

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Confidentiality Statement

ABBREVIATIONS

µg	microgram
µL	microliter
5-FU	5-fluorouracil
ACE	angiotensin-converting-enzyme
AE	adverse events
α	alpha
αDC	alpha Dendritic Cell
ALT	Alanine transaminase
ANA	antinuclear antibodies
ANC	absolute neutrophil count
AST	Aspartate transaminase
β	beta
BUN	blood urea nitrogen
CBER	Center for Biologics Evaluation and Research
cc	cubic centimeter
CD3	cluster of differentiation 3
CDER	Center for Drug Evaluation and Research
CEA	carcinoembryonic antigen
°C	degrees Celsius
CKM	chemokine modulatory
Cl	chloride
cm	centimeter
CO2	carbon dioxide
COX2	Cyclooxygenase-2
CRC	colorectal cancer
CRCLM	CRC liver metastasis
CRCM	metastatic colorectal cancer
CRS	Clinical Risk Score
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
dL	deciliter
DLT	Dose Limiting Toxicity
DSMC	Data Safety Monitoring Committee
(ds)RNA	Double-stranded RNA
ECOG	Eastern Cooperative Oncology Group
EKG	Electrocardiography
°F	degrees Fahrenheit
FDA	F
g	gram
γ	gamma
GI	gastrointestinal

GMP	good Manufacturing Practices
HCG	Human Chorionic Gonadotropin
HDI	High Dose Interferon
HPRT	hypoxanthine phosphoribosyl transferase
ICH	immunohistochemistry
IFN	interferon
IL-2	interleukin-2
im	intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
ISH	in situ hybridization
IU	International Units
IV	intravenous
kg	kilogram
LPS	lipopolysaccharide
m2	meters squared
mg	milligram
min	minute
mL	milliliter
MRI	Magnetic Resonance
mRNA	Messenger RNA
NCI	National Cancer Institute
ng	nanogram
NSAID	non-steroidal anti-inflammatory drug
PET	Positron emission tomography
PGE2	prostaglandin E2
po	by oral route of administration
RPCI	Roswell Park Cancer Institute
RP2D	recommended Phase 2 dose
SAE	severe adverse events
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvate Transaminase
SOC	standard of care
ssRNA	single-stranded RNA
Teff	effector T cells
TLR	toll-like receptor
Treg	regulatory T cells
U	unit
ULN	upper limit of normal
UPCI	University of Pittsburgh Cancer Institute

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1 OBJECTIVES

1.1 Study Objectives

1.1.1 Primary Objectives

- To determine the impact of a chemokine-modulatory regimen on the immune microenvironment of colorectal liver metastases, specifically the changes in the ratio between CTL marker (CD8a gene expression) to Treg markers (FoxP3 gene expression)

1.1.2 Secondary Objectives

- Estimate the objective response rate of a chemokine-modulatory regimen in metastatic colorectal cancer (per RECIST 1.1)
- Examine the safety and tolerability profile of the combination of Interferon Alpha-2b, rintatolimod, and celecoxib when given as chemokine modulation to colorectal cancer patients prior to surgical resection using the CTEP NCI Common Terminology Criteria for Adverse Events (CTCAE Version 4.0).

1.1.3 Exploratory Objectives

- Estimate the median progression free survival of a chemokine-modulatory regimen in metastatic colorectal cancer.
- Estimate overall survival in participants with recurrent and/or metastatic unresectable colorectal cancer who received the chemokine-modulatory regimen
- To conduct correlative science studies including:
    - Comparison (using RT-PCR, Immunofluorescence [IF] and immunohistochemistry [IHC] on serial sections) of the metastatic tissue specimen with regard to total numbers of infiltrating T cells, their CD4/CD8 ratios, frequencies of FoxP3 cells, and the expression of chemokine receptors on $CD4^+$ and $CD8^+$ T cells (CXCR3, CCR5, CCR4, CCR6, and CXCR4).
    - Evaluate the local expression of T_{eff}-attracting chemokines (CCR5, CXCL9, CXCL10 and CXCL11) and T_{reg}-favoring chemokines (CCL22 and CXCL12) using IF and RT-PCR.

2 BACKGROUND

2.1 Metastatic Colorectal Cancer

Cancer of the colon and rectum (colorectal cancer, CRC) is the third leading site of cancer diagnosed annually in the United States and the most common form of gastrointestinal malignancy. It accounts for approximately 150,000 new cases annually. Hepatic metastases as the sole or dominant site of life-limiting disease occur in approximately 20-50% of all patients. Median survival for patients with untreated hepatic metastases ranges from 4-12 months [1]. Complete surgical resection of isolated hepatic metastases has been demonstrated in numerous series to result in 5-year overall survival up to 58% in some series [2]. However, most patients are not candidates for resection and systemic therapy remains the mainstay of treatment. Systemic chemotherapy with

5-FU based regimens is associated with modest improvements in survival as compared with supportive care alone (8-14 months) [3]. The addition of oxaliplatin or irinotecan can increase survival up to 24 months. The addition of further biologic agents, including bevacizumab and cetuximab, further improves median survival to approximately 30 months [4]. However, gains in outcomes have petered off with the most recently approved agents. In the last 5 years, while three new drugs have been approved, 2 of 3 confer a median survival benefit of less than two months [5, 6]. Novel therapies and approaches are needed to alter the treatment paradigm in this disease. On the basis on compelling activity in MSI-H (micro-satellite instability-high)/MMR (mismatch repair) deficient cancer, PD-1 targeting therapy has recently been approved in MSI-H tumors and offers new hope for these patients [7]. However, MSI-H tumors represent just 3% of all stage IV colorectal cancers [8]. For the other 97%, great need remains. Therapies that might alter the tumor microenvironment and expand the therapeutic spectrum of immune checkpoint inhibitors would be practice changing.

2.2 Critical Role of the Chemokine-Driven T Cell Migration in Colorectal and Other Cancers

The numbers of effector T cells (T_{eff}) in CRC tissues have been shown to be an independent prognostic marker in CRC, with their high numbers predicting a delayed time to tumor recurrence [9-12]. In contrast, regulatory T cells (T_{reg}) in CRC patients are associated with poor tumor responses [13-16]. Chemokines and their respective receptors are critical for T cell migration and homing into tissues during homeostasis and inflammation [17-22]. Several studies have shown that chemokines are equally important for migration and homing in cancer tissues. For example, the chemokine receptors CXCR3 and CCR5 are critical for T cell entry into tumors, a process which is guided by intra-tumoral expression of their respective chemokines. High levels of the chemokine ligands for CCR5 (CCL5/RANTES) and CXCR3 (CXCL9/MIG, CXCL10/IP10, CXCL11/I-TAC) in tumor tissue have been associated with infiltration of CD8 T cells in CRC [23], malignant melanoma [24] and gastric cancer [25]. High $CXCR3^+$ T cells in the peripheral circulation for example have been associated with increased survival of stage III melanoma patients [26]. In contrast, high levels of CCL22 (ligand for CCR4) expression by dendritic cells (DC) or macrophages in ovarian ascites leads to recruitment of regulatory T cells; a pattern associated with decreased patient survival [27].

As shown by the above studies, selective enrichment of T cells with specific chemokine receptors in patients with cancer may impact tumor response and patient survival. But most tumors do not have T cell infiltration or express the right chemokines for effector T cell (T_{eff}) entry. Colon tumors frequently show elevated CCL22 levels induced by a Prostaglandin E_2 ($PGE_{2)}$ rich tumor microenvironment [28, 29] permitting the influx of regulatory T cells (T_{reg}) rather than T_{eff} . This leads to a highly biased and unfavorable T_{reg}/T_{eff} ratio in tumors.

This study attempts to correct the above mentioned chemokine bias in the colorectal tumor microenvironment and analyze whether such modulation can help $T_{eff,}$ entry while simultaneously preventing T_{regs} influx into tumors.

2.3 Past experience with IFNα, Rintatolimod and Celecoxib

Interferon–alpha (Intron® A) is currently FDA approved for the adjuvant treatment of stage III melanoma. It has been examined by multiple investigators as an immune modulator in the setting of vaccine therapy with demonstrated safety [30]. It has been extensively evaluated in the setting

of metastatic CRC (as single agent or in combination with chemotherapy or IL-2), but was shown to be largely ineffective [31-48] at daily doses ranging from 3-20 million units/m^2, administered subcutaneously or intravenously. In the treatment of melanoma, a small study by Astsaturov and colleagues [49] administered IFNα–2b in previously vaccinated melanoma patients, and was shown to convert immunization into objective clinical responses, suggesting its ability to amplify the effectiveness of local T cells infiltration and/or function at the tumor site. Vaishampayan et al examined IFNα-2b 5 million units subcutaneously three times a week following vaccination with a melanoma vaccine (Melacine) [50]. Treatment at this dose was well tolerated. Similarly Mitchell at al recently completed a large trial examining IFNα-2b, 5 million units subcutaneously three times a week for one year [51]. Di Pucchi and Pilla similarly examined the combination of a melanoma peptide vaccine with IFNα-2b, 3 million units subcutaneously three times a week, with little to no toxicity.

We propose to include a short course of high dose Interferon Alpha-2b (20 million units/m^2, IV, daily for 3 days), a dose similar or lower than the previously evaluated dose in melanoma patients [52-54]. In this group of patients, in accordance with previous reports, we expect to consistently observe flu-like symptoms but no regimen limiting toxicities, particularly taking into account the short duration of this administration.

Celecoxib has been extensively studied in long term clinical trials in patients predisposed to the development of colorectal cancer [55, 56]. However, there is to our knowledge, no clinical data examining its use as a short term immune modulator in the setting of neoadjuvant therapy. Several studies in murine models have suggested improved efficacy of anti-tumor vaccination with Cox-2 inhibition [57-59]. Recent literature suggests increased risk of adverse cardiovascular events associated with the administration of some selective Cox-2 inhibitors [60-62].The literature regarding the precise risk with celecoxib remains controversial, with reported cardiovascular side effects observed mainly in long-term users. It has been suggested that lower doses and shorter lengths of administration may decrease the risk of adverse cardiovascular events. This trial will involve a short course of celecoxib, consisting of 200 mg twice a day for 3 days during administration of the chemokine modulation.

Rintatolimod (Selective toll-like receptor-3 [TLR3] Ligand; analog of poly IC with reduced toxicity for i. v. use).

Rintatolimod is a TLR3 specific Poly IC analog in which the cytidylic acid chain has uridylic acid substitutions at a molar ratio of 12:1. Chemokine modulation in temporal association with type I interferon administration is observed with this agent.

Rintatolimod has been studied extensively in multiple clinical settings including cancer, chronic viral infection, vaccination protocols, and in chronic treatment of chronic fatigue syndrome.

Rintatolimod (Ampligen®) has been generally well tolerated with only a low incidence of clinical toxicity. Clinical experience with Rintatolimod now totals over 800 patients with more than 200 patients receiving Rintatolimod for up to one (1) year, over 50 patients up to two (2) years, and more than 20 patients over two (2) years.

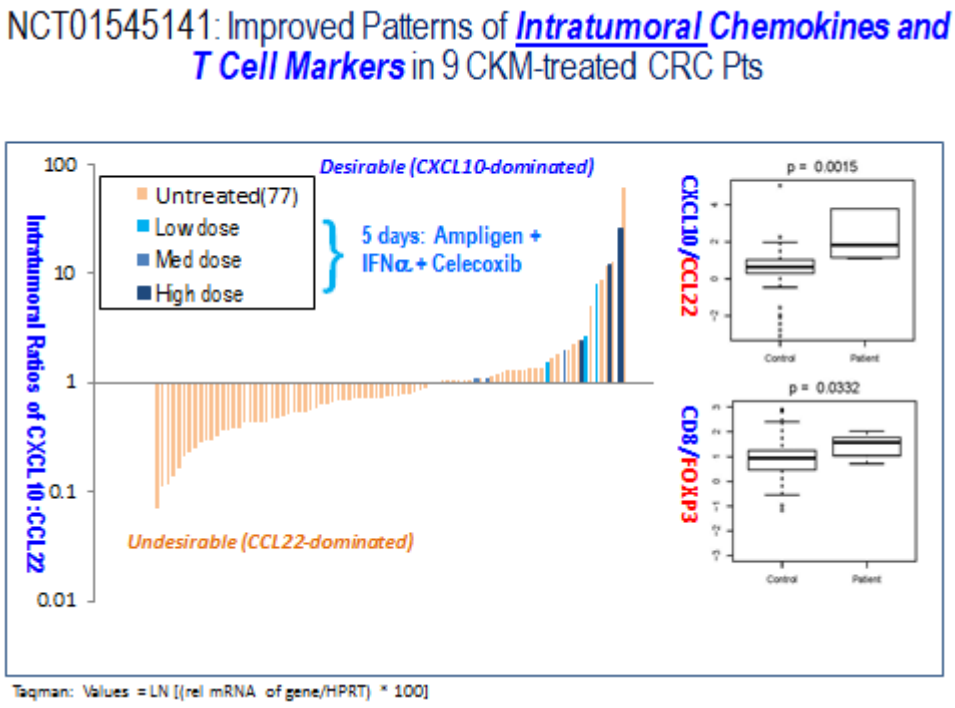
The proposed dosage of 200 mg IV is the starting dose used in chronic treatment of Chronic Fatigue Syndrome (CFS). That dose is escalated to 400 mg IV twice weekly for the CFS indication; however for use as a chemokine modulator, pharmacologic peak serum levels are achieved at 200 mg; thus, 200 mg daily for 3 days will be incorporated for the immune modulation regimen.

Preclinical evidence demonstrates the synergy between IFNα and Rintatolimod in inducing the production of the effector T cell-attracting chemokines.

2.4 Clinial experience with Rintatolimod (Ampligen®) in combination with Interferon Alpha-2b and Celecoxib.

NCT01545141 (UPCI 10-131) was a phase I study which evaluated the combination of rintatolimod, interferon-Alpha 2b, and Celecoxib administered for one course over 5 days, prior to tumor resection. The phase I study was completed, with escalation to the maximal planned dose, without the emergence of undue toxicity. A 5 day regimen consisting of rintatolimod 200 mg IV daily, Interferon Alpha-2b (20 million units/ m^2) IV daily and celecoxib 200 mg po bid was deemed tolerable and suitable for further investigation.

In addition to the demonstrated safety, preliminary evidence of biologic efficacy was observed. Individuals who were treated with CKM prior to resection, demonstrated an improvement in intratumoral CD8/FOXP3 ratios as well as CXCL10/CCL22 ratios when compared to nonrandomized controls, treated with standard care at the University of Pittsburgh. The design (neo-adjuvant treatment) and numbers of patients treated do not permit any meaningful estimate of clinical outcomes or radiographic response at this time.



In addition to the evaluation of rintatolimod, interferon Alpha-2b and Celecoxib administered i. v. for one course over 5 days, as a stand-alone treatment, the same combinations (but different administration regimens) are being evaluated as a part of combinatorial regimens in additional patients with peritoneal carcinomatosis (NCT02151448 / UPCI 12-110) and recurrent ovarian cancer (NCT02432378 / UPCI 11-128). Clinical trial NCT02151448 (over 50 patients evaluated

so far) involve multiple cycles of DC vaccines followed by 4 day long cycles of IFN-a/celecoxib regimen, with rintatolimod administered on days 2 and 4 of each cycle). Clinical trial NCT02432378 (5 patients evaluated so far) involve multiple cycles of intraperitoneal (i.p.) chemotherapy followed (with 1 day delay) with single i.p. doses of rintatolimod (with increasing i.p. doses of IFNα and a standard dose of celecoxib (orally).

No DLTs have been observed in either of these trials.

2.5 Rationale and significance

As detailed above, a previously-demonstrated correlation between the density of CRC-infiltrating effector T cells and long-term outcomes [11, 12] has been established. In our preclinical ex vivo studies performed using explants of resected metastatic CRC, the combination of IFNα with nonselective or COX2-selective inhibitors of prostaglandin synthesis resulted in elevated production of the effector T cell-attracting chemokines CXCL10 and CCL5. This was associated with concomitant suppression of the intratumoral expression of CCL22, a T_{reg}-attracting chemokine [63, 64]. However, in a subset of patients, the optimal results, particularly with regard to CCL5 induction, required additional stimulation by a third agent, a TLR3 Ligand. NCT01545141 demonstrated the safety of the CKM regimen administered over 5 days, without the emergence of new adverse effects or with compromise of surgical outcomes. However, the brief course, limited patient numbers, and lack of pre-treatment biopsies did not allow for an objective estimate of clinical efficacy nor did it allow for a robust evaluation of the impact of this CKM regimen on the tumor microenvironment. If solid evidence of a beneficial biologic effect on colorectal were demonstrated, this would strongly support further investigation, as a self-standing treatment in adjuvant settings or in combination with checkpoint blockade, vaccination or adoptive T cell transfer, which all depend on the presence of T cells in tumor microenvironments (or the ability of T cells to enter tumors).

We seek to establish the immunologic impact of an abridged 3 day course of a chemokine regimen, consisting of Interferon Alpha-2b, celecoxib, and rintatolimod, administered in repeated sessions over 3 weeks. A 3 day regimen will be used preferentially over the 5 day regimen due to concerns of chronic toxicity with multiple cycles and also for ease of implementation and participant scheduling. We further seek to evaluate clinical activity and to confirm the observed safety profile with repeated dosing. We hypothesize that the proposed chemokine modulation treatment in recurrent/metastatic CRC patients may increase the density of tumor infiltrating lymphocytes (TILS) and provide anti-cancer benefit.

Treatment with serial liver biopsies will allow us the comparative analysis of the effect of chemokine modulation on the local recruitment of effector-type T cells **and** the de-recruitment of T_{reg} within affected tumor tissues; helping us to determine the "preferred" chemokine-modulating regimen for subsequent extended studies. Such prospective studies will focus on using combinations of chemokine modulation and cancer vaccines or immune checkpoint inhibitors in patients with CRC. We have, for example, recently observed that αDC1, a new type of DC vaccine [65, 66] is particularly effective in inducing the effector pathway of T cells differentiation. This was manifested by the induction of tumor-killing function and the induction of effector-type chemokine receptors (CXCR3 and CCR5) [65, 67]. Combining the αDC1vaccine to a safe, tolerable and efficacious CKM regimen may hold promise for patients with poor prognostic CRC.

Pre-clinical data similarly demonstrates significant benefit with the addition of anti-PD-1 therapy to a CKM regimen, making this another natural combination.

3 INCLUSION AND EXCLUSION CRITERIA

3.1 Inclusion Criteria:

To be included in this study, participants must meet the following criteria:

1. Recurrent and/or metastatic unresectable colorectal cancer with hepatic metastases.
2. Hepatic metastases present which are amenable to biopsy.
3. Prior treatment with, contra-indication to or refusal of a fluoropyrimidine, irinotecan, oxaliplatin and an anti-EGFR targeted therapy (if RAS wt), as well as a PD-1 or PD-L1 targeted drug if MSI-H/dMMR.
4. No chemotherapy, radiotherapy, major surgery, or biologic therapy within 3 weeks of protocol treatment.
5. An ECOG performance status of 0, 1, or 2. Refer to Appendix C.
6. Have measurable disease per RECIST 1.1 criteria present.
7. Ability to swallow and retain oral medications.
8. Participants of child-bearing potential must agree to use adequate contraceptive methods (e.g., hormonal or barrier method of birth control; abstinence) prior to study entry. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.
9. Age equal to 18 years or older.
10. Must have normal organ and marrow function as defined below:
    - Platelet $\geq$ 75,000/μL
    - Hemoglobin $\geq$ 9 g/dL
    - Hematocrit $\geq$ 27%
    - Absolute Neutrophil Count (ANC) $\geq$ 1500/μL
    - Creatinine $\leq$ institutional upper limit of normal (ULN)

    OR

    - Creatinine clearance $\geq$ 50 mL/min for patients with creatinine levels greater than ULN (refer to Appendix I for Cockcroft-Gault Equation)
    - Total bilirubin $\leq$ 1.5 X institutional ULN or for patients with known Gilbert's Syndrome total bilirubin $\leq$ 3 x ULN
    - AST (SGOT) and ALT(SGPT) $\leq$ 2.5 X institutional ULN
    - Plasma amylase and lipase $\leq$1.5 X institutional ULN.

11. Participant or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.

Please refer to Appendix D for the Investigator Study Eligibility Verification Form: Inclusion Criteria.

3.2 Exclusion criteria

Participants will be excluded from the study for the following:

1. Patients currently treated with systemic immunosuppressive agents, including steroids, are ineligible until 3 weeks after removal from immunosuppressive treatment.
2. Patients with active autoimmune disease requiring ongoing immunosuppressive therapy or, history of transplantation.
3. Patients who are pregnant or nursing. Women of childbearing potential (WOCBP) will have to undergo a urine pregnancy test as part of screening.
4. Untreated CNS metastases.
5. Cardiac risk factors including:
    - Patients experiencing cardiac event(s) (acute coronary syndrome, myocardial infarction, or ischemia) within 3 months of signing consent
    - Patients with a New York Heart Association classification of III or IV (Appendix A)
6. History of upper gastrointestinal ulceration, upper gastrointestinal bleeding, or upper gastrointestinal perforation within the past 3 years. Patients with ulceration, bleeding or perforation in the lower bowel are not excluded.
7. Prior allergic reaction or hypersensitivity to celecoxib, NSAIDs, or any study agents which would prevent completion of protocol-therapy.
8. Patients are ineligible if they plan on regular use of NSAIDs at any dose more than 2 times per week (on average) or aspirin at more than 325 mg at least three times per week, on average. Low-dose aspirin not exceeding 100 mg/day is permitted. Patients who agree to stop regular NSAIDs or higher dose aspirin are eligible and no wash out period is required.
9. Received an investigational agent within 30 days prior to enrollment.
10. Unwilling or unable to follow protocol requirements.
11. Patients with known serious mood disorders.
12. Any additional condition which in the Investigator's opinion deems the participant an unsuitable candidate to receive the study drugs

Please refer to Appendix E for the Investigator Study Eligibility Verification Form: Exclusion Criteria.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this study.

4 LOCAL AND STUDY-WIDE NUMBER OF SUBJECTS

Estimated number of RPCI subjects: The number of subjects required for this study is approximately 15 (12 evaluable for the primary endpoint), but will depend upon the number of additional accruals required to replace subjects who fail to meet the criteria for efficacy evaluation. Accordingly, the subject accruals cannot be known ahead of time but can only be estimated. All participants will be enrolled from RPCI. There will be no network site participation.

4.1 Target Accrual

Approximately 15 patients (12 evaluable for the primary endpoint) will be needed to estimate the biologic impact of this regimen and establish a preliminary estimate of efficacy. Total accrual time is expected to take approximately 48 months.

5 LOCAL AND STUDY-WIDE RECRUITMENT METHODS

5.1 Recruitment

Potential participants will be identified and recruited during scheduled visits to the RPCI Gastrointestinal (GI) Center. Non-investigator RPCI physicians and community physicians may also refer potential subjects to the investigator for evaluation. There will be no "cold calling". Informed consent will be obtained on all subjects by the investigator/co-investigator prior to all study specific procedure (including screening procedures). No subject will be entered into this clinical trial without having a signed written consent form.

6 MULTI-SITE RESEARCH

N/A

7 STUDY TIMELINES

15 participants from RPCI will be enrolled to the study over approximately 48 months. Patients will be followed for PFS and survival for at least an additional 12 months.

8 STUDY ENDPOINTS

8.1 Primary Endpoint

- Immunologic efficacy will be assessed by the increase in the total number of tumor-infiltrating CD8+ T cells in the CRC lesions (measured as the ratio between the CD8 mRNA message and the expression of the housekeeping gene HPRT which will be provided by the study statistician in a SAS compatible excel formatted electronic file), and comparing the total number of tumor-infiltrating CD8+ T cells in pre-treatment vs post-treatment biopsies.

8.2 Secondary Endpoints

- Objective response rate by RECIST v1.1
- Overall safety profile characterized by type, frequency, severity (according to CTCAE Version 4.0), timing, seriousness and relationship to study treatment

8.3 Exploratory Endpoints

- Median progression free survival by RECIST v1.1
- Median overall survival

9 DESIGN

9.1 Study Design

The intratumoral efficacy of the combination of Interferon Alpha-2b, rintatolimod, and celecoxib will be evaluated by administering fixed doses of each drug. Subjects will receive 3 weeks of the Chemokine Modulation (CKM) regimen, administered on days 1, 2, 3, 8, 9, 10, 15, 16, and 17 of protocol treatment, according to the safe established dosing levels (see Table 1). During this time, subjects will be monitored continuously for safety, based on Bayesian analysis.

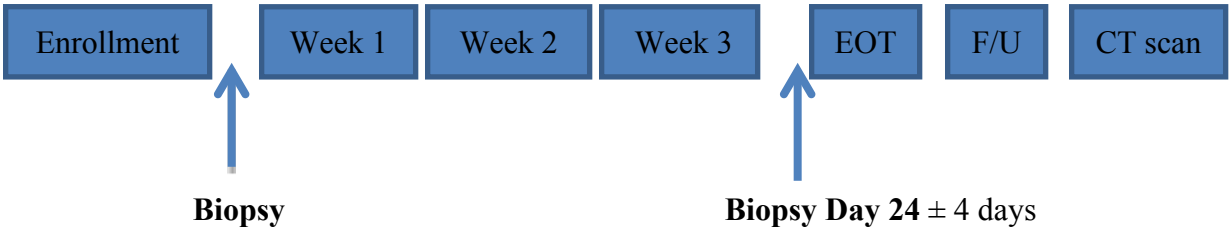
Table 1 Dosing

Components of the chemokine modulation regimen		
Interferon Alpha-2b (20 million units/M^2) IV over 20 minutes once a day for 3 consecutive days, repeated weekly over 3 weeks (days 1,2,3,8,9,10,15,16,17).	Rintatolimod (200 mg) IV once a day for 3 consecutive days, repeated weekly over 3 weeks (days 1,2,3,8,9,10,15,16,17). Initial administration should begin at a slow rate of infusion (approximately 20 cc/ hour). Increase rate to 40cc/hour after 30 minutes.	Celecoxib (200 mg, orally, twice a day) for 3 consecutive days, repeated weekly over 3 weeks (days 1,2,3,8,9,10,15,16,17). Doses should be given approximately 12 hours apart.

9.2 Treatment Phase

A total of three weeks of therapy will be planned. Serial liver biopsies will be obtained, with the first performed prior to initiating therapy and the second performed on Day 20 (± 2 days). Following completion of the 3 consecutive weeks of treatment, the study treatment period will conclude. In order to secondarily assess clinical benefit, repeat CT imaging will be assessed at day 46 (± 3 days). If a patient completes therapy and a decision is made to pursue a further alternate treatment, the subject will come off study; however, in this circumstance, assessment of the next clinical staging study will be requested for determination of RECIST parameters.

Schema



9.3 Follow-up Phase

Patients will be seen for an end of treatment visit to review adverse events, clinical outcome of therapy. If stable disease/response is demonstrated during repeat CT imaging, patients will continue in follow-up with CT imaging every 8 weeks until progression, clinical deterioration or withdrawal from the study. If in the clinical judgement of the treating team, it is in the best interest of the patient to pursue another therapeutic option, the patient will come off study and CT imaging will be obtained only as per SOC/protocol. 12 months of survival follow-up will be pursued by chart audit or if necessary phone contact. If the patient comes off study during the follow-up period to start a new treatment, the new treatment information will be captured in the database.

10 TREATMENT

10.1 Dosing and Administration

10.1.1 Chemokine modulating regimen

For dosing, refer to Table 1. The chemokine modulating regimen will be administered on an outpatient basis at Roswell Park Cancer Institute over 3 consecutive days, every week x 3 weeks (Days 1, 2, 3, 8, 9, 10, 15, 16, 17) in the following order:

- Pretreatment: 500 mL Normal saline IV over 60 minutes
- Pre-meds: Acetaminophen (Tylenol) 650 mg by mouth x 1 dose; Prochlorperazine (Compazine) 10 mg by mouth x 1 dose – administered 30 minutes (±5 minutes) after starting pre-treatment hydration

- Celecoxib: 200 mg orally, administered along with pre-meds
- Interferon Alpha-2b : (20 million units/M^2) IV over 20 minutes
- Rintatolimod: 200 mg IV, initial administration should begin at a slow rate of infusion (approximately 20 cc/ hour) and increase to 40 cc/ hour after 30 minutes. Tubing should be flushed with 30 to 50 mL of normal saline solution upon completion. Administration will be followed by 1 hour of observation and vital signs at 30 and 60 minutes post infusion (± 5 minutes)
- Celecoxib: 200 mg orally to be taken by the patient at home approximately 12 hours following the initial dose.

Pretreatment and pre-meds listed above are recommendations and may be modified based upon institutional SOC requirements and at the discretion of the investigator. Treatment is intended for an outpatient setting. However, at the investigator's/physician's discretion, the participant may receive treatment as an inpatient, if deemed necessary.

Each 3 day treatment will be initiated after interval history and physical examination is performed and once the relevant laboratory studies have been confirmed to be within clinically acceptable ranges, specifically: ANC ≥ 1000/mm3, total bilirubin ≤ 1.5 x ULN or ≤ 3 x ULN (for patients with Gilbert's Syndrome), AST and ALT < 3 x ULN (or < 150% of values at baseline). Treatment may be delayed up to 3 weeks to allow for resolution of any intercurrent illness or for logistical reasons. Delays beyond 3 weeks will require discussion with the study PI to review appropriateness of continuing therapy on study.

10.2 Dose Modification

The following dose modification rules will be used with respect to potential toxicity. Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0).

Therapy will be immediately discontinued for any grade IV treatment-related toxicity that becomes apparent. The exception to this is for grade 4 neutropenia of < 7 days duration. Study treatment will be reinitiated for any grade treatment-related III toxicity pending the reversal of such toxicity after withholding treatment (i.e. the toxicity resolves to the subject's baseline level). In the event of any adverse effect, appropriate medical treatment will be instituted and study treatment will be discontinued if the above toxicity remains.

Table2. Treatment Related Toxicity Management

Treatment-related AEs	Toxicity grade or conditions (CTCAE5.0)	Action taken for Interferon Alpha-2b	Action taken for Rintatolimod	Action taken for Celecoxib	Additional actions
Chills/Fever	Grade 3	None	None	None	Consider additional acetaminophen dosing, supportive care
Pancreatitis	Grade 3	Hold Decreased future doses by 50%	Hold	Hold	Supportive measures as clinically indicated
AST / ALT elevation or Increased bilirubin	Grade 3	Hold until improved to grade 1; Decrease future doses by 50%	Hold	Hold	Advise evaluation for other possible etiologies; consider imaging
Vomiting	Grade 3	Hold until resolved to grade 1; Decrease future doses by 50%	Hold	Hold	
Neuropathy	Grade 2 or greater	Hold until decreased to patient's baseline; Decrease future doses by 50%	None	None	
Depression or other clinically relevant mood disorder	Grade 3	Permanently discontinue; appropriate supportive care/intervention	None	None	
Neutropenia	Grade 3 or greater	Hold until ANC > 1000; Decrease future doses by 50%	Hold	Hold	
Hemorrhage	Grade 2 or greater	None	None	Permanently discontinue	
Myocardial infarction or other arterial thrombotic event	Grade 2 or greater	None	None	Permanently discontinue	

Treatment-related AEs	Toxicity grade or conditions (CTCAE5.0)	Action taken for Interferon Alpha-2b	Action taken for Rintatolimod	Action taken for Celecoxib	Additional actions
All other clinically significant AEs[1]	Intolerable Grade 2 or Grade 3	Hold therapy until resolved; reduce future doses by 50%	Hold therapy until resolved; reduce future doses by 50%	Hold therapy until resolved	
	Grade 4	Permanently Discontinue	Permanently Discontinue	Permanently Discontinue	
1. Holding of any therapies here for intolerable grade 2 or grade 3 toxicities will be at the discretion of the investigator based upon the nature of the AE and whether one drug can be clearly implicated or not; in the case that one drug can be clearly implicated, only that drug should be dose reduced in the future; in the case that the causative agent is unclear, it is recommended that interferon and rintatolimod are reduced as directed					

10.2.1 Celecoxib Non-Hematologic Toxicities

Considered to be possibly, probably, or definitely related to Celecoxib (Interferon Alpha-2b and rintatolimod will be continued). **See Table 2**. Celecoxib will be discontinued for any attributable grade ≥2 toxicity, except for nausea, vomiting, or a similar readily manageable condition.

10.2.2 Interferon Alpha-2b and/or rintatolimod Non-hematologic toxicities

Considered to be possibly, probably, or definitely related to Interferon Alpha-2b and rintatolimod:

- Interferon Alpha-2b **:**

  For grade 3 toxicity:

  - 1st episode: dose will be held and restarted at the next study visit at a dose reduction of 50%. The dose can be re-escalated if toxicity is reduced to ≤ grade 1 or baseline.
  - 2nd episode: Treatment will be discontinued

  For grade 4 toxicity (except neutropenia of < 7 days duration):

  - Treatment will be discontinued

- **Rintatolimod:**

  For grade 3 toxicity:

  - 1st episode: dose will be held and restarted at the next study visit at a dose reduction of 33%.The dose can be re-escalated if toxicity is reduced to ≤ grade 1 or baseline
  - 2nd episode: dose will be held and (if toxicity is resolved to ≤ grade 1 or baseline) restarted at the next study visit at a dose reduction of 33%. The dose cannot be re-escalated

For grade 4 toxicity:

- Treatment will be discontinued

10.2.3 Toxicity Management

The toxicity of high dose interferon (20million units/m²/d) has been established by Kirkwood et al in a number of trials. Most notable was the E1684 trial [68] where HDI (20 million units/m²/d) was administered daily for 5 days x 4weeks. In that trial (n=143), grade 3 toxicities were 67%, grade 4 toxicities were 9% (mainly constitutional and neurologic), and there were 2 treatment related mortalities (grade 5) due to hepatoxicity. The proportion of Grade 3 and 4 toxicities in that trial were 48.2% for constitutional toxicities (defined as 'worst grade of any constitutional toxicity, including fever, chills, flu-like symptoms, fatigue, malaise, and diaphoresis), and 66% for non-constitutional toxicities (23.8% for myelosuppression, 13.9% for hepatotoxicity, 28% for neurological toxicity).

While the combination of HDI and rintatolimod may lead to synergistic additive side effects, we will monitor subject toxicities for unexpected increases in constitutional symptoms, myelosuppression, hepatotoxicity, and neurological toxicity. Moreover, the presence of celecoxib may improve the side effect profile. Rather than a separate phase of the study to characterize safety, we will use continual Bayesian monitoring of SAEs with a stopping rule that permits suspension of the trial by the investigator for review by the Data Safety and Monitoring Committee (DSMC). We will focus on monitoring for grade 3 or greater treatment related hematologic toxicities, and grade 4 or greater treatment related non-hematologic toxicities. A non-informative prior distribution of SAEs will be utilized to develop the safety stopping rules (details in Section 19.4).

- **Gastrointestinal Toxicity**:
  - Nausea and/or vomiting should be controlled with adequate antiemetic therapy. Prophylactic antiemetic therapy can be used at the discretion of the investigator/sub-investigator. Subjects are encouraged to take plenty of oral fluids.
  - Diarrhea should be managed with appropriate antidiarrheal therapy. Subjects should be encouraged to take plenty of oral fluids. If symptoms do not decrease to grade 1 or less with adequate antidiarrheal therapy, all protocol drugs should be held until resolved to < grade 1.
- **Pain**
  - For fever or mild local pain, acetaminophen will be utilized at the discretion of the investigator/sub-investigator or designee.
- **Hypersensitivity Reactions**

Caution: Subjects who had a mild to moderate hypersensitivity reaction have been successfully re-challenged, but careful attention to prophylaxis and bedside monitoring of vital signs is recommended. Hypersensitivity reactions to Interferon Alpha-2b and/or rintatolimod will be managed as follows:

- Mild symptoms (e.g., mild flushing, rash, pruritus): Complete infusion. Supervise at bedside. No treatment required.

- Moderate symptoms (e.g., moderate rash, flushing, mild dyspnea, chest discomfort): Stop infusion. Give intravenous diphenhydramine 25 mg and intravenous dexamethasone 10 mg. Resume infusion after recovery of symptoms at a low rate, then, if no further symptoms, at full dose rate until infusion is complete. If symptoms recur, stop infusion. The subject should receive no additional Interferon Alpha-2b or rintatolimod for that week's three-day treatment, but may be retreated after discussion with the investigator. Record toxicity.
- Severe life threatening symptoms (e.g., hypotension requiring pressor therapy, angioedema, respiratory distress requiring bronchodilation therapy, generalized urticaria): Stop infusion. Give intravenous diphenhydramine and dexamethasone as above. Add epinephrine or bronchodilators if indicated. If wheezing is present, that is not responsive to bronchodilators, epinephrine is recommended. Subject should be removed from further protocol therapy. Report as serious adverse event.

10.3 General Concomitant Medication and Supportive Care

Additional chemo-, immune-, and radiotherapies are not permitted during the active treatment period of this trial. Palliative radiotherapy may be permitted for symptomatic control of pain from bone metastases following discussion with the Principal Investigator, provided that the radiotherapy does not affect target lesions.

10.4 Duration of study treatment

A total of 3 weeks of treatment is planned. A repeat liver biopsy will be performed at Day 24 (± 4 days) after completion of protocol therapy and the study biopsy; the patient will then proceed onto the follow-up period.

The participant may stop treatment earlier than planned in the event that the following occurs:

- Disease progression
- Unacceptable toxicity
- Withdrawal from study
- Intercurrent illness that prevents further administration of treatment
- Participant non-compliance with study requirements

10.5 Compliance

Interferon Alpha-2b, Rintatolimod and the morning dose of Celecoxib will be administered in the Clinical Research Center (or other clinical area) and documented by the nurse on the Medication Administration Record. The evening dose of Celecoxib will be self-administered at home and documented by the participant on the patient diary. The participant will be asked to bring the diary with him/her to each clinical visit. Refer to Appendix H

11 PROCEDURES INVOLVED

11.1 Description of Procedures

The study-specific assessments are detailed in this section and outlined in Appendix F (Study Calendar). Baseline and/or Screening assessments must be performed within 14 days prior to the first dose of investigational product unless otherwise noted. Any results falling outside of the reference ranges may be repeated at the discretion of the investigator. All on-study visit procedures are allowed **a window of ± 1 day** unless otherwise noted.

Unless otherwise defined in the written protocol text, all procedures/assessments will be conducted in accordance with RPCI Clinical Research Services Standard Operating Procedures.

Informed consent ***MUST*** be completed prior to receiving any study related procedures.

11.1.1 Screening (performed between days -14 through day -1 unless otherwise specified)

- Recording of concomitant medications
- Medical and Surgical History
- Complete Physical Exam including height and weight
- Vital Signs
- ECOG Performance Status Assessment
- Hematology
- Chemistry
- CEA Level
- ANA testing
- Serum HCG pregnancy testing (within 7 days of Day 1 in women of child-bearing potential only)
- CT and/or MRI (within 28 days of start of treatment)
- EKG
- Liver Biopsy

11.1.2 Evaluations Performed on day 1 of each treatment week (day 1, 8, 15):

- Recording of concomitant medications
- Recording of Adverse Events
- Targeted Physical Exam including weight
- Vital Signs (temperature, blood pressure, pulse rate and respiratory rate) will be performed pre- treatment and at 30 and 60 minutes post rintatolimod
- (± 5 minutes).
- ECOG Performance Status Assessment
- Hematology
- Chemistry
- Chemokine Modulating regimen
- Blood (60cc) for correlative studies on Day 1 and Day 15 only (within 1 hour after end of infusion treatment)

11.1.3 Evaluations Performed on days 2 and 3 of each treatment week(days 2,3, 9, 10, 16, 17):

- Recording of concomitant medications
- Recording of Adverse Events
- Vital Signs (temperature, blood pressure, pulse rate and respiratory rate) will be performed pre-treatment and at 30 and 60 minutes post rintatolimod (± 5 minutes).
- Chemokine Modulating regimen
- Blood (60cc) for correlative studies on Day 3 only (within 1 hour after end of infusion treatment)

11.1.4 Evaluations Performed on Day 20 (+/- 2 days):

- Recording of Adverse Events
- Liver Biopsy

11.1.5 End of Treatment Visit on Day 29 (+/- 10 days):

- Recording of concomitant medications
- Recording of Adverse Events
- Targeted Physical Exam including weight
- Vital Signs
- ECOG Performance Status
- Hematology
- Chemistry
  NOTE: In the event patient cannot tolerate treatment with all 9 days of treatment, then Blood 60cc for correlative will be collected at time that the patient recovers from toxicities (ideally within 1 week of the last infusion). Liver biopsy will also be collected provided there is not an overriding safety concern for the patient.

11.1.6 Follow-up (patients will be followed for 12 months after EOT visit)

- Survival status (phone contact/chart review is acceptable)
- CT scan and/or MRI: to be performed at day 46 (± 3 days). If reassessment scan shows stable disease/response, patients will continue in follow-up with CT imaging every 8 weeks until progression, clinical deterioration or withdrawal from study. If in the clinical judgment of the treating team, it is in the best interest of the patient to pursue another therapeutic option, the patient will come off study and CT imaging will be obtained only as SOC/protocol. In this instance, review of the subsequent disease assessment study will be requested for determination of RECIST parameters.

11.2 Correlative studies (blood samples)

Five (10 mL) green-top heparinized tubes of blood will be collected via venipuncture for biomarker analysis at multiple time points. These will be collected as outlined in section 11.1. All tubes will be sent to the RPCI Hematologic Procurement Shared Resource Laboratory.

Hematologic Procurement Shared Resource
Attn: I 52917 Specimens
Basic Science Building 5th Floor Room 528
Elm & Carlton Streets
Buffalo, New York 14263
716-845-7656

In addition 1 (10 mL) green-top heparinized tube will be sent to Dr. Kalinski's Lab in Cancer Cell Center Room 502F to the attention of Melissa Grimm, PhD contact phone 716-845-7353 or cell 716-353-5384. This will be for preliminary analyses, which may include the pre- and post-treatment cytokine levels and immune cell subsets, via RT-PCR, flow cytometry assays, or alternative laboratory methods.

NOTE: All six tubes will be sent to RPCI Hematologic Procurement Shared Resource Laboratory first for sample tracking. The Procurement Shared Resource will then forward 1 (10mL0 green-top to Dr. Kalinski's Lab

Plasma samples are to be processed at 4°C using a refrigerated centrifuge at approximately 3000 rpm for about 10 minutes with the plasma being aliquoted into 2 cryovials per time-point. The samples will immediately be frozen at - 70ºC or below until analyzed. PBMC's will then be obtained by Ficoll gradient as per the lab's standard procedure. Two of the tubes will be pooled and the PBMC's will be frozen in Gibco Cell culture freezing media and will be stored in LN2 until time of analysis. The remaining tube of PBMC's will be frozen in RNA later and will be stored in -70 degrees or below until analyzed. The screw cap polypropylene cryogenic tubes will be labeled with the clinical study number, participant's MR number, participant's study number, protocol time point, protocol day, date and time of draw.

As a component of exploratory aims, blood may be analyzed to assess pre- and post- treatment cytokine levels and immune cell subsets, via ELISA, RT-PCR and flow cytometry assays.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current **Temperature Logs** and study-specific **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

11.3 Pathology

Fresh Biopsy Samples

At each liver biopsy collection time point, 6 total cores will be obtained, including at least 5 tumor cores and 1 additional core of surrounding liver tissue (to enhance the stringency of our assays). 4 cores of tumor and 1 core of surrounding liver tissue will be placed in Dulbecco's Phosphate-Buffered saline, and 1 core of tumor will be placed in formalin, and processed to FFPE block per institute standard.

Pre- and post-treatment cores should be obtained from the same lesion. If non-necrotic lesions are present at baseline, they should be prioritized for biopsy, with the necrotic lesions being

avoided per the interventional radiologists' discretion. Any biopsy core which appears as necrotic should be discarded.

Label the samples with study-specific subject ID number, clinical study number, protocol time point and, protocol day. Phosphate-Buffered saline, will be supplied by Dr. Kalinski's lab and sample will be kept at ambient temperature. Samples in Dulbecco's Phosphate-Buffered Saline will be sent to Dr. Pawel Kalinski's lab upon completion of processing (preferably ASAP within 2 hours of collection, but within 4 hours of collection is allowed). Once the remaining core has been processed to FFPE block, it will be delivered to OmniSeq

- The changes in the ratio between CTL marker (CD8a gene expression) to Treg markers (FoxP3 gene expression), using RT-PCT will be considered as a primary endpoint of efficacy.

Additional correlative studies may be performed on the same material:

- Comparison (using RT-PCR, Immunofluorescence [IF] and immunohistochemistry [IHC] on serial sections) of the metastatic tissue specimen with regard to total numbers of infiltrating T cells, their CD4/CD8 ratios, frequencies of FoxP3 cells, and the expression of chemokine receptors on $CD4^+$ and $CD8^+$ T cells (CXCR3, CCR5, CCR4, CCR6, and CXCR4).
- Evaluate the local expression of T_{eff}-attracting chemokines (CCR5, CXCL9, CXCL10 and CXCL11) and T_{reg}-favoring chemokines (CCL22 and CXCL12) using IF and RT-PCR.
- Additional tissue evaluations, including OmniSeq Immune Report card, NanoString or similar, may be performed, depending on the availability of funding.

12 WITHDRAWAL OF SUBJECTS

12.1 Treatment Discontinuation

Upon treatment discontinuation all end of treatment evaluations and tests will be conducted. All participants who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the participant's medical records and the appropriate eCRF.

Reasons for treatment discontinuation should be classified as follows:

- Death
- Progressive disease
- Toxicity; treatment related or unrelated
- Investigator judgment
    - The Investigator may discontinue a participant if, in his/her judgment, it is in the best interest of the participant to do so.
- Noncompliance
- Participant voluntary withdrawal

  - A participant may withdraw from the study at any time, for any reason. If a participant discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal.
- Sponsor decision.

13 RISKS TO SUBJECTS

13.1 Celecoxib

Prolonged use of celecoxib may cause dyspepsia, headaches (including migraines) and borderline elevated liver function tests (which could indicate liver damage). Recently, information from three long-term studies of celecoxib has become available. In the first study, a cancer prevention study, an increased risk of heart attacks, strokes, and/or deaths resulting from heart or blood vessel disease was reported among people taking celecoxib. Approximately 1 in 100 subjects enrolled in this study receiving the placebo treatment had one of these serious events. In contrast, between 2 and 3 in 100 subjects taking celecoxib (between 400 and 800 mg daily) had one of these serious events. Another clinical cancer prevention study found no increased risks in subjects taking celecoxib 400 mg daily. The third study, an Alzheimer's disease prevention study, did not find increased risks with celecoxib. Dosing has been suspended in all three of these studies based on the findings of the first cancer prevention study. As a result, the FDA is now evaluating the possibility that celecoxib increases the risk of heart attack, stroke, and or death resulting from heart or blood vessel disease. Known infrequent side effects of celecoxib include nausea and/or vomiting, diarrhea, flatulence, abdominal and stomach pain, bleeding ulcer, upper respiratory tract infection, pharyngitis, rhinitis, sinusitis, peripheral edema, back pain, dizziness, insomnia, and skin rash. Rare risks include sudden death (unexpected or instant death that occurs within minutes or hours from any cause other than violence), vasculitis, hepatitis, liver failure, kidney failure, blood dyscrasia, hypoglycemia, hyponatremia, viral meningitis, severe allergic reaction, visual changes, and transient ischemic attack. Warnings/Precautions: Stomach problems may be more likely to occur if patients drink alcoholic beverages while taking this medicine. Taking two or more of the nonsteroidal anti-inflammatory drugs together on a regular basis may increase the chance of unwanted effects. Also, taking acetaminophen, aspirin or other salicylates, or ketorolac (e.g., Toradol) regularly, while taking a nonsteroidal anti-inflammatory drug, may increase the chance of unwanted effects. The risk will depend on how much of each medicine is taken every day and how long the medicines are taken together. Therefore, patients should not take acetaminophen or aspirin or other salicylates or ketorolac (e.g., Toradol). Clinical studies with celecoxib have identified potentially significant interactions with fluconazole and lithium. Experience with nonsteroidal anti-inflammatory drugs (NSAIDs) suggests the potential for interactions with furosemide and ACE inhibitors. Celecoxib is contraindicated in patients with known hypersensitivity to celecoxib. Celecoxib should not be given to patients who have demonstrated allergic-type reactions to sulfonamides. Celecoxib should not be given to patients who have experienced asthma, urticaria, or allergic-type reactions after taking aspirin or other NSAIDs. Severe, rarely fatal, anaphylactic-like reactions to NSAIDs have been reported in such patients.

For more risk information reference the Investigator’s Brochure or package insert.

13.2 Interferon Alpha-2b

Interferon Alpha-2b may cause fever, chills and flu-like symptoms; loss of appetite; nausea; vomiting, diarrhea and abdominal pain; fatigue; lowered white blood count may increase risk of infection; lowered platelets may lead to an increase in bruising or bleeding; hair loss. Other risks which may be common in cases of prolonged administration of Interferon Alpha-2b include drowsiness; temporary confusion; anxiety, amnesia, irritability, confusion, delusions and depression which can be severe; numbness and/or tingling in the hands and/or feet, skin rashes and inflammation of the pancreas. Inflammation of the pancreas is swelling or irritation of the pancreas which may result in tenderness or pain in the stomach and/or back. When the pancreas is inflamed, the body is not able to absorb all the nutrients it needs.

For more risk information reference the Investigator's Brochure or package insert.

13.3 Rintatolimod

Clinical experience with rintatolimod totals over 800 patients with more than 400 patients receiving Rintatolimod for at least six (6) months, greater than 200 patients for one (1) year, over 50 patients up to two (2) years, and with 20 or more patients over two (2) years at doses as high as 1200 mg i. v. twice weekly. No evidence of dose-limiting organ toxicity, including hematologic, liver, or renal toxicity, has been observed.

Adverse events related to infusion such as mild flu-like symptoms, transient headache, fever, myalgia, arthralgia, and fatigue/malaise which were seen, usually occur during the initial weeks of treatment and tend to subside on repeated administration. These side events were seen in Chronic Fatigue Syndrome patients, cancer patients, chronic hepatitis B infected patients and individuals infected with HIV at doses of 200 and 400 mg and higher. Patients that experience these minor side effects can continue on Rintatolimod and as noted, these signs and symptoms typically subside after several weeks of continued treatment. Specific symptoms of note include a flushing reaction, characterized by at least one occurrence of erythema of the face, neck and chest, which has been observed in approximately 10% of patients treated in various studies. Usually the flushing is both mild and transient and disappears with repeated dosing. Occasionally, it can be accompanied by a tightness of the chest, tachycardia, anxiety, shortness of breath, subjective reports of "feeling hot", diaphoresis and nausea. The reaction is usually infusion-rate dependent and can generally be controlled by slowing the infusion rate. An antihistamine (diphenhydramine hydrochloride) can be helpful in controlling and reducing the response in the occasional patient for whom the symptom persists. Other less frequently occurring adverse effects include nausea, diarrhea, itching, urticaria, bronchospasm, transient hypotension, photophobia, rash, bradycardia and transient visual disturbances. A severe unexpected local reaction to extravasation of rintatolimod (Ampligen®, Poly I:Poly $C_{12}U$) at the infusion site in the dorsum of the left hand was reported in a Chronic Fatigue Syndrome patient with chilblains. Several patients experienced liver enzyme level elevations while receiving Rintatolimod associated with chronic dosing over many weeks.

Rintatolimod has been dosed in combination with alpha interferon in investigator initiated studies under investigator IND applications during the period between December 1985 and April 1994. A total of 24 patients received combination treatments. Clinical conditions included renal cell carcinoma, chronic myelogenous leukemia, melanoma, and ovarian cancer. Rintatolimod was given as an IV infusion at a dose of 300 mg twice weekly. The starting dose was sometimes as low

as 1-10 mg. The interferons were administered at a dose of 3 million Units daily, with some doses of 0.75 million units at the low side and up to 6 million units at the higher side.

The therapy with rintatolimod in combination with alpha interferon was generally well tolerated without evidence of dose-limiting or cumulative toxicities. The most frequent adverse reactions were considered minor in severity and duration. Most of them were flu-like symptoms such as chills, cold feeling, fatigue, decreased appetite, fever, muscular aches. Also shortness of breath has been seen as well as hypotension, nausea, anemia, dyspnea, numbness, itching and blurred vision. These adverse events were judged possibly related to the condition of the patients, but also possibly related to the administration of rintatolimod or interferon. In some cases worsening of the patient's condition has been seen due to tumor progression, but in general favorable clinical patterns were observed in these patients with advanced disease. Such combinations were not intended to be immune modulating and were evaluated in the context of clinical cancer care.

For more risk information reference the Investigator's Brochure or package insert.

14 POTENTIAL BENEFITS TO SUBJECTS

Patients enrolled in this study have either been treated with standard therapies which provide substantial beneficial or are ineligible for or refusing such therapies. This treatment has potential to provide anti-tumor benefit (regression/stabilization). The probability of such a benefit is unknown.

15 DATA AND SPECIMEN BANKING

After processing, all samples for correlative analysis (blood and tissue) will be sent to Dr. Kalinski's laboratory for storage and analysis (CCC, C-502). Samples will be used for planned study assays as well as for future analysis for other, yet to be identified biomarkers that may be related to clinical outcome. Any clinical data that is associated with the samples will be stored on a secured server in the Department of Medicine, will be accessible only by the PI, co-investigators and PI-designated data manager and, will be password protected. All computer entry and networking programs will be done using PIDs only.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

16 MEASUREMENT OF EFFECT

16.1 Solid Tumors

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eur J Ca 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

For the purposes of this study, patients should be re-evaluated for response on Day 46 (± 3 days). In addition to a baseline scan, confirmatory scans should also be obtained 8 weeks (not less than 4) weeks following initial documentation of objective response.

Please refer to Appendix G for a summary of tumor response assessment according to RECIST 1.1 criteria.

17 SAFETY EVALUATION

17.1 Adverse Events

17.1.1 Definition

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite').

An AE is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

- **Diagnosis Versus Signs and Symptoms**

  If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

- **Adverse Events Occurring Secondary to Other Events**

  In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.

  However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

- **Abnormal Laboratory Values**

  Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated blood potassium level of 7 mEq/L should be recorded as "hyperkalemia"

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

- **Preexisting Medical Conditions (Baseline Conditions)**

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

17.2 Grading and Reporting Adverse Events

- **Grading and Relationship to Drug**

The descriptions and grading scales found in the CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 4 of the CTCAE is identified and located at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 4.

The relationship of event to study drug will be documented by the Investigator as follows:

Unrelated: The event is clearly related to other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs administered to the participant.

Unlikely: The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.

Possible: The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs.

Probable: The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs.

Definite: The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

- **Reporting Adverse Events**: Routine AEs occurring between the start date of intervention until 30 days after the last intervention, or until the event has resolved, the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible, will be reported. New information will be reported after it is received.

Table 2 Guidelines for Routine Adverse Event Reporting (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

17.3 Serious Adverse Events

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a participant or participants, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does NOT include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Reporting Serious Adverse Events

All new SAEs occurring from the date the participant signs the study consent until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported. The RPCI SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAEs occurring after the 30 day follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention should be reported.

SAEs identified as an Unanticipated Problem by the Investigator must be reported. Please refer to Section 17.5 for details on reporting Unanticipated Problems.

17.4 Follow-Up for Serious Adverse Events

All related SAEs should be followed to their resolution, until the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

17.5 Unanticipated Problems

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
    - The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant privacy or confidentiality of data.
    - The characteristics of the participant population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed **Serious** per **Section 17.3.**

Reporting Unanticipated Problems:

The Reportable New Information (RNI) Form will be submitted to the CRS Compliance Office within 1 business day of becoming aware of the Unanticipated Problem. After review, CRS Compliance will submit the RNI to the IRB.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to CRS Compliance with an updated Reportable New Information Form. The site Investigator or designated research personnel will report all unanticipated problems, whether related or unrelated to the investigational agent(s) to the IRB in accordance with their local institutional guidelines.

17.6 FDA Reporting

When RPCI is the IND holder the following describes the FDA reporting requirements by timeline for AEs and new safety findings that meet the criteria outlined below:

Within 7 Calendar Days

Any adverse event that meets **ALL** the following criteria:

Related or possibly related to the use of the study drug;

Unexpected; and

Fatal or life-threatening.

Within 15 Calendar Days

Any adverse event that meets **ALL** the following criteria:

Related or possibly related to the use of the study drug;

Unexpected; and

Serious but not fatal or life-threatening;

Or, meets **ANY** of the following criteria:

- A previous adverse event that is not initially deemed reportable but is later found to fit the criteria for reporting (report within 15 days from when event was deemed reportable).
- Any findings from other studies, including epidemiological studies, pooled analysis of multiple studies, or other clinical studies conducted with the study drug that suggest a significant risk in humans exposed to the drug.
- Any findings from animal or in vitro testing that suggest a significant risk for human participants including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.
- Any clinically important increase in the rate of occurrence of a serious, related or possibly related adverse event over that listed in the protocol or investigator brochure.

Sponsors are also required to identify in IND safety reports, all previous reports concerning similar adverse events and to analyze the significance of the current event in the light of the previous reports.

Reporting Process

The principal investigator or designee will complete and submit a FDA Form 3500A MedWatch for any event that meets the above criteria. Forms will be submitted to the CRS Compliance Office via email to CRSCompliance@RoswellPark.org.

18 DATA MANAGEMENT AND CONFIDENTIALITY

18.1 Data Collection

Full build studies are managed by RPCI CRS Data Management for analysis by RPCI Biostatisticians. All electronic case report form (eCRF) data are captured for these studies.

Data management activities are performed using a CTMS system that enables the collection, cleaning and viewing of clinical trial data. CRS data management designs the study-specific database and facilitates development by the Information Technology team. Once the database design is approved by the Investigator, Statistician, and Clinical Research Coordinator, the database is put into production and data entry can begin. Data can be entered and changed only by those with the rights to do so into the eCRFs.

18.2 Maintenance of Study Documents

Essential documents will be retained per RPCI's policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with RPCI.

18.3 Revisions to the Protocol

RPCI may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

18.4 Termination of the Study

It is agreed that, for reasonable cause, either the RPCI Investigators or the Sponsor, may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, RPCI may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of participants enrolled in the study.

18.5 Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

19 STATISTICAL PLAN

19.1 Study Design/Endpoints

This is a Phase 2A study of the chemokine-modulating regimen Celecoxib, IFNα, and rintatolimod. The regimen will be evaluated via serial biopsies comparing the quantity of tumor infiltrating CD8+ cells prior to therapy with that seen post-therapy. Tumor imaging will be repeated at day 46 (+/- 3 days) to assess objective response to therapy. Tumor infiltrating CD8+ cells in the biopsied tumor will be measured by quantitative RT-PCR and results will be expressed as a ratio to a housekeeping gene.

19.2 Power and Sample Size

Sample Size Determination: We consider a five-fold increase in TIL expression as clinically meaningful. In a series of 55 *ex-vivo* CRC tumor specimens from patients treated surgically for recurrent CRC, the log (base 10) transform of the TIL expression yielded an estimated mean = 1.2

and standard deviation = 0.9. A fivefold increase due to chemokine modulation would increase the mean from 1.2 to 1.9 (a 0.77 SD shift).

The sample size calculations are based on the primary analysis, where the change in TILs is evaluated using a one-sided permutation t-test. With a sample size of n=12 *evaluable patients*, the study design has 90% power to detect a 0.77 standard deviation increase (pre- to post-treatment) at a significance level of 0.1. Therefore we have an adequate design to detect a clinically meaningful increase in TIL expression.

Analysis Cohorts and Patient Replacement: Patients missing pre-treatment and/or post-treatment TILs are consider unevaluable for the primary endpoint, and will be replaced. Patients that receive at least one study dose will be included in the *safety analysis cohort*. Patients that are considered 'evaluable' for the primary endpoint will be included in the *primary analysis cohort*. All patients that receive at least one study dose and are followed for clinical outcomes will be included in the *secondary analysis cohort*.

Demographics: Patient characteristics will be summarized for each analysis cohort using the appropriate descriptive statistics – mean, standard deviation, and percentiles for continuous variables; and frequencies and relative frequencies for categorical variables.

19.3 Proposed Data Analysis

Primary Analysis: The *primary objective* is to determine the impact of a chemokine-modulatory regimen on the immune microenvironment of colorectal liver metastases. The *primary endpoint* is the change in TILs, as measured by CD8= transcript, which is treated as a continuous variable.

The TILs will be summarized by time-point (pre-/post-treatment) using the mean, median, standard deviation; and graphically using dot-plots. A 90% confidence interval for the mean change in TILs will be obtained using standard methods. Using a one-sided permutation paired t-test, the following hypotheses about the change in TILLs will be evaluated:

$$H_0: \mu_d \leq 0 \text{ versus } H_A: \mu_d > 0,$$

where μ_d is the true change in TILs (post-treatment – pre-treatment).

This analysis will be limited to subjects who are considered evaluable for efficacy. Therefore the recommended sample size for efficacy is 12 *evaluable subjects.*

Secondary Analysis: The secondary objectives are to evaluate the clinical outcome of objective response and safety of the proposed therapy. The responses (based on RECIST v1.1 criteria) will be treated as binary data and summarized using frequencies and relative frequencies; with the objective response rate (ORR) estimated using a 90% confidence interval obtained using Jeffrey's prior method. The safety analysis is described in Section 9.4.

Exploratory Analyses: The exploratory objectives are to evaluate the clinical outcomes of overall survival (OS) and progression-free survival (PFS). The OS and PFS will be treated as bivariate time-to-event data and will be summarized using standard Kaplan-Meier methods. OS will be defined as the time from the start of treatment until death due to any cause or last follow-up. PFS will be defined as the time from the start of treatment until disease progression (defined by RECIST 1.1) or last-follow-up.

Interim Analysis: As part of amendment #8, an interim analysis will be utilized to examine whether refinement of patient selection (i.e. earlier line) and tumor selection (i.e. selection of lesion and number of tumor cores) will improve: 1) the intra-subject variability observed in the first patients (likely due to choice of lesion and abundance of necrotic tissue), and efficacy with respect to the primary end-point (change in TILs).

The intra-class correlation coefficient (ICC) as an interim evaluation of the intra-subject variability associated with TILs. The conditional power for the one-sided paired t-test about the change in TILs will be calculated as an interim evaluation of efficacy.

The research team will meet to discuss these results and determine whether to: 1) continue enrollment in this patient cohort at the given dose level; or 2) incorporate a dose escalation scheme. If there is reduced intra-patient variability and potential evidence of efficacy (i.e. high ICC and conditional power), then the study enrollment will be completed as currently described. Otherwise, we may consider increasing the dose of Rintatolimod using an accelerated titration design (detailed in a subsequent amendment).

19.4 Safety Monitoring

Safety Evaluation: A previous Phase I study utilized the same dosing strength (at a different dosing schedule: 5 days versus the proposed 3 days), but only gave one cycle; whereas the current study purposes 3 consecutive weeks of 3-day treatments. As such, a continuous toxicity monitoring plan will be implemented to quickly identify the emergence of any new AEs or SAEs.

Subject safety will be monitored continuously using Bayesian methods with the following decision rule: if the posterior probability is .80 or greater that 33% or more of treated subjects experience a treatment related serious adverse event (SAE), then the study will be suspended pending review. This posterior probability will be calculated from the study's accumulating data and a weakly informative prior distribution. If π denotes a random variable representing the proportion of subjects who will experience a treatment related SAE, we assume π has a beta distribution with parameters a = 1 and b = 2.

Selected values of the posterior distribution $PP(\pi \geq .33 \mid \text{SAEs and prior})$ are shown in the table below. Also shown are the binomial probabilities of suspending the study for observed count of SAE's.

Table X Number of Subjects Observed to Have Treatment-Related SAEs and Corresponding Posterior and Binomial Probabilities Needed to Suspend the Study

Subjects	Treatment-Related SAEs	$PP(\pi > 33\%)$*	$Pr(X \geq r \mid p = .33)$
2	2	.892	.109
3	3	.956	.036
4	3	.903	.108
5	3	.832	.205
6	4	.915	.097
7	4	.860	.168
8	5	.927	.085
9	5	.883	.140
10	5	.829	.206

11	6	.901	.117
12	6	.857	.171
13	6	.805	.233
14	7	.880	.143
15	7	.836	.195

*π is the SAE rate. The minimum acceptable upper bound of a treatment-related SAE is 33%. PP($\pi > 33\%$) is the posterior probability that the SAE rate exceeds this 33% upper bound. This posterior probability of an SAE is calculated from the prior distribution, the number of subjects treated and the observed number of treatment-related SAEs.

It is anticipated that 15 patients will be enrolled to this study. The table above presents the minimum number of subjects experiencing treatment-related SAEs (i.e., those SAEs judged to be possibly, probably, or definitely related to the study regimen) that would dictate suspension of the trial in accordance with the stopping rule. Using these assumptions the trial will be suspended if:

- 2 subjects experience treatment related SAE among the first 2 subjects enrolled
- 3 subjects experience treatment related SAE among 3 – 5 subjects or,
- 4 subjects experience treatment related SAE among 6 – 7 subjects or,
- 5 subjects experience treatment related SAE among 8 – 10 subjects or,
- 6 subjects experience treatment related SAE among 11 – 12 subjects,
- 7 subjects experience treatment related SAE among 13 – 15 subjects.
- Any death not clearly due to the underlying disease or extraneous causes

At the completion of the study, all AEs will be summarized by weekly treatments and grade using frequencies and relative frequencies.

Treatment Related SAEs for Safety Monitoring

Treatment Related SAE s will be defined as the following events that occur during the period of Day 1 through the End of Treatment visit:

- ≥ Grade 3 treatment-related non-hematologic toxicities, except:
    - The following grade ≥ 3 toxicities (lasting <7 days)

      Flu-like symptoms/constitutional symptoms (i.e., fever, chills, arthralgias, myalgias)
      Depression, anxiety, restlessness, or insomnia
      GI symptoms (i.e., nausea, diarrhea)
      AST/ALT (unless accompanied by Grade 2 increased bilirubin)
    - Please note, if the above grade 3 toxicities last ≥ 7 days, they will be defined as a DLT
- ≥ Grade 4 treatment related non-hematologic toxicity
- Hy's law criteria:

- An elevated alanine transaminase (ALT) or aspartate transaminase (AST) lab value that is greater than or equal to three times (3X) the upper limit of normal (ULN) and
- An elevated total bilirubin lab value that is greater than or equal to two times (2X) ULN and, at the same time, an alkaline phosphatase (ALP) lab value that is less than 2X ULN,
- As a result of within-protocol-specific testing or, unscheduled testing

Safety Review and Study Suspension

The Roswell Park Data Safety and Monitoring Committee (DSMC) will assess the progress of the study, the safety data, and critical efficacy endpoints. The DSMC will review the study annually and will make recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design (c) or termination of the study.

Additionally, should the study be suspended due to safety; members of the research team, study statistician, and DSMC will meet to discuss safety concerns and possible adjustments to the study or study closure.

20 PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF SUBJECTS

This study will be reviewed at the scheduled RPCI Early Phase Clinical Trials (EPCT) meetings and the minutes are forwarded to the IRB for review.

21 VULNERABLE POPULATIONS

- N/A

22 COMMUNITY-BASED PARTICIPATORY RESEARCH

- N/A

23 SHARING OF RESULTS WITH SUBJECTS

Individual response data is shared with the participant as a part of their clinical care.

24 SETTING

Potential subjects with recurrent colorectal cancer will be identified and recruited during scheduled visits to the RPCI Gastrointestinal Center. Potential subjects, who are referred by their RPCI or community physicians, will be scheduled with an investigator in the clinic for evaluation. All participants related research procedures will be conducted at RPCI.

25 PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF SUBJECTS

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

26 RESOURCES AVAILABLE

- N/A

27 PRIOR APPROVALS

- N/A

28 COMPENSATION FOR RESEARCH-RELATED INJURY

Refer to informed consent document related to this study.

29 ECONOMIC BURDEN TO SUBJECTS

Refer to informed consent document related to this study.

30 CONSENT PROCESS

This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each participant (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific procedure. It is the responsibility of the investigator to ensure that the participant is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the participant log and participant records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The investigator is also responsible for obtaining participant authorization to access medical records and other applicable study specific information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the participant is treated. The clinical trial should be conducted in accordance with the ethical principles embodied in the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, consistent with good clinical practice and the applicable regulatory requirements and according to the guidelines in this protocol, including attached appendices.

Informed consent will be obtained according to SOP: Informed Consent Process for Research (HRP-090).

31 PROCESS TO DOCUMENT CONSENT IN WRITING

The Investigator (or IRB specified designee) is responsible for obtaining written consent from each participant or the participant's legally authorized representative in accordance with GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the participant according to applicable GCP guidelines, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or

institutionally mandated requirements for informed consent must also be adhered to. The participant should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the signed consent form to the participant and the signed original shall be maintained in the Investigator File. A copy of the signed consent form must be filed in the participant file. At any stage, the participant may withdraw from the study and such a decision will not affect any further treatment options.

SOP: Written Documentation of Consent (HRP-091) will be followed.

32 DRUGS OR DEVICES

RPCI will hold the IND for this study.

32.1 Celecoxib

A sulfa non-steroidal anti-inflammatory drug (NSAID) used in the treatment of osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation and menstrual symptoms, and to reduce numbers of colon and rectum polyps in patients with familial adenomatous polyposis.

32.1.1 Other Names

Celebrex, Celebra or Onsenal (commercially available)

32.1.2 Formulation and packaging

Celecoxib as capsules in the following dosages: 100 mg and 200 mg.

32.1.3 Drug shipment

Celecoxib will be provided by RPCI.

32.1.4 Drug administration

Celecoxib 200mg will be administered orally twice a day approximately 12 hours apart on Days 1-3, 8-10, and 15-17. The morning dose will be given to the participant at the same time as the other pre-medications for the CKM regimen. The evening dose of Celecoxib will be self-administered by the participant at home and documented on the patient diary. See Appendix H.

32.1.5 Drug storage and accountability

The Investigator or designate will be responsible for ensuring that the investigational product is securely maintained in a locked, limited access facility in accordance with the applicable regulatory requirements.

Store Celecoxib at room temperature at 77 °F (25 °C) away from light and moisture. Brief storage between 59-86 °F (15-30 °C) is permitted.

Drug storage temperature will be maintained and recorded, as applicable.

32.2 Interferon Alpha-2b

Interferon Alpha-2b is approved around the world for the treatment of chronic hepatitis C, chronic hepatitis B, hairy cell leukemia, chronic myelogenous leukemia, multiple myeloma, follicular lymphoma, carcinoid tumor, and malignant melanoma. Interferon alpha-2b has many drug classifications including anti-infective, anti-neoplastic, antiproliferative, antiviral and immunological agent.

32.2.1 Formulation and packaging

50 million units/mL, lyophilized powder, which must be reconstituted prior to administration.

- Vial size: 50million units/vial
- Diluent: Compatible with normal saline, Ringer's injection, lactated Ringer's, and 5% sodium bicarbonate injection. Interferon Alpha-2b should be reconstituted with 1 mL to reach a final concentration of 10:1. IV dose should be diluted in sodium chloride 0.9%/100 mL and given over 20 minutes. The final concentration of INTRON A should not be less than 10 million IU/100 mL
- Source: Schering Plough Corp.

32.2.2 Drug Shipment

Interferon Alpha-2b will be provided by RPCI.

32.2.3 Preparing and dispensing

The lyophilized product is reconstituted as directed by the manufacturer. Investigational Drug Service Pharmacy (IDS) will prepare and dispense.

For IV injection, it is recommended that Interferon Alpha-2b be administered as a 100,000 U/mL solution to minimize adsorption of the drug to glass and plastic containers.

32.2.4 Drug administration

Interferon Alpha-2b (20 million units/M^2) will be administered intravenously over 20 minutes once a day on Days 1-3, 8-10, and 15-17.

32.2.5 Drug storage and accountability

The Investigator or designate will be responsible for ensuring that the investigational product is securely maintained in a locked, limited-access facility in accordance with the applicable regulatory requirements.

Powder for injection should be stored at 2 to 8°C (36-46°F). After reconstitution, the solution should be used immediately but may be stored up to 24 hours at 2-8°C (36-46°F).

Drug storage temperature will be maintained and recorded, as applicable.

32.2.6 Concomitant medications

Interactions between Interferon Alpha-2b and other drugs have not been fully evaluated. Caution should be exercised when administering Interferon Alpha-2b therapy in combination with other

potentially myelosuppressive agents such as zidovudine. Concomitant use of Interferon Alpha-2b and theophylline decreases theophylline clearance, resulting in a 100% increase in serum theophylline levels. Concomitant Interferon Alpha-2b and REBETOL (Ribavirin) use is contraindicated. A more detailed guide concerning drug interactions can be found in Appendix B.

32.3 Rintatolimod (poly IC analog)

A substituted double stranded poly-ribonucleic acid (polyI:poly$C_{12}U$), rintatolimod preserves activity of poly-IC with a much improved systemic toxicity profile. The product has been studied extensively for use as a vaccine adjuvant and for its direct antiviral activity, as well in several cancer studies as a monotherapy, but most extensively in chronic fatigue syndrome (CFS).

32.3.1 Other names

poly$IC_{12}U$, Ampligen®, poly I: poly$C_{12}U$; Polyinosinic: polycytidylic-polyuridylic acid; polyriboinosinic-polyribocytidylic (uridylic) acid.

32.3.2 Formulation and packaging

Rintatolimod is supplied as a liquid solution in glass bottles containing 200 mg (100 mg in case of toxicity) per 80 mL. Rintatolimod is a colorless solution containing 2.5 mg/mL in physiological salts (0.15 M NaCl, 0.01 M phosphate, 0.001 M Mg++). The product does not contain preservatives or antioxidants.

32.3.3 Drug Shipment

Rintatolimod will be provided by Hemispherx and shipped to the participating site.

The date of receipt and the amount of drug received will be documented. Drug shipment records will be retained by the investigational pharmacist or designee.

32.3.4 Preparing and Dispensing

A vial of rintatolimod is suitable for direct IV infusion. IDS will prepare and dispense. Each vial should be taken from the refrigerator and allowed to equilibrate to room temperature.

32.3.5 Drug administration

Rintatolimod 200 mg will be administered by intravenous infusion after Interferon Alpha-2b on Days 1-3, 8-10, and 15-17. Additional details on the procedures for receiving, storing and using rintatolimod (Ampligen®) can be found in a separate document entitled “Procedures for Receiving, Storing, and Using Ampligen® (Poly I:Poly $C_{12}U$) Liquid Solution”.

The initial administration should begin at a slow rate of infusion (approx. 20 cc/hour) and increase to 40cc/hour after 30 minutes. Tubing should be flushed with 30 to 50 mL of normal saline solution upon completion.

32.3.6 Drug storage and accountability

The Investigator or designate will be responsible for ensuring that the investigational product is securely maintained in a locked, limited-access facility, as specified by Hemispherx and in accordance with the applicable regulatory requirements.

Rintatolimod should be stored at 2 to 8°C, but should be infused at room temperature. Used vials should be accounted for and destroyed according to institutional procedure.

Drug storage temperature will be maintained and recorded, as applicable.

32.4 Handling and Disposal

The Investigator or designee will be responsible for dispensing and accounting for all investigational drug provided by Hemispherx exercising accepted medical and pharmaceutical practices. Study drugs must be handled as cytotoxic agents and appropriate precautions taken per the institution's environmentally safe handling procedures. All investigational drugs will be dispensed in accordance with the Investigator's prescription or written order.

All products dispensed will be recorded on a product accountability record. Records of product lot numbers and dates received will be entered on a product accountability form. This record will be reviewed by the Sponsor's staff or representative during periodic monitoring visits. It is the Investigator's responsibility to ensure that an accurate record of investigational drug issued and returned is maintained.

Used vials (excess drug) will be destroyed according to standard practices after properly accounting for the dispensing. Partially used vials of study drug will not be re-used for other participants.

Under no circumstances will the Investigator supply investigational drug to a third party or allow the investigational drug to be used in a manner other than as directed by this protocol.

In regards to drug receipt, accountability and storage, SOP IDS-601 will be followed.

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34 APPENDICES/ SUPPLEMENTS

Appendix A NYHA CLASSIFICATION

NEW YORK HEART ASSOCIATION CLASSIFICATION OF CARDIAC DISEASE

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Appendix B DESCRIPTION OF INTRON A POTENTIAL INTERACTIONS

Aldesleukin: Interferons (Alfa) may enhance the adverse/toxic effect of Aldesleukin. In particular, risks of myocardial and renal toxicity may be increased by this combination. *Risk D: Consider therapy modification*

Methadone: Interferons (Alfa) may increase the serum concentration of Methadone. *Risk C: Monitor therapy*

Ribavirin: Interferons (Alfa) may enhance the adverse/toxic effect of Ribavirin. Hemolytic anemia has been observed. *Risk C: Monitor therapy*

Telbivudine: Interferon Alfa-2b may enhance the adverse/toxic effect of Telbivudine. Specifically, the risk for peripheral neuropathy may be increased. *Risk X: Avoid combination*

Theophylline Derivatives: Interferons may decrease the metabolism of Theophylline Derivatives. **Exceptions:** Dyphylline. *Risk C: Monitor therapy*

Zidovudine: Interferons may enhance the adverse/toxic effect of Zidovudine. Interferons may decrease the metabolism of Zidovudine. *Risk C: Monitor therapy*

Appendix C

ECOG Performance Status Scores

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

Appendix D

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM: INCLUSION CRITERIA

Participant Name : _____

Medical Record No. : _____

Title: Phase 2A Study Evaluating a Chemokine-Modulatory Regimen in Patients with Colorectal Cancer Metastatic to the Liver

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. Recurrent and/or metastatic unresectable colorectal cancer with hepatic metastases.	
☐	☐	☐	2. Hepatic metastases present which are amenable to biopsy	
☐	☐	☐	3. Prior treatment with, contra-indication to or refusal of a fluoropyrimidine, irinotecan, oxaliplatin and an anti-EGFR targeted therapy (if RAS-wt), as well as a PD-1 or PD-L1 targeted drug if MSI-H/dMMR.	
☐	☐	☐	4. No chemotherapy, radiotherapy, major surgery, or biologic therapy within 3 weeks of protocol treatment	
☐	☐	☐	5. An ECOG performance status of 0, 1, or 2. Refer to Appendix C.	
☐	☐	☐	6. Have measurable disease per RECIST 1.1 criteria present.	
☐	☐	☐	7. Ability to swallow and retain oral medications.	
☐	☐	☐	8. Participants of child-bearing potential must agree to use adequate contraceptive methods (e.g., hormone or barrier method of birth control; abstinence) prior to study entry. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.	
☐	☐	☐	9. Age equal to 18 years or older.	
☐	☐	☐	10. Must have normal organ and marrow function as defined below: o Platelet ≥ 75,000/μL o Hemoglobin ≥ 9 g/dL	

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
			○ Hematocrit ≥ 27% ○ Absolute Neutrophil Count (ANC) ≥ 1500/μL ○ Creatinine ≤ institutional upper limit of normal (ULN) OR Creatinine clearance ≥ 50 mL/min for patients with creatinine levels greater than ULN (refer to Appendix I for Cockcroft-Gault Equation) ○ Total bilirubin ≤ 1.5 X institutional ULN or for patients with known Gilbert's syndrome total bilirubin ≤ 3 x ULN ○ AST(SGOT) and ALT(SGPT) ≤ 2.5 X institutional ULN ○ Plasma amylase and lipase ≤1.5 X institutional ULN.	
☐	☐	☐	11. Participant or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.	

Investigator Signature: _____ Date: _____

Printed Name of Investigator: _____

Appendix E

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM: EXCLUSION CRITERIA

Participant Name: _____

Medical Record No. : _____

Title: Phase 2A Study Evaluating a Chemokine-Modulatory Regimen in Patients with Colorectal Cancer Metastatic to the Liver

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. Patients currently treated with systemic immunosuppressive agents, including steroids, are ineligible until 3 weeks after removal from immunosuppressive treatment.	
☐	☐	☐	2. Patients with active autoimmune disease, requiring ongoing immunosuppressive therapy or history of transplantation.	
☐	☐	☐	3. Patients who are pregnant or nursing. Women of childbearing potential (WOCBP) will have to undergo a urine pregnancy test as part of screening.	
☐	☐	☐	4. Untreated CNS metastases	
☐	☐	☐	5. Cardiac risk factors including: o Patients experiencing cardiac event(s) (acute coronary syndrome, myocardial infarction, or ischemia) within 3 months of signing consent o Patients with a New York Heart Association classification of III or IV (Appendix A)	
☐	☐	☐	6. History of upper gastrointestinal ulceration, upper gastrointestinal bleeding, or upper gastrointestinal perforation within the past 3 years. Patients with ulceration, bleeding or perforation in the lower bowel are not excluded.	
☐	☐	☐	7. Prior allergic reaction or hypersensitivity to celecoxib ,NSAIDs, or any study agents which would prevent completion of protocol-therapy.	
☐	☐	☐	8. Patients are ineligible if they plan on regular use of	

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
			NSAIDs at any dose more than 2 times per week (on average) or aspirin at more than 325 mg at least three times per week, on average. Low-dose aspirin not exceeding 100 mg/day is permitted. Patients who agree to stop regular NSAIDs or higher dose aspirin are eligible and no wash out period is required.	
☐	☐	☐	9. Received an investigational agent within 30 days prior to enrollment.	
☐	☐	☐	10. Unwilling or unable to follow protocol requirements.	
☐	☐	☐	11. Patients with known serious mood disorders.	
☐	☐	☐	12. Any additional condition which in the Investigator's opinion deems the participant an unsuitable candidate to receive the study drugs	

Participant meets all entry criteria: ☐ **Yes** ☐ **No**
If "NO", do not enroll participant in study.

Investigator Signature: _____ **Date:** _____

Printed Name of Investigator: _____

Appendix F

Study Calendar

Baseline and/or Screening assessments must be performed within 14 days prior to the first dose of investigational product unless otherwise stated. Any results falling outside of the reference ranges may be repeated at the discretion of the investigator. All on-study visit procedures are allowed **a window of ± 1 day** unless otherwise noted.

Evaluation[1]	**Informed Consent**	**Screening (Day -14 through Day -1)**	**Day 1**	**Days 2 & 3**	**Day 8**	**Days 9, 10**	**Day 15**	**Days 16 & 17**	**Day 20 (± 2 days)**	**EOT Day 29 (± 10 days)**	**F/U Day 46 (± 3 days)**
Written Informed Consent prior to any Screening Procedures	X										
Recording of Concomitant Medications		X	X	X	X	X	X	X		X	
Recording of Adverse Events			X	X	X	X	X	X	X	X	X[9]
Survival Follow-up											X[11]
Clinical Assessments											
Medical & Surgical History		X									

Evaluation[1]	**Informed Consent**	**Screening (Day -14 through Day -1)**	**Week 1 Day 1**	**Week 1 Days 2 &3**	**Day 8**	**Days 9, 10**	**Day 15**	**Days 16 & 17**	**Day 20 (± 2 days)**	**EOT Day 29 (± 10 days)**	**F/U Day 46 (± 3 days)**
Physical Examination including height and weight[2]		X[2]	X[2]		X[2]		X[2]			X[2]	
Vital Signs[8]		X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]		X[8]	
ECOG Performance Status		X	X		X		X			X	
Laboratory Procedures											
Hematology[3]		X	X		X		X			X	
Chemistry[4]		X	X		X		X			X	
Serum hCG[5]		X[5]									
CEA level		X									
ANA testing		X									
Blood (60cc) for in vitro assays[6]			X	X[6a]			X			X	
Imaging/ Other Procedures											
12-Lead ECG		X									
Liver Biopsy		X							X		

Evaluation[1]	**Informed Consent**	**Screening (Day -14 through Day -1)**	**Week 1 Day 1**	**Week 1 Days 2 &3**	**Day 8**	**Days 9, 10**	**Day 15**	**Days 16 & 17**	**Day 20 (± 2 days)**	**EOT Day 29 (± 10 days)**	**F/U Day 46 (± 3 days)**
Tumor/Disease Assessment (CT and/or MRI)		X[7]									X[10]
Study Drug Administration											
CKM regimen administration			X	X	X	X	X	X			

1. Assessments to be performed at the times stipulated in the table and as clinically required in the management of the subject.
2. At each time point, Physical exam to include assessment of Head/Ears/Eyes/Nose/Throat (HEENT), Skin, Chest/Lungs, Heart, Neck/lymph nodes, Abdomen including liver/spleen, Extremities and weight. Height to be collected at screening only. Physical exam does not need to be repeated on Day 1 if performed within 3 days prior to Day 1.
3. CBC with auto differential.
4. Glucose, BUN, creatinine, sodium, potassium, chloride, CO_2, calcium, total protein, albumin, alkaline phosphatase, AST, ALT, total bilirubin, amylase, lipase.
5. Within 7 days of Day 1 and as clinically indicated once on treatment in women of child-bearing potential only.
6. Blood (60cc) for in vitro assays will be drawn as outlined in section 11.2.

6[a]. Day 3 only within 1 hour after end of infusion

7. Within 28 days prior to Day 1.
8. Vital Signs (blood pressure, pulse, respiratory rate and temperature) will be obtained at screening and end of treatment visits. On dosing days, vital signs will be obtained pre-treatment, 30 and 60 minutes post Rintatolimod (± 5 minutes).
9. Patients will be followed for survival status for 12 months after end of treatment visit. Phone contact/chart review is acceptable.
10. Restaging imaging CT/MRI to be performed on day 46 ± 3 days. If this scan demonstrates stable disease/response, patients will continue in follow-up with CT imaging every 8 weeks until progression, clinical deterioration or withdrawal from study. If in the clinical judgement of the treating team, it is in the best interest of the patient to pursue another therapeutic option, the patient will come off study and CT imaging will be obtained only as SOC/protocol.
11. Once every 3 months for up to 12 months after EOT visit to ascertain survival – both chart review and/or phone contact are acceptable

Appendix G

RECIST 1.1 Criteria

Objective Tumor Response

All protocol-defined imaging studies must be performed at the investigative site or sponsor-approved facility using protocol-defined parameters. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. RECIST 1.1 will be used to assess objective tumor response.

Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, will be identified as target lesions and recorded and measured at baseline. Target lesions will be selected on the basis of their size. Lesions with the longest diameter (short axis for lymph nodes) and are ≥ 10 mm (CT and MRI), ≥ 15 mm lymph nodes, > 20 mm CXR and are for accurate repetitive measurements (either by imaging techniques or clinically) will be chosen. A sum of the longest diameter (short axis for lymph nodes) of all target lesions will be calculated and reported as the baseline sum diameters. This will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

- **Complete Response (CR):** Disappearance of all target lesions. Any lymph nodes must have a reduction in short axis to < 10 mm. Changes in tumor measurements must be confirmed by repeat studies performed no less than 6 weeks after the criteria for response are first met.
- **Partial Response (PR):** At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters. Changes in tumor measurements must be confirmed by repeat studies performed no less than 6 weeks after the criteria for response are first met.
- **Progressive Disease (PD):** At least a 20% increase in the sum of diameters of target lesions, taking as references the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions is also considered progression.
- **Stable Disease (SD):** Neither a sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameter while on study. Participants having a documented response with no confirmation of the response will be listed with stable disease.

Non-Target Lesions

All other small lesions (longest diameter < 10 mm or lymph nodes ≥ 10 mm to < 15 mm short axis) and non-measurable lesions (i.e., leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, blastic bone lesions, or abdominal masses / abdominal organomegaly identified by physical exam that is not measurable by imaging) should be identified as non-target lesions and indicated as present in the

source documents at baseline. The general location will also be documented on the images drawing a regularly-shaped Region of Interest. Measurements of the non-target lesions will not be performed, but the presence or absence of each should be noted throughout follow-up and evaluation.

Complete Response: Disappearance of all non-target lesions and normalization of tumor marker level, if applicable. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Non-Complete Response/Non-Progressive Disease: Persistence of 1 or more non-target lesion(s) and/or maintenance of tumor marker level above the upper limits of normal.

Progressive Disease: Appearance of 1 or more new lesions or the unequivocal progression of existing non-target lesions. Although a clear progression of non-target lesions is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed at a later time.

Evaluation of Response

Time point response assessments will be performed every 8 weeks [starting with Day 46 (± 3 days)]. To determine time point response, refer to the tables below:

Time Point Response Criteria: target (± non-target disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Time Point Response Criteria: non-target disease only

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[1]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

1 Non-CR/non-PD is preferred over SD for non-target disease since SD is used as endpoint for assessment of efficacy in trials so to assign this category when no lesions can be measured is not advised.

The best overall response is the best response recorded from the start of study treatment until disease progression, taking into account any requirement for confirmation. In general, the

participant's best response assignment will depend on the achievement of both measurement and confirmation criteria and will be determined by combining the participant's status of target lesions, non-target lesions, and new lesions.

- Symptomatic Deterioration: Participants with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not related to study treatment or other medical conditions should be reported as progressive disease due to "symptomatic deterioration." Every effort should be made to document objective progression even after discontinuation of treatment due to symptomatic deterioration. Symptomatic deterioration that may lead to discontinuation of treatment include, but is not limited to, symptoms such as:
- Weight loss > 10% of body weight.
- Worsening of disease-related symptoms (e.g., worsening dyspnea, increasing pain/increasing requirement for narcotic analgesics).
- Decline in performance status of > 1 level on ECOG scale.

Confirmation Measurement

If Day 46 CT scan demonstrates stable disease/response, patients will continue in follow-up with CT imaging every 8 weeks until progression, clinical deterioration or withdrawal from study.

Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

- **Clinical Lesions:** Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.
- **Chest x-ray:** Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- **Conventional CT and MRI:** This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning

sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

- **Ultrasound:** Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.
- **Endoscopy, Laparoscopy:** The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.
- **Tumor Markers:** Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response.
- **Cytology, Histology:** These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

- **FDG-PET:** While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG PET imaging can be identified according to the following algorithm:
- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

- FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PROTOCOL TITLE: Phase 2A Study Evaluating a Chemokine-Modulatory Regimen in Patients with Colorectal Cancer Metastatic to the Liver

Appendix H

Celecoxib Patient Diary

Protocol # _____ Patient Name: _____
Drug Name: Celecoxib
Treatment Week: _____ Med. Record #: ___ ___ ___ ___ ___ ___

Study Medication Calendar

You are participating in a study that requires Celecoxib 200 mg to be given twice daily, approximately 12 hours apart for three consecutive days. This dose will be given three days a week for three consecutive weeks. Your morning dose of Celecoxib will be given to you along with your pre-medications while you are in clinic. Please complete this calendar to document your evening doses of Celecoxib. Your nurse will let you know what time you receive your morning dose of Celecoxib so you can take your evening dose approximately 12 hours after.

Day			
Dose			
Time			

Please remember to bring this calendar and your pill bottle (including any unused pills) with you to your next study appointment.

Coordinator use only
Date of return: _____
# of pills
(dispensed: _____ -- returned: _____)
_____ x 100 = % adherence: _____
# of pills scheduled _____

Patient signature: _____ Date: _____

Investigator signature: _____ Date: _____

Appendix I

Creatinine Clearance Calculation

Cockcroft-Gault Equation*

Men: CrCl = [(140-YR) x weight in kg] / (PCr x 72)

Women: CrCl = 0.85 x [(140-YR) x weight in kg] / (PCr x 72)

Where:

CrCl is creatine clearance (mL/min)

PCr is plasma creatinine (mg/dL)

YR is age (years)