Abbreviated Title: Phase 1 IFN + monocytes

NIH Protocol #: 17-C-0011

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Title: Phase 1 Study of Intraperitoneal Infusion of Autologous Monocytes with SYLATRON® (Peginterferon alfa-2b) and ACTIMMUNE® (Interferon gamma-1b) in Women with Recurrent or Refractory Ovarian Cancer, Fallopian Tube Cancer or Primary Peritoneal Cancer

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Drug Name:	Sylatron® (Peginterferon alfa-2b)	Actimmune® (Interferon gamma-1b)	Autologous monocytes
BB-IND Number:	17040	17040	17040
Sponsor:	Center for Cancer Research, NCI	Center for Cancer Research, NCI	Center for Cancer Research, NCI
Manufacturer:	Merck	Horizon Pharma, Inc.	Department of Transfusion Medicine (DTM)

Commercial Agents: None

PRÉCIS

Background:

- Monocytes can differentiate into classic M1 macrophages inhibiting tumor proliferation and promoting natural killer (NK) cell differentiation.
- Human alpha interferons (interferon alfa, IFNα), interferon gamma (IFNγ) and monocytes have strong anti-neoplastic response in vitro and in vivo
- IFNα and IFNγ have been shown in early phase clinical trials to be safely administered intraperitoneally.
- Intraperitoneal monocytes alone or activated with IFNγ are safe and feasible as demonstrated in phase I clinical studies.
- We have shown that the combination of human monocytes, IFN-α2a and IFN-γ1b, or pegylated IFN-α, act synergistically against tumor cells in vitro and in mouse models.

Objectives:

- To identify a maximum tolerated dose of intraperitoneal autologous monocytes and Sylatron® (Peginterferon alfa-2b) and Actimmune® (Interferon gamma-1b).

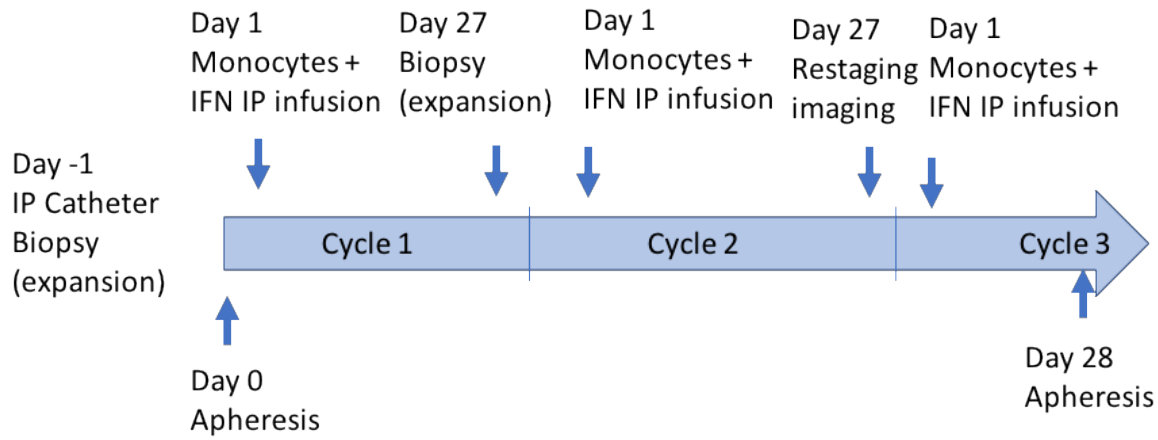
Eligibility:

- Advanced metastatic or unresectable epithelial ovarian cancer, primary peritoneal cancer or fallopian tube cancer that is relapsed and resistant or refractory to prior platinum-based standard care systemic regimen.
- Patients must be off prior chemotherapy, radiation therapy, hormonal therapy or biological therapy for at least 4 weeks.
- ECOG performance status 0-1 and adequate organ and marrow function.

Design:

- This is an open label single arm phase I trial to determine the maximum tolerated dose of intraperitoneal monocytes and Sylatron® (Peginterferon alfa-2b) and Actimmune® (Interferon gamma-1b).
- Autologous monocytes obtained through apheresis will be mixed with Sylatron® (Peginterferon alfa-2b) and Actimmune® (Interferon gamma-1b) in a 3 + 3 dose escalation and administered intraperitoneally once every 28 days. A new product will be manufactured for each treatment cycle.
- Once the maximum tolerated dose is obtained, an expansion cohort will be enrolled to better quantify response rate and time to disease progression.
- Research samples including peripheral blood and ascites will be obtained prior to the initiation of study therapy and prior to the start of each cycle. Tissue biopsies will be obtained in the dose expansion phase to characterize the immune infiltration.
- Patients will be evaluated every 2 cycles for response using RECIST v1.1 and every cycle for safety using CTCAE v4.0.

Study Schema



Total Volume (250 mL)

Dose Level	Monocytes total number	SYLATRON ® Peginterferon alfa2b, mcg (final concentration)	ACTIMMUNE ® Interferon gamma-1b, mcg (final concentration)
1	0	25 mcg (0.1 µg/mL)	5 mcg (0.02 µg/mL)
2	75 x 10^6 ± 20%	25 mcg (0.1 µg/mL)	5 mcg (0.02 µg/mL)
3	750 x 10^6 ± 20%	25 mcg (0.1 µg/mL)	5 mcg (0.02 µg/mL)
3b*	0	250 mcg (1 µg/mL)	50 mcg (0.2 µg/mL)
4	750 x 10^6 ± 20%	250 mcg (1 µg/mL)	50 mcg (0.2 µg/mL)

*DL3b will enroll if ≥2 subjects enrolled on DL3 or DL4 experience DLT

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

1.1.1.1 To identify a maximum tolerated dose of intraperitoneal autologous monocytes and Sylatron® (Interferon alfa-2b) and Actimmune® (Interferon gamma-1b).

1.1.2 Secondary Objectives

1.1.2.1 To quantify the response rate and time to disease progression

1.1.3 Exploratory Objectives

1.1.3.1 To characterize the immune infiltration into ascites and tumor (expansion cohort)

1.1.3.2 To analyze circulating leukocyte populations

1.2 BACKGROUND AND RATIONALE

1.2.1 Ovarian Cancer

Ovarian cancer is the leading cause of death due to gynecological malignancies, and the fifth leading cause of death due to cancer in women. Patients are diagnosed late in the course of disease (Stage 3 or 4) due to lack of early symptoms and reliable non-invasive testing. Because of the late diagnosis and aggressiveness of the disease, ovarian cancer results in approximately 14,000 deaths per year. Standard of care for the treatment of ovarian cancer is tumor cytoreductive surgery followed by administration of platinum and taxane based chemotherapy [1]. The disease course is characterized by a high rate of relapse despite an initial good response to the therapy [2]. Patients are classified as chemotherapy sensitive, resistant or refractory. Sensitive patients – who relapse more than 6 months after initial treatment – are re-treated with platinum-based combinations. Without curative second line treatment for patients with resistant or refractory epithelial ovarian cancer (EOC), median survival is 16 months, with most of the population dying within the first two years [3].

Ovarian cancer is largely retained in the peritoneal cavity, with metastases outside of the peritoneum occurring late in disease if at all. In 2006 there was a major advance in the treatment of a portion of the population of women diagnosed with EOC [4] . Patients with stage III optimally resected cancer were given cisplatin IP and paclitaxel IV and IP, compared to standard IV administration of both drugs. There was a 15-month increase in overall survival. This study showed that IP delivery of agents was a viable therapeutic option, and that IP therapy could increase efficacy of treatment.

Adoptive cell therapy was pioneered and developed in the Intramural Research Program. Autologous adoptive cell therapy of tumor infiltrating lymphocytes (TILs) is under development for the treatment of melanoma [5]. TIL therapy is based upon the observations that lymphocytes recognize and kill cancer cells. Based on these observations it was posed that the anti-inflammatory environment of the tumor inhibited a *de novo* immune response. Clinical trials have tested numerous strategies for re-activating lymphocytes, and other leukocyte subsets.

The generation of pharmaceutical grade IFNs in the 1980s generated a large number of human trials aimed at using the anti-viral and anti-neoplastic properties of IFNs [6]. The FDA licensed IFNα-2a and IFNα-2b for treatment of melanoma, hairy cell leukemia, follicular lymphoma, chronic myelogenous leukemia and AIDS related Kaposi's sarcoma. The trials did show greater efficacy than standard of care, and are used as second and third line treatments. Furthermore, it has been shown that activated monocytes are capable of killing malignant cells [7]. While different combinations of IFNs and monocytes were tested in human clinical trials for their tumoricidal properties, the three have never been combined (**Table 1**).

Experimental agents	References
IFNα-2a,2b	[8, 9],[10-12]
IFNγ	[13-16]
Monocytes	[17, 18]
IFNγ + monocytes	[19]

Table 1. Clinical development of interferons and monocytes as anti-cancer therapy

1.2.2 Intraperitoneal Interferon

During the late 1980s and 1990s phase one trials were completed testing IP infusion of immune modifying agents, including, but not limited to IFNα or IFNγ for treatment of cancers involving organs in the peritoneal cavity. The large-scale production of cytokines and the production of therapeutic grade lymphocytes through counter-elutriation paved the way for infusion of the cytokines, lymphocytes, and cytokine stimulated lymphocytes for the treatment of malignancies of the peritoneal cavity. Studies focused on, but were not limited to, the treatment of ovarian cancer.

1.2.2.1 Phase I clinical studies

Intraperitoneal (IP) IFNα was used in the first intraperitoneal immunotherapy studies conducted in patients with ovarian cancer. The promising results of these studies, however, were eclipsed by the emergence of taxane based therapies at the time, and have not been taken further than early phase clinical trials. The first Phase 1 study of IP immunotherapy for ovarian cancer evaluated infusion of high dose IFNα-2a as salvage therapy for patients with high grade epithelial ovarian cancer. Of 14 patients receiving IFNα-2a over sixteen weeks, 6 patients (36%) had complete response, one patient (1%) had partial response, and 6 patients (55%) had disease progression [8, 9]. There was found to be an increase in NK cell activity and antibody dependent cell cytotoxic activity in response to IFNα-2a in 11 of the patients that were treated [11].That trial was followed by a study of 20 patients treated for 8 weeks. Eleven patients (55%) responded, and five of those patients (25%) had complete response. The median duration of complete response was 11 months. The most common adverse effects not requiring dose reduction were fever (80%), abdominal pain (32%), and leukopenia (52%) [12]. In a study with 39 patients with ovarian cancer given intraperitoneal IFNα-2b in combination with chemotherapy, 14/35 patients achieved a pathological CR with only one patient discontinuing treatment due to severe fatigue [10]. In a pharmacokinetics study, IP and blood levels of IFNα-2b were measured after administration. All patients had metastatic disease within the peritoneal cavity and were given a range of IFNα-2b from $5x10^6$ units to $15x10^6$ units. The bioavailability of IP IFNα-2b was found to be 30 fold higher intraperitoneally compared to in the peripheral blood, with a slower elimination half-life (10-32hr IP compared to 5-13 hours in the peripheral blood) [20].

The first study of high dose IP IFNγ for the treatment of ovarian cancer enrolled 27 patients with relapsed, chemo-resistant disease. Unfortunately, none of the patients on this small trial showed objective response, with 14 patients having progressive disease during the protocol [15]. A follow up of the first IP IFNγ study was published in 1996. A total of 109 patients enrolled. All patients had stage three or greater epithelial ovarian cancer and were given IFNγ either by subcutaneous injection or IP injection through an implanted catheter. Twenty-three patients (23.5%) had a complete response, while 8 (8%) patients had a partial response [16]. In a study with 7 patients treated with IP IFNγ, there was an increase in NK cell activity and tumor-associated lymphocytes and macrophages but no objective responses [14]. A similar study with 8 patients treated with IP IFNγ found similar rises in tumor associated lymphocytes and macrophages: 1 patient with a complete response, 2 with partial responses and 2 with stable disease [13]. Pharmacokinetic studies showed that IFNγ was retained within the peritoneum for longer than 24 hours with up to a 150-200 fold increase in peak levels above that in plasma. All patients who received IP IFNγ 4–8 $x10^6$ IU/m^2 developed transient fever ≥38°F. Release into the blood was found only in the higher doses (>$2x10^6$ IU/m^2), with a peak level reached in the serum at 6 hours. The peak levels in the peritoneal cavity increased as IFNγ dose increased, with 30.7 IU/mL with $0.05x10^6$ IU/m^2 up to 1,720 IU/mL with $8x10^6$ IU/m^2) and levels persisted in both the blood and peritoneum for up to 24hr [15]. One of the most important observations from the studies was the tolerable side effect profile, and prolonged intraperitoneal concentrations of IFNs. No published studies report the co-administration of IFNs IP. Together these studies show that IP administration of IFNα or IFNγ is a potentially testable therapeutic treatment.

1.2.3 Intraperitoneal Monocytes

The anti-tumor effect of activated monocytes is well characterized. Monocytes are precursors of resident tissue macrophages, and their functions in normal homeostasis include antigen presentation, phagocytosis of apoptotic cells, and tissue development. [21] Monocytes adapt to their microenvironment and differentiate based on cytokine, chemokine and metabolite expression [22]. In the presence of a tumor, monocytes differentiate into classical M1 macrophages that inhibit tumor proliferation and secrete proinflammatory cytokines and chemokines. They are also able to promote natural killer (NK) cell differentiation, providing a rapid cytotoxic response to tumor cells [18, 23, 24]. This response is further potentiated by the release of IFNs by NK cells, promoting the tumoricidal effects of these cells [25]. Macrophages differentiated towards an M1 phenotype can potentially be exploited in the clinic to selectively target tumor cells without damaging normal tissue [26]. Monocytes can also differentiate into M2 (alternative macrophages) that promote tumor proliferation [27-29] and are associated with a poor prognosis in advanced epithelial ovarian cancer [30]. Therefore, the success of monocytes as an anti-tumor treatment approach may depend on the ability to maintain the M1 phenotype and avoid M2 differentiation in the tumor microenvironment.

Several studies have shown that monocytes can be isolated from the peripheral blood in large numbers (1 billion) and at relatively high purity (80-95%) [31, 32]. Two phase 1 safety trials studied intraperitoneal infusion of autologous monocytes activated with IFNγ or muramyl tripeptide phosphatidylethanolamine (MTP-PE) [19]·[17]. These studies showed that IP administration of IFN stimulated monocytes was safe and feasible for the treatment of peritoneal carcinoma of colorectal origin. Indium-111 (^{111}In) labeled monocytes were visualized using whole-body Gamma Camera Imaging, and were found to be confined to the peritoneal cavity and not present in the lungs, heart or liver after up to 5 days of monitoring [19]. A subsequent study assessed autologous transplantation of monocytes stimulated with liposomal-muramyl tripeptide phosphatidyl ethanolamine (L-MTP-PE), a potent adjuvant, for the treatment of carcinomas of the peritoneum. Patients' abdomens were imaged at 30 minutes and 7 days post-infusion. Once again, the ^{111}In was confined to the peritoneal cavity with no measurable activity in liver or lungs. Patients experienced low grade toxicities of fevers, chills and abdominal pain. [31]. Response rates were not reported.

1.2.4 Preclinical studies of monocytes combined with both Interferons alpha and gamma

Given the recent successes of immunotherapy in other malignancies, intraperitoneal immunotherapy is a promising area for exploration in cancers predominantly confined to the peritoneal cavity, such as ovarian cancer. Our experimental approach is based on previous observations that IFNs themselves are potent anti-neoplastic proteins [6]. We have shown *in vitro* and in animal models that monocyte stimulation with IFN-α and IFN-γ achieves M1 differentiation, important for anti-tumor activity. These human monocytes, in combination with human alpha interferons (IFNα-2a or pegylated IFNα-2b) and interferon gamma (IFNγ-1b) are potent killers of cancer cell lines in vitro [33, 34]. Our studies included 6 ovarian cancer cell lines, and demonstrated that the presence of monocytes additionally decreased viability of some cell lines at low doses of interferons (**Figure 1**) [35]. Our most recent, unpublished work suggests that the mechanism of killing is partially mediated by surface TNF-related apoptosis-inducing ligand (TRAIL) on monocytes that induces cell death via extrinsic apoptosis.

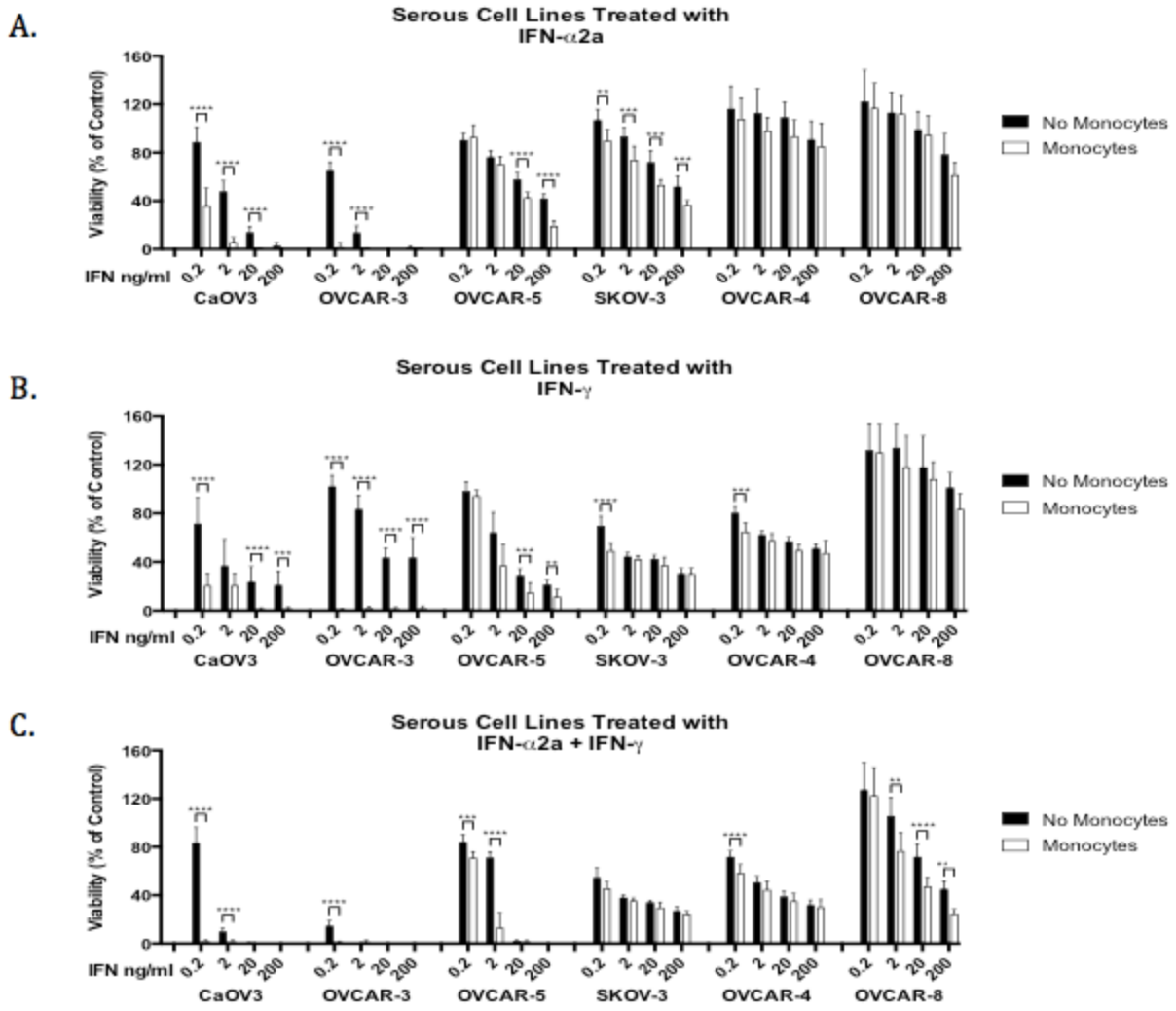


Figure 1. IFN activated monocytes are potent killers of ovarian cancer cell lines. Ovarian cancer cells were treated with IFNα-2a and/or IFNγ with or without monocytes. Cell line CaOV3 showed the greatest effect of monocyte addition with either IFN. OVCAR-3 was the most sensitive to the combination of IFNs without monocytes. OVCAR-5 killing was most dependent on the presence of both IFNs and monocytes. Other cell lines showed variable response to the tested combinations. * p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

We expanded this observation to demonstrate that intra-tumoral injection of monocytes with IFNs decreased human ovarian cancer xenograft size (**Figure 2**) [36]. Importantly, the monocytes introduced into the tumor differentiated into inflammatory M1 macrophages when in the presence of both IFNs (**Figure 3**).

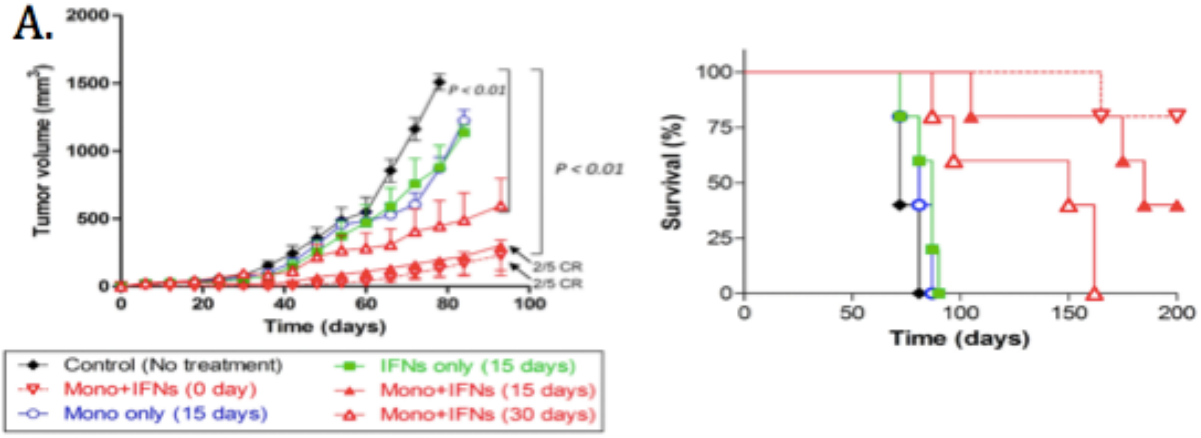


Figure 2 Intratumoral administration of IFNs activated Monocytes inhibited tumor growth and prolonged survival (A) Mice were injected SC with 10 × 10^6 OVCAR-3 cells and treated with monocytes (3 × 10^7) or IFN-α2a and IFN-γ (20 ng each), or combination by i.t. injection on day 0, 15 or 30 post-tumor implantation. Mice receiving combination therapy showed significant inhibition of tumor growth compared to IFNs alone, monocytes alone and PBS. (B) Kaplan-Meier survival curves of mice with OVCAR-3 tumors are shown (n=5 per group). CR, complete regression. Tumor volumes measured by Vernier caliper; survival calculated based on sacrifice

when tumors size reached > 2 cm in diameter. Experiments were repeated twice; bars, SD.

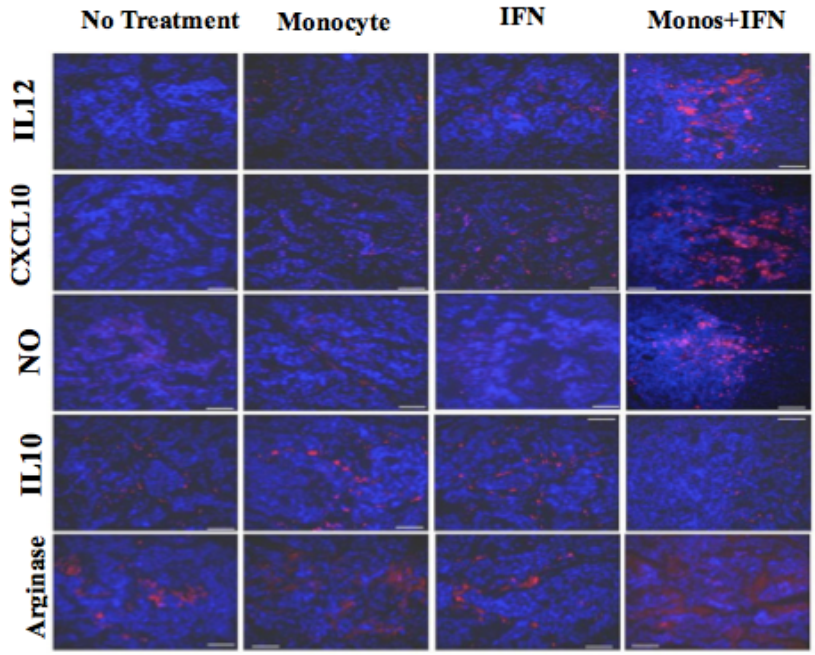


Figure 3. IFNs activate differentiation of monocytes to classical (M1) macrophages in OVCAR-3 tumors The tumor samples were collected on day 17 (2 days after 15 day post inoculation treatment, respectively) from the mice in Fig. 1A and the immunofluorescence microscopy were done using antibodies specific for IL-12, CXCL10, NOS2, IL-10, and Arginase-1. Bars, 50 μm, Magnification X400.

We have shown *in vitro* and in the nude mouse that while IFNα-2a or IFNγ-1b work with monocytes to kill tumor cells, and the combination of all three can achieve the greatest amount of tumoricidal activity. Intratumoral injection of these cells in mice resulted in a reduction in tumor burden and increased overall survival [36]. Additional unpublished data show that IP administration of human monocytes primed with alpha and gamma interferons reduces both tumor burden and ascites volume in a xenograft model of human ovarian cancer (OVCAR3). Furthermore, we demonstrated that monocytes and IFNs improve tumor shrinkage with carboplatin and paclitaxel and significantly increase tumor cell death [35]. Both unpublished and published data strongly suggest that the monocytes must be in contact with the tumor cells for killing. Thus, the rationale for IP immunotherapy is not only to deliver higher concentrations of the drug directly to the tumor, but also to skew monocytes to the M1 phenotype and to activate NK cells in the tumor microenvironment, potentiating tumor cell death.

1.2.5 Monocytes from women with ovarian cancer

We have shown that monocytes isolated through elutriation at the NIH blood bank and monocytes isolated from anti-coagulated peripheral blood from healthy women from the NIH blood bank are capable of killing tumor cells (**Figure 4**). We have also shown that patients with epithelial ovarian cancer have normal populations of monocytes compared to age matched controls, and the patient monocytes are as effective as healthy age matched control monocytes in killing ovarian cancer cell lines.

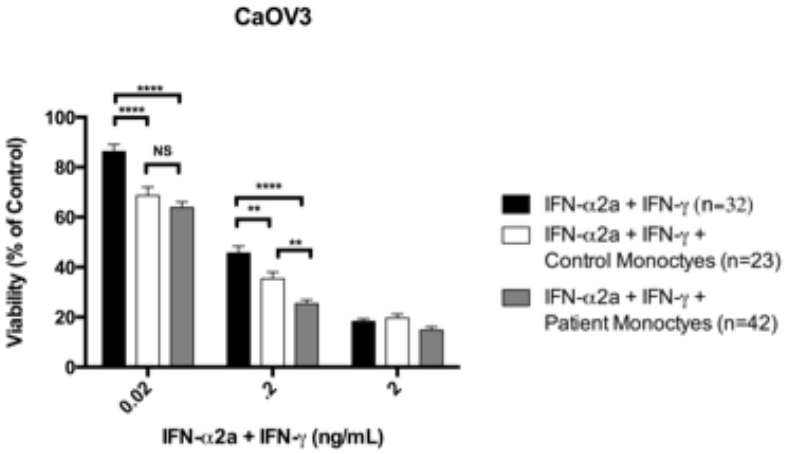


Figure 4. Monocytes from women with ovarian cancer are equally effective as healthy donors. Monocytes were isolated from women with ovarian cancer (n=42) or healthy donors (n=23). Viability of ovarian cancer cell line CaOV3 was assessed in the presence of interferons alone or in combination with monocytes from individual sources. *p<0.05, **p<0.01, ****p<0.0001.

Based upon the observations that: (1) the immune system can mount a long and durable response to tumors, (2) human IFNα-2 and IFNγ-1b and monocytes have strong anti-neoplastic response in vitro and in vivo, (3) the IP route of administration is safe, and (4) ovarian cancer is largely confined to the peritoneal cavity, it is likely that the administration of IFNs and monocytes IP will create a strong anti-tumor environment and can overcome the immunosuppressive environment of epithelial ovarian cancer. We hypothesize that the monocyte and interferon administration will be tolerable to women with relapsed ovarian cancer. If successful, this regimen can create a backbone on which to add novel agents such as TRAIL agonists or PD-L1 blockade, in order to increase immune-mediated killing of ovarian cancer.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histologically or cytologically confirmed advanced metastatic or unresectable epithelial ovarian cancer, primary peritoneal cancer or fallopian tube cancer that is relapsed and resistant (recurred less than 6 months after chemotherapy) or refractory (progressed on chemotherapy) to prior platinum- and taxane-based standard care systemic regimen. Or patients who are not eligible for additional platinum therapy. Histopathologic diagnosis must be confirmed in the Laboratory of Pathology (LP), NCI.

2.1.1.2 Patients must have measurable or evaluable disease. Measurable disease is defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan. See Section **6.3** for the evaluation of measurable disease. Evaluable disease includes CA125, ascites, peritoneal carcinomatosis, as defined in Section **6.3.1.**

2.1.1.3 Patients must be at least 4 weeks from previous therapy (chemotherapy, hormonal therapy, and radiation therapy, immunotherapy and monoclonal antibodies, alternative therapy or investigational therapeutic agents). There is no limitation on the amount of prior therapies allowed. Patients with ovarian cancer 4 weeks from previous therapy have been found to have normal monocyte function (unpublished).

2.1.1.4 Patients who have had cranial radiation therapy need to have completed it ≥8 weeks prior to enrollment.

2.1.1.5 Patients are permitted to receive investigational imaging agents while on study.

2.1.1.6 Patients who have had major surgery must be fully recovered and require a recovery period of ≥4 weeks prior to enrolling on study.

2.1.1.7 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of intraperitoneal monocytes, IFNα2 or IFNγ in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

2.1.1.8 ECOG performance status ≤1 (Karnofsky ≥70%, see **Appendix A**).

2.1.1.9 Adequate renal function, defined as serum creatinine ≤ 1.5 X upper limit of normal (ULN), or measured creatinine clearance ≥ 60 mL/min/1.73m^2.

2.1.1.10 Adequate hepatic function, defined as AST and ALT levels ≤ 3 X ULN and total bilirubin < 1.5 X ULN, unless known diagnosis of Gilbert's syndrome, where bilirubin ≤ 5 mg/dl will be permitted. Gilbert's syndrome will be defined as elevated unconjugated bilirubin, with conjugated (direct) bilirubin within the normal range and less than 20% of the total. Total bilirubin will be permitted up to 5 mg/dl, if patients have historical readings consistent with the definition of Gilbert's syndrome prior to entering study.

2.1.1.11 Adequate bone marrow function, defined as absolute neutrophil (ANC) ≥ 1,500/mm3 (≥1.5 X106/L), platelet count ≥ 75,000/mm3 (≥ 75 X106/L), and hemoglobin ≥ 8 g/dL (transfusion to obtain hemoglobin ≥ 8 g/dL is allowed).

2.1.1.12 The effects of intraperitoneal monocytes, IFNα2, and IFNγ on the developing human fetus are unknown. For this reason and because interferons based on animal data may cause fetal harm, women of child-bearing potential (excludes women with recurrent ovarian cancer) and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.

2.1.1.13 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who are receiving any other investigational agents (with exception of imaging agents as indicated above in section **2.1.1.5**).

2.1.2.2 Patients cannot have previously been treated with interferons (e.g., for chronic active hepatitis).

2.1.2.3 Lack of recovery of prior adverse events to Grade ≤1 severity (National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE] v 4.03) (except alopecia) due to therapy administered prior to the initiation of study drug dosing. Stable

persistent grade 2 peripheral neuropathy may be allowed as determined on a case-by-case basis at the discretion of the Investigator as interferon has not been shown to cause or exacerbate peripheral neuropathy.

2.1.2.4 Patients with active infection will not be eligible, but may become eligible once infection has resolved and at least 7 days have elapsed after antibiotics use was completed.

2.1.2.5 Concomitant chronic (daily or almost daily for ≥1 month prior) use of steroids or non-steroidal anti-inflammatory drugs (NSAIDS).

2.1.2.6 Patients with a recent history (within last 5 years) of autoimmune disease or inflammatory diseases will be excluded, because interferons may worsen these conditions. Exceptions will be allowed for vitiligo and hypothyroidism that has been stable on thyroid replacement medications for >6 weeks.

2.1.2.7 Impaired cardiac function or clinically significant cardiac disease including the following:

- New York Heart Association class III or IV congestive heart failure
- Myocardial infarction within the last 12 months
- Subjects known to have impaired LVEF according to institutional standards

2.1.2.8 History of allergic reactions attributed to compounds of chemical or biologic composition similar to interferons or other agents used in the study.

2.1.2.9 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations within the last 12 months that would limit compliance with study requirements. Patients with history of neuropsychiatric disorders or Major Depressive Disorder (DSM 5 definition: http://dsm.psychiatryonline.org/doi/full/10.1176/appi.books.9780890425596.dsm04) requiring medical treatment will not be eligible to enroll, based on the black box warning (SYLATRON™ (peginterferon alfa-2b) for injection, for subcutaneous use. Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Whitehouse Station, NJ). Exception to this is if patients experienced transient post-partum depression that resolved and patient has been off treatment for >10 years. Patients who are taking oral anti-depressants for normal sadness, bereavement, or grief will not be excluded.

2.1.2.10 Pregnant women are excluded from this study because interferons based on animal data may cause fetal harm. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with interferons, breastfeeding should be discontinued if the mother is treated with intraperitoneal interferons. These potential risks may also apply to other agents used in this study.

2.1.2.11 Patients on combination antiretroviral therapy for the treatment of HIV are ineligible because of the potential for pharmacokinetic interactions with interferons alfa and gamma.

2.1.2.12 Patients receiving any medications or substances that are potent inhibitors or inducers of CYP1A2 or CYP2D6 are ineligible. Lists including such medications and substances are provided in **Appendix C**.

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk pre-screening activities that may be performed before the subject has signed a consent for this study include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

A waiver of consent for these activities has been requested in section **12.6.1**.

2.2.2 Screening activities performed after a consent for screening has been signed (Registration Part 1)

The following activities will be performed only after the subject has signed the consent for this study for screening. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent. For baseline evaluations, please see section **2.5**.

Screening Assessments – Part 1 The following screening activities to determine eligibility may be performed any time prior to Registration Part 2.
Medical history, including age, performance status, and dates of prior treatment regimens.
Confirmation of tumor histology in NCI Laboratory of Pathology (Archival tumor samples will be requested; if unavailable, the subject may choose to undergo fresh biopsy)
Assessment of disease by physical exam and imaging

Screening Assessments – Part 1 The following screening activities to determine eligibility may be performed any time prior to Registration Part 2.
(may include CT chest, abdomen and pelvis or MRI; report from outside institution is acceptable).

Screening Assessments – Part 2	
Once patient eligibility has been established according to the results of the Screening Assessments Part 1 above, the following Screening Part 2 activities will be performed to determine final patient eligibility prior to Registration Part 2. Scans and x-rays must be done within 28 days (- 7 days) prior to the initiation of study therapy. All other studies should be performed within 7 days prior to initiation of study therapy, unless otherwise specified.	
Assessment	**Timeframe (calendar days)**
	Within 7 days prior to treatment (unless otherwise noted)
History and physical examination	X
Assessment of disease by imaging (may include CT chest, abdomen and pelvis)	Within 28 days prior to treatment
Ophthalmic evaluation including visual acuity and funduscopy performed by an ophthalmologist	Within 180 days (-14 days) prior to treatment
Assessment of venous access in Department of Transfusion Medicine	Anytime prior to apheresis
Intraperitoneal catheter placement in Interventional Radiology, if not already placed OR surgical implantation of intraperitoneal port by Gynecologic Oncology if not already placed OR verification by clinicians of functional IP catheter or IP port (if pre-existing)	X
CBC with differential and platelet count	X
Metabolic panel (magnesium, calcium, albumin, phosphorus)	X
Acute care panel (electrolytes, glucose, BUN, creatinine)	X

Assessment	Timeframe (calendar days)
	Within 7 days prior to treatment (unless otherwise noted)
Hepatic function tests (AST/ALT/total bilirubin)	X
CA125	X
β-HCG in women of childbearing potential	X

2.3 REGISTRATION PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) may be rescreened at the discretion of the PI. Rescreened participants should be assigned a different participant number as for the initial screening.

2.4 TREATMENT ASSIGNMENT AND RANDOMIZATION/STRATIFICATION PROCEDURES

Cohorts

Number	Name	Description
ES1	*Phase I, Dose Escalation Cohort 1*	*Patients with epithelial ovarian cancer, primary peritoneal cancer or fallopian tube cancer that is relapsed and platinum resistant or refractory*
EX1	*Dose Expansion Cohort 1*	*10 additional patients will be treated at the MTD*

Arms

Number	Name	Description

ES1	*Phase 1, Dose Escalation Arm*	*The autologous monocytes cell product in 250ml total volume of investigational combination (monocytes + ACTIMMUNE + SYLATRON) is infused via the intraperitoneal catheter or port over 30-60 min +/- 10 min every 28 days +/- 7 days until disease progression, limiting toxicity, intercurrent medical issues, or patient withdrawal.*
EX1	*Dose Expansion Arm*	*10 additional patients will be treated at the MTD, defined as Dose Level 4: autologous monocytes cell product in 250ml total volume of investigational combination: Monocytes (*750 x $10^6 \pm 20\%$) + *ACTIMMUNE* 50 mcg (0.2 µg/mL) + *SYLATRON* 250 mcg (1 µg/mL) *is infused via the intraperitoneal catheter or port over 30-60 min +/- 10 min every 28 days +/- 7 days until disease progression, limiting toxicity, intercurrent medical issues, or patient withdrawal.*

Randomization and Arm Assignment

Randomization and stratification procedures do not apply to this trial.

Patients in Cohort ES1 will be directly assigned to Arm ES1.

Patients in Cohort EX1 will be directly assigned to Arm EX1.

2.5 BASELINE EVALUATION

Scans and x-rays must be done within 28 days (- 7 days) prior to the initiation of study therapy. All other studies should be performed within 7 days prior to initiation of study therapy, unless otherwise specified. If the activities performed during Screening Part 2 fall within these designated timeframes, they will not need to be repeated at baseline.

Baseline Assessments	**Timeframe (calendar days)**
	Within 7 days prior to treatment (unless otherwise noted)
History and physical examination	X
Assessment of disease by imaging (may include CT chest, abdomen and pelvis)	Within 28 days prior to treatment
Assessment of venous access in Department of Transfusion Medicine	Anytime prior to apheresis
CBC with differential and platelet count	X
Metabolic panel (magnesium, calcium, albumin, phosphorus)	X

Baseline Assessments	**Timeframe (calendar days)**
	Within 7 days prior to treatment (unless otherwise noted)
Acute care panel (electrolytes, glucose, BUN, creatinine)	X
Hepatic function tests (AST/ALT/total bilirubin)	X
CA125	X
β-HCG in women of childbearing potential	X

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a single arm dose escalation study including an expansion cohort at the MTD.

A 3+3 phase I trial of monocytes with Sylatron® (Peginterferon alfa-2b) and Actimmune® (Interferon gamma-1b) dose escalation will proceed to determine the MTD. The dose-limiting toxicity (DLT) period will be during the first cycle (28 days). For each dose level, three patients will be enrolled. There will be a 2 week wait from the time of cell infusion from one patient until apheresis and cell infusion of the next patient. If any patient assigned to a particular dose level experiences DLT, then up to three additional patients (up to a total of six patients) will be enrolled at that dose level. All patients on a dose-level must complete the 28-day cycle prior to beginning enrollment on the next dose level. If any patient experiences DLT-like adverse events related to experimental agents or study procedures in subsequent cycles, enrollment will be placed on hold until evaluated by the IRB and FDA.

Following Registration Part 2, patients will have intravenous access assessment in the Department of Transfusion Medicine (DTM) Dowling Clinic and a tunneled IP catheter will be placed in interventional radiology (IR) using ultrasound guidance. The tunneled IP catheter will be placed prior to start of the protocol therapy. If the patient already has an existing IP port for prior IP chemotherapy, this can be used for IP infusion, and a new tunneled catheter will not be placed. The patient will also be given the option to have an IP port placed instead of a tunneled catheter. If she prefers the IP port, she will be evaluated and if the procedure is deemed safe and feasible, it will be performed using direct visualization in the operating room (laparascopic camera). Research blood will be collected, and ascites will be collected if present. In the expansion cohort, solid tumor will be biopsied if amenable at the time of catheter or port placement. The purpose of the biopsy is to assess monocyte and other immune cell infiltration and differentiation related to treatment.

On Day 0, the patient will undergo intravenous line placement and apheresis in DTM. PBMCs will be stored according to standard operating procedures of DTM (**Appendix B**). For patients on DL1 and DL3b, there will be no Day 0 in the calendar as they will not be apheresed.

On Day 1, monocytes will be elutriated, quantified, and mixed with interferons. The final product will be infused in the inpatient setting through the IP catheter/port, as per standard operating procedure described in **Appendix D**. Patient will be observed overnight for adverse events.

Patient will be discharged on Day 2, if well, with IV lines removed. IP catheter can be left in place or removed at physician discretion. Cycle will be repeated every 28 days. A new product will be manufactured for each treatment cycle.

In the expansion cohort, solid tumor will be biopsied again, if amenable, on C2D1 and at the time of disease progression. The purpose of these biopsies is to assess monocyte and other immune cell infiltration and differentiation related to treatment.

After 2 cycles, the patient may be treated as an outpatient per physician discretion.

Imaging will be performed at baseline and every 2 cycles.

3.1.1 Dose Limiting Toxicity

Dose-limiting toxicity (DLT) evaluation period is the first cycle. DLT is defined as:

- ≥Grade 3 fatigue (defined as fatigue not relieved by rest, limiting self-care ADLs) lasting > 7 days
- ≥Grade 3 cytopenias lasting >7 days
- ≥Grade 3 transaminitis lasting >7 days
- ≥Grade 3 depression lasting >7 days
- ≥Grade 3 abdominal pain lasting >7 days
- Any other grade ≥ 3 toxicity lasting >72 hours despite maximal medical intervention

3.1.2 Dose Escalation

Patients who do not complete treatment in cycle 1 for reasons other than study drug-related toxicity will be replaced. If 1 of 3 patients experiences DLT, the dose level will be expanded to 6 patients. If 2 patients in a dose level of 3 to 6 patients experience DLT, then the MTD will have been exceeded and the next lower dose level will be expanded to 6 patients. MTD will be the highest dose at which 0-1 of 6 patients experience DLT. If the planned highest dose level is reached and determined to be safe after expansion to 6 patients, it will be considered the MTD. An expansion cohort of 10 additional patients will be treated at the MTD and the results combined with those of patients at the MTD to obtain improved estimates of safety and toxicity as well as to perform analyses to address secondary objectives. If either dose level 3 or dose level 4 exceeds the MTD (≥2 patients on either dose level experience a DLT) then we will enroll 3-6 patients on dose level 3b.

Total Volume (250 mL)

Dose Level	**Monocytes total number**	**SYLATRON ® Peginterferon alfa2b, mcg (final concentration)**	**ACTIMMUNE ® Interferon gamma-1b, mcg (final concentration)**
1	0	25 mcg (0.1 µg/mL)	5 mcg (0.02 µg/mL)

2	75 x 10^6 ± 20%	25 mcg (0.1 µg/mL)	5 mcg (0.02 µg/mL)
3	750 x 10^6 ± 20%	25 mcg (0.1 µg/mL)	5 mcg (0.02 µg/mL)
3b*	0	250 mcg (1 µg/mL)	50 mcg (0.2 µg/mL)
4	750 x 10^6 ± 20%	250 mcg (1 µg/mL)	50 mcg (0.2 µg/mL)

*DL3b will enroll if ≥2 subjects enrolled on DL3 or DL4 experience DLT

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter up to 3 patients at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter up to 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. UP to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

3.1.3 Maximum Tolerated Dose (MTD)

The Dose Escalation Phase has concluded, and the MTD is defined as Dose Level 4 (Amendment I, version date 02/11/2020).

3.2 DRUG ADMINISTRATION

Monocytes are collected, elutriated and mixed with interferons according to **Appendix B**.

The cell product is delivered to the inpatient ward, and infused within 4 hours after preparation. The cell product in 250 mL is infused via the intraperitoneal catheter/port over 30-60 min +/- 10 min.

After 2 cycles, per physician discretion, the infusion can be given as an outpatient in the day hospital/infusion center.

The administration is repeated every 28 days +/- 7 days to accommodate for holidays, inclement weather, or other scheduling conflicts.

Interactions between interferon gamma-1b and other drugs have not been fully evaluated. Caution should be exercised when administering interferon gamma-1b in combination with other potentially myelosuppressive agent. Preclinical studies in rodents using species-specific interferon-gamma have demonstrated a decrease in hepatic microsomal cytochrome P-450 concentrations. This could potentially lead to a depression of the hepatic metabolism of certain drugs that utilize this degradative pathway.

Peginterferon alfa-2b inhibits CYP1A2 and CYP2D6 activity. Monitor for potential increased toxicities of drugs metabolized by CYP1A2 and/or CYP2D6 with a low therapeutic index or a narrow therapeutic range when they are administered concomitantly with peginterferon alfa-2b.

Patients will continue therapy until documentation of progressive disease, limiting toxicity, intercurrent medical issues, or patient withdrawal

Participants will be contacted remotely or assessed in person at least once weekly during the first cycle of treatment. Subsequent follow up will be at least monthly, with the start of each new cycle, and then during follow up according to the defined schedule (see Study Calendar, Section **3.4**).

Pre-cycle and off-treatment AE assessments will be done at a scheduled clinic visit. Post-therapy follow-up assessments will be performed within 30 days of stopping study treatment over the phone or at a clinic visit.

3.3 DOSE MODIFICATIONS

3.3.1 General Management of Toxicities

- In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).
- Once a patient has a dose reduction for toxicity, the dose will not be increased.
- Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.
- For AEs that are unrelated to the study drugs, study drug may be held for up to 14 days at the discretion of the PI.

3.3.2 General Recommendations for Dose Modification and Management of Hematologic Adverse Events occurring after the DLT period

Table 2. Dose Modification and Management of Hematologic Adverse Events

Observation	Action

Absolute neutrophil count (ANC) ≥ 1000/mcL **AND** Platelets ≥ 75,000/mcL **AND** Hemoglobin ≥ 8 g/dL	No interruption.
ANC < 1000/mcL **OR** Platelets < 75,000/mcL **OR** Hemoglobin < 8 g/dL	*On first occurrence*, hold treatment for up to 14 days until ANC ≥ 1000/mcL, platelets ≥ 75,000/mcL, and hemoglobin ≥ 8 g/dL. Initiate appropriate medical therapy (per Section **3.3.2.1**) and no change in dose upon re-initiation. *On second occurrence*, hold treatment for up to 14 days until ANC ≥ 1000/mcL, platelets ≥ 75,000/mcL, and hemoglobin ≥ 8 g/dL. Initiate appropriate medical therapy (per Section **3.3.2.1**). Treatment may be restarted at one dose level (DL) lower for the drug(s) causing the toxicity. Patients whose counts have not recovered to ANC ≥ 1000/mcL, platelets ≥ 75,000/mcL, and hemoglobin ≥ 8 g/dL after 14 days should be removed from treatment.
Grade 4 hematologic AE	*On first occurrence,* hold treatment for up to 14 days until ANC ≥ 1000/mcL, platelets ≥ 75,000/mcL, and hemoglobin ≥ 8 g/dL. Initiate appropriate medical therapy (per Section **3.3.2.1**). Treatment may be restarted at one DL lower for the drug(s) causing the toxicity. *On second occurrence,* discontinue all three experimental agents and follow the patient for adverse events as described in section **3.5** until resolution/stabilization of toxicity.

3.3.2.1 Hematologic toxicities

- Treatment may be delayed for a maximum of 2 weeks after holding the treatment for toxicities that develop and do not resolve as defined above (exemptions: lymphopenia, or leukopenia in the absence of grade 3 or higher neutropenia).

3.3.2.2 Neutropenia

- Growth factors to prevent neutropenia will not be administered prophylactically, but can be used to assist the recovery.
- Filgrastim/Pegfilgrastim will be used only if the patient has neutropenia with sepsis for the purpose of facilitating recovery of the neutropenic sepsis.

3.3.2.3 Thrombocytopenia

- Thrombocytopenia will be treated conservatively. In the absence of bleeding, or a necessary invasive procedure, platelet transfusions should be given for a platelet count ≤ 10,000/mcL.
- If invasive procedure(s) is (are) planned, or the patient develops bleeding, platelet transfusions should be administered in accordance with the standard of practice, usually maintaining a platelet count above 50,000/mcL.

3.3.2.4 Anemia

- Symptomatic anemia should be treated with red blood cell transfusion and is recommended if the hemoglobin falls below 8 g/dL or the patient is symptomatic.
- The initiation of erythropoietic therapy for the management of chemotherapy-induced anemia follows the American Society of Hematology/ASCO clinical practice guidelines (http://www.asco.org).

3.3.3 General Recommendations for Dose Modification and Management of Non-Hematologic Adverse Events

- The management of general AEs not otherwise specified should be as per **Table 3**.
- At the discretion of the investigator, the study drugs or monocytes may be held or dose modified independently if the observed toxicity is attributed to only one of the agents, while the patient continues to receive the agent(s) not associated with the observed toxicity.
- Dose modifications for nausea, vomiting, and diarrhea will be made only if they are refractory to treatment. The time a given drug is held should not exceed 14 days.
- Asymptomatic electrolyte abnormalities with optimal repletion will not require dose reduction if resolution to grade 1 or less is documented within 72 hours.

Table 3. General Management of Adverse Events (Non-Hematologic)

Observation	Action
Grade 1 or 2 AEs resolves promptly (within 48 hours) with supportive care	Maintain dose level (DL)
Any ≥ grade 3 non-hematologic >72h (excluding those mentioned in Section **3.1.1**, Dosing Limiting Toxicity)	*During DLT evaluation period:* Patient will be removed from treatment *At any other time* Hold study regimen for up to 14 days until toxicity resolves to ≤ grade 1. Treatment may be restarted at one DL lower according to PI discretion.

Any grade 2 non-hematologic AE lasting > 7 days despite maximal support.	Hold study regimen for up to 14 days until toxicity resolves to ≤ grade 1. Treatment may be restarted at one DL lower according to PI discretion Patients whose toxicity has not resolved after 14 days will be removed from treatment.

3.3.4 Safety Protocol Stopping Rules

The study may be halted if any of the following safety conditions are met:

1. If one or more deaths occur, we will immediately stop accrual and we will promptly discuss this with the NCI IRB and the FDA. In addition, the study will be halted if any death occurs within 30 days of treatment regimen.
2. If 2 DLTs occur on dose level 1.

3.4 STUDY CALENDAR

Baseline evaluations are to be conducted within 7 days prior to start of protocol therapy, unless otherwise specified. Scans and x-rays must be done within 28 days (- 7 days) prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

1 cycle = 28 days

		Cycle 1[1]						Cycle 2		Subsequent cycles	Post Therapy Follow-up[2]
Procedure	**Screening/ Baseline[15, 17]**	**Day -2 or -1**	**Day 0[5]**	**Day 1[14]**	**Day 2[12]**	**Day 27[4]**		**Day 1**	**Day 2[12]**	**D1**	
Clinical Assessment	X									X	X
Physical Exam		X		X	X	X		X	X	X	X
Vital signs	X	X	X	X	X	X		X	X	**X**	X
Performance Score	X	X				X				**X**	X
Offer NIH Advanced Directives Form[10]	X										
Ophthalmic evaluation[6]	X										
Tunneled IP catheter or IP port placement[7]	X										
Venous access assessment	X										
Establish venous access prior to each apheresis			X							X	
Apheresis[13]			X							X	
Autologous monocytes mixed with Sylatron® (Interferon Alfa-2b) and Actimmune® (Interferon Gamma-1b), IP infusion				X				X		X	
Labs (See Section **2.2** and **2.5**)[11]	X	X		X	X	X		X	X	X	X

Research blood collection [8]		X			X	X[3]			X	X[3]	
Ascites collection[8]		X				X[3]					
Biopsy (expansion) [8]		X				X		X			X
Radiological Assessments (may include CT-C/A/P or MRI; report from outside institution is acceptable)[16]	X										
Restaging imaging[9]			Every 2 cycles								X
Adverse Events		X →									X
Concomitant Medications		X →									X

[1]Weekly phone calls will be made during cycle 1.

[2]Post therapy follow up will be at one month following the last dose of treatment; however, AEs due to toxicity or research will be followed until resolution to ≤ grade 1 . If the patient cannot return to the Clinical Center for this visit, a request will be made to collect required clinical labs (specify as needed) from a local physician or laboratory. If this is not possible, patients may be assessed by telephone for symptoms. If patient has begun another treatment regimen or palliative care, follow up may be done by telephone, and exam/labs will be omitted.

[3]Must be done prior to administration of study agents.

[4]Flexibility +/- 7 days permitted for Days 27-28 of each cycle.

[5] Day 0 procedures do not apply for patients in the Dose Level 1 or Dose Level 3b cohort [6]Ophthalmic evaluation including visual acuity and funduscopy by an ophthalmologist within 180 days (-14 days) prior to treatment.

[7] IP catheter or port can be placed at any time prior to the start of protocol therapy. If patient has pre-existing IP catheter or IP port, this will be assessed by the clinician for determination of function within the same time frame as other baseline studies. See Section **3.1** for additional information.

[8] Research blood, ascites and biopsy testing will be performed in the NIH laboratory, Building 10, RM 4B55. Phone: 617-905-0922.

[9] Restaging should be performed within +/- 7 days of completion of previous cycle.

[10] As indicated in section **12.3**, all subjects ≥ age 18 will be offered the opportunity to complete an NIH advanced directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

[11] CA125 lab will be drawn at the beginning of each cycle. Day 2 labs will include only CBC with differential and platelet count, unless other labs are clinically indicated.

[12] Day 2 activities apply to the first two cycles only.

[13] Apheresis collection will be done every 1-3 months provided there is an adequate monocyte population at the time of apheresis collection. If the apheresis collection is inadequate, apheresis can be done as frequently as each cycle to obtain the desired number of monocytes.

[14] Do not repeat labs if already done within 1 week.

[15] Performance score (ECOG) will be performed at the beginning of each cycle.

[16] MRI isperformed only as clinically indicated if patient cannot tolerate CT scan with IV contrast.

[17] Screening and Baseline activities will be performed according to Sections **2.2** and **2.5** respectively.

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.5.1 Criteria for removal from protocol therapy

- Progressive disease
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined in Section **3.1**
- Investigator discretion
- Positive pregnancy test
- Procedure failure (incapacity to do apheresis or obtain intraperitoneal access)
- Lost to follow-up
- Intercurrent illness

3.5.2 Off-Study Criteria

- Completed study follow-up period
- Participant requests to be withdrawn from study
- Death
- Starting alternative therapy
- Refused follow-up
- Procedure failure
- Screen failure
- Lost to follow-up

3.5.2.1 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for two scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant within 72 business hours in order to reschedule the missed visit and will counsel the participant on the importance of

maintaining the assigned visit schedule and ascertain if the participant wishes to and/or is eligible to continue in the study.

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 PROHIBITED MEDICATIONS

- No concomitant use of alternative, complementary therapies or over-the-counter agents will be allowed without approval of the PI. All medications must be recorded in the case report form and be reviewed by the treating physician at each visit.
- Oral anticoagulants such as warfarin are not contraindicated provided that there is increased vigilance with respect to monitoring INR. If medically appropriate, low molecular weight heparin may be considered preferable to warfarin, due to shorter half-life and more predictable anticoagulant effect.
- Caution should be exercised in the concomitant use of any medication that may markedly affect renal or hepatic function. Such medications may be used with caution as deemed essential for treatment, or if already in use prior to entry in the study without any effect on renal or hepatic function.
- Potent inhibitors or inducers of CYP1A2 or CYP2D6 (wash out period 7 days) – see **Appendix C**.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

5.1.1 Peripheral blood and ascites

Blood will be acquired to study both the functionality of the patient's monocytes (*in vitro* killing assay), and to analyze the peripheral blood mononuclear cell (PBMC) compartment. 20 mL of whole blood will be collected into 10 mL EDTA lavender-top tubes. Blood will be diluted 1:1 in RPMI and placed on a Ficoll density gradient, and the PBMC buffy coat will be removed. Further isolation of monocytes will be performed using negative magnetic bead based isolation, which will result in negatively isolated (untouched) monocytes. Based on our experiences isolating monocytes from the peripheral blood of patients with ovarian cancer (20 mL), we usually isolated between 2-4 million monocytes. 1.7 million monocytes are needed to assay them for cytotoxicity of target ovarian cancer cell lines (CaOV3 and OVCAR5) at four different concentrations of Sylatron® (Peginterferon alfa-2b) and Actimmune® (Interferon gamma-1b).

Another 20 mL of whole blood will be collected for the immune-phenotyping of the patients circulating immune system and PBMC will be isolated as stated above. Plasma will be stored for cytokine analysis and interferon antibodies. In our previous studies 20 mL of peripheral blood resulted in a range of 13 million to 27 million total PBMC. Using multi-parameter flow

cytometry (FC) we will analyze T cell subsets, B cell subsets, myeloid compartment (macrophages, monocytes, dendritic cells), and NK cells (**Table 4**). We will use 5 separate panels with 10-12 antibodies per panel, with special emphasis on the detection of molecules associated with checkpoint blockade (PD1, PD2, PDL1, PDL2, CTLA4) to establish hypotheses for future clinical trials. In total, the panels will allow for the analysis of 137 distinct populations of immune cells. Blood from healthy donors acquired from the DTM will also be analyzed. The analysis of PBMC from healthy controls will allow comparisons between women affected with ovarian cancer and those without cancer. Using fluorescence minus one (FMO) gating strategy we will quantify the number of background events counted. These events will be subtracted from the total events counted to produce a quantification of true events. Measurement of 100,000 events per sample will enable us to perform statistical comparison of changes between healthy and affected, and changes within patients due to treatment. Counting a large number of events and using healthy age matched controls will allow us to statistically determine if there is a significant change between healthy donors, treatment groups, and between patients. When available, cells from ascites will be analyzed similarly.

Table 4. Cell Populations Analyzed by Flow Cytometry

Cell Type	Subsets
T cell	CD4, CD8, naïve, central memory, peripheral memory, effector, regulatory
B Cell	Naïve, memory, plasma
Monocyte	Classical, intermediate, non-classical
Dendritic Cell	cDC1, cDC2, pDC
Macrophages	M1, M2
NK Cell	Cytokine producers, cytotoxic

Excess apheresis fractions and ascites (if available) will be viably frozen for future functional studies.

Testing will be performed in the NIH laboratory, Building 10, RM 4B55. Specimen processing and storage will be performed by the Blood Processing Core (BPC). See Section **5.2**.

5.1.2 Plasma

5.1.2.1 Anti-interferon antibodies

At baseline and at the last cycle on study, patients will be monitored for the development of antibodies against interferons. Although IFNα and IFNγ are recombinant human proteins, repeated administration of these drugs can result in the development of antibodies against them [37, 38]. We will use two assays in our study to measure in participants the amount of: (a) binding antibodies to Sylatron® (Peginterferon alfa-2b) and Actimmune® (Interferon gamma-

1b), and (b) neutralizing antibodies to both IFNs (antibodies that block the antiviral and antiproliferative function of the IFNs).

We will assay for IFN binding antibodies using a high-throughput multiplex assay [39]modified ELISA technique [38]. Briefly, plasma will be diluted 1:100 in PBS and incubated for 30 minutes with differentially fluorescing magnetic beads that have been covalently coupled to 2.5ug recombinant human IFNa (PBL Biomedical Laboratories; catalog 11101-2) or IFNg (R&D Systems; catalog 285-IF-100/CF). Beads will be washed then incubated with 1ug/mL PE-labeled goat anti-human IgG (eBioscience; catalog 12-4998) for an additional 30 minutes before being washed again and run in a multiplex assay on the BioPlex X200 instrument (BioRad). Serum and ascitic fluid isolated from the patients and serum isolated from healthy age matched controls will be added and serially diluted as a negative control. For positive controls antibodies (WHO reference reagents) to IFNα-2b and IFNγ-1b will be used.

To detect IFN neutralizing antibody, plasma or ascites (when present) samples which are positive for IFN binding antibodies will be mixed with serial dilutions of Sylatron or Actimmune and then added to 96-well tissue culture plates seeded with $1x10^4$ target cells (immortalized lung epithelial alveolar cells A549) [37]. WHO IFN international standards for IFNs α and γ and WHO neutralizing IFN antibody reference reagents will be used as controls. After 15-24 h incubation, encephalomyocarditis virus (EMCV) will be added to the cultures and incubated for an additional 24 h. If IFNs are present the virus will be unable to replicate allowing the cells to grow. If IFNs are not present (neutralized by antibody) the EMCV will kill the target cells. Percent cell viability of cell control can then be correlated to the WHO IFN international standards to calculate how much neutralizing antibody is present.

5.1.2.2 Multiplex Arrays

Plasma will be used for analysis of circulating cytokines. We will use multiplex cytokine arrays to analyze fluids compartment of blood and ascites. Multiplex allows for the analysis of up to 45 proteins including cytokines, chemokines, and growth factors with relatively small volumes of samples. We will use the Human ProcartaPlex Human Panel from eBioscience, or similar product.

Human Cytokine/ Chemokine/ Growth Factor Panel 1 (45 plex)	EPX450-12171-901	BDNF, EGF, Eotaxin (CCL11), FGF-2 (FGF basic), GM-CSF, GROα (CXCL1), HGF, IFNγ, IFNα, IL-1RA, IL-1β, IL-1α, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-9, IL-10, IL-12 p70, IL-13, IL-15, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, IP-10 (CXCL10), LIF, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, βNGF, PDGF-BB, PLGF, RANTES/CCL5, SCF, SDF1α/CXCL12, TNFα, TNFβ/LTA, VEGF-A, VEGF-D

5.1.3 Tissue biopsies (expansion phase)

Biopsies will be requested from all study participants in the expansion phase and will be obtained if clinically feasible. Tumor amenable to biopsy is not required for enrollment. If determined to be amenable to biopsy, however, tissue will be harvested by 18g core needle by an interventional radiologist. Up to 4 cores will be stored – 2 will be frozen immediately in OCT medium, and 2 will be formalin fixed and paraffin embedded. An amendment to this protocol (Amendment I, version date 02/11/2020) was submitted for Radiation Safety Committee and IRB approval prior to implementation of the expansion phase.

5.1.3.1 Multiparameter Microscopy

Correlative studies will include evaluation for in vivo evidence that interferons promote differentiation of monocytes to M1 macrophages resulting in tumor infiltration and death. Current advances in histology have enabled us to analyze up to 14 different proteins on a single histology slide. This technology can be used to study different cell populations and their interactions on the same section of tissue, instead of protein expression on serial sections. We will section biopsies and stain for: CD68, IL-12, CXCL10, NOS2, IL-10, and Arginase-1 (macrophage/monocyte); TRAIL, CD3, CD4, CD8 (T cell markers); FoxP3, PDL1, and PDL2 (Lymphocyte Inhibitory Markers). This analysis will allow us to quantify the presence and phenotype of immune cells in tissue before and after exposure to study agents.

5.1.4 Sampling Schedule

Test/assay	**Volume blood (approx) or ascites**	**Type of tube**	**Collection point (+/- 48hrs)**	**Location of specimen analysis**
Cellular component:	40 mL	EDTA * (10 mL tubes x 3)	D2 (cycles 1 and 2 only), Final visit	Women's Malignancies Branch (WMB; formerly Dr. Annunziata's lab)
Plasma or ascites fluid: Anti-IFN antibodies	3 mL	EDTA ** (from above tubes)	C1D1, Final Visit	Dr. Steve Holland's lab, NIAID
Multiplex cytokine arrays	10 mL	(from above tubes)	D1 each cycle	FNLCR clinical services program
Biopsy (expansion cohort only)	NA	NA	D1 cycles 1 and 2, and at progression	WMB (formerly Dr. Annunziata's lab)

* first 2 cycles, collect 30 mL (3 x 10-mL tubes)
** subsequent cycles, collect 1 x 10-mL tube only

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Research samples will be obtained from patients, as described in the protocol. Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

5.2.1 Figg Lab Blood Processing Core (BPC)

5.2.1.1 Sample Collection

Samples will be collected as described above. Record the date and exact time of draw on the tube. Please page 102-11964 (Figg lab) for sample pick-up.

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

5.2.1.2 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.2.1.3 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All

researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.2.2 NCI-Frederick Leidos Biomedical, Inc. Laboratory

Blood and tissue collected during the course of this study will follow storage, handling and labeling procedures to ensure that security, confidentiality and sample integrity are maintained. All samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Thus, samples will be coded and linked, with access to personal data restricted to the study investigators.

All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in a locked freezer at -70°C according to stability requirements. These freezers are located offsite at NCI-Frederick, at the Leidos Biomedical, Inc. Lab in Frederick, MD. Samples will be stored until requested by a researcher named on the protocol. All use and requests for use will be recorded by the Leidos Biomedical, Inc. Lab. Any unused samples must be returned.

Some samples as indicated below may be stored in monitored freezers/refrigerators in the investigator's laboratory at specified temperatures with alarm systems in place.

At the termination of this protocol, samples will remain in storage as detailed above. If additional studies are to be performed on any samples retaining patient identifiers, obtained during the conduct of this trial, a Request to Conduct Research for Stored Human Samples Specimens, or Data Collected in a Terminated NIH-IRB Protocol will be submitted. Otherwise, access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material. If specimens are to be discarded at any point, they will be disposed of in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland.

5.2.3 Women's Malignancies Branch (formerly Annunziata Laboratory)

Barcoded samples will be processed and stored by the Blood Processing Core (BPC) immediately after collection, and will remain with the BPC until requested for research use by the laboratory for planned analyses.

5.2.4 Holland Laboratory, NIAID

Access to patient identifying information will be restricted to appropriately trained members of the lab; samples will be deidentified by the lab before transfer to the Holland laboratory. Samples will be processed immediately by the Holland laboratory using validated SOPs in order to ensure specimen quality. All laboratory personnel will be trained to adhere to SOPs and will be

monitored for high-quality performance. Using a computerized inventory system and a backup hardcopy process, all specimen collection and processing steps will be documented and the specific location of each specimen will be tracked. Each new specimen collected will be assigned a unique barcode identifier that can be linked to the original specimen collected and other relevant information within the inventory system. After processing, samples will not be stored in the Holland lab, but will be transferred back to the Women's Malignancies Branch (WMB) for storage.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events will be collected as required in accordance with FDA regulations.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Document AEs from the first study intervention, Study Day 0, through 30 day*s* after the agent/intervention was last administered. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Coded, linked or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository: clinicaltrials.gov.
- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.3 RESPONSE CRITERIA

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

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)[40]. 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.

6.3.1 Definitions

Evaluable for toxicity: All patients who signed consent will be evaluable.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray$\geq$20 mm
- By CT scan:

  - Scan slice thickness 5 mm or under: as ≥10 mm
  - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam ≥ 10 mm

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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

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

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.3 Methods for Evaluation of Measurable Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. 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.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

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 patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [41-43]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [44].

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.

6.3.4 Response Criteria

6.3.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the

smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

6.3.4.2 Evaluation of Non-Target Lesions

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

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

6.3.4.3 Evaluation of Best Overall Response

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

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	

SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

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

6.3.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.3.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.4.2 Data Safety Monitoring Board (DSMB)

This protocol does not require monitoring by a DSMB.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore 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 related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

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

- Death,
- A life-threatening adverse event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.

- Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.1.6 Adverse Events of Special Interest (AESI)

Not applicable.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4**.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Waiver of expedited reporting to CCR

Not applicable.

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

8.5.1 Horizon Pharma, Inc.

All events listed below must be reported in the defined timelines to OSROSafety@mail.nih.gov.

OSRO will send all reports to the manufacturer as described below.

8.5.1.1 To be reported by the Office of Sponsor and Regulatory Oversight (OSRO)

Copies of all safety reports will be sent to Horizon concurrently with their submission to the FDA, along with any other information affecting patient safety, including the following:

- Information on any pregnancy and its outcome occurring in a subject participating in the Study within two working days after becoming aware of the information.

8.5.1.2 To be reported by study team

- Any premature termination of the study for safety reasons;
- Any urgent safety measure (e.g. stop/hold recruitment);
- Any amendment of the protocol for safety reasons; and
- Any communication of safety concern from competent authorities and a Data Safety Monitoring Board (DSMB), if applicable.

All safety communication to Horizon will be sent to:

Email: AdverseEvents@horizonpharma.com

Fax: 1-800-860-7836

8.5.2 Merck Sharp & Dohme Corp.

All safety communications will be sent to Merck within two business days of but not longer than three calendar days of receipt of the information, regardless of whether or not a causal relationship with the Merck product exists. All FDA communications, including annual reports, will be sent to Merck concurrently with their submission to the FDA, along with any other information affecting patient safety, including the following:

8.5.2.1 To be reported by the Office of Sponsor and Regulatory Oversight (OSRO)

All events listed below must be reported in the defined timelines to OSROSafety@mail.nih.gov.

The OSRO will send all reports to the manufacturer as described below.

- All reports of Merck study drug exposure during pregnancy or lactation (including a female partner of a male study subject, whether associated with an AE or not) within the same timeline for an SAE. The Principal Investigator will follow to term to obtain the outcome of the pregnancy, which will also be reported to Merck.

8.5.2.2 To be reported by study team

- Notification of a possible defect in the Merck product within one business day of first becoming aware of the possible defect.
- Notification within 24 hours of first becoming aware when the FDA or any other regulatory authority notifies the site of a pending inspection.

8.5.2.3 All safety communication to Merck will be sent to:

Email: aer_mailbox@merck.com

Fax: 215-661-6229

8.6 REPORTING PREGNANCY

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.6.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known.

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.6.2 Paternal exposure

Not applicable to this trial, as men are not included in the patient population.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in expedited manner to the FDA in accordance to 21 CFR 312.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING PLAN

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6, and SOPs; and human subjects' protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 STATISTICAL CONSIDERATIONS

Patients will be accrued in standard 3+3 dose escalation design, with expansion to 6 patients at the MTD and 10 additional patients at the MTD to allow for sufficient patients to perform correlative studies.

The 16 patients treated at the MTD (6 from dose escalation /MTD determination plus 10 additional patients) will be used to evaluate biologic correlates. With 16 patients, there would be 80% power to detect a change (either actual change or fold/relative change as appropriate) in the number of activated monocytes from baseline to after one cycle of treatment which would be equal to ¾ of a standard deviation of the difference measured (effect size 0.75) with a two-tailed 0.05 significance level paired t-test. If there are usable paired results from 10 patients instead of 16, this would result in the ability to detect a change with a 1.0 effect size based on the same parameters. In addition, a large number of other parameters will be obtained via flow cytometry and the changes from baseline to after one cycle of therapy will be determined. Given the large number of possible tests performed and the exploratory nature of the evaluation, there will not be any formal adjustment for multiple comparisons for these parameters. However, with 16 patients, there would be 80% power for any of the tests comparing baseline to post-treatment to detect a one SD difference between the two time points (effect size 1.0) with a two-tailed 0.01 significance level paired t-test; if there are 10 patients, there would be 80% power to detect a 1.0 effect size with a two-tailed 0.05 significance level paired t-test. The results of these exploratory tests will be reported in the context of the number of such tests performed.

The clinical response rate (in patients with measurable disease) and time to progression (in all patients) will also be estimated in a preliminary fashion. Appropriate confidence intervals will be provided along with fractions responding and a Kaplan-Meier curve of TTP.

It is estimated that up to 24 patients will be required to complete all 4 dose levels plus 10 additional patients will be needed at the MTD. Thus up to 34 evaluable patients may be required. In order to allow for a small number of inevaluable patients, the accrual ceiling will be set at 40 patients. Expected accrual rate is 1-2 patients per month. The DLT period is one cycle.

11 COLLABORATIVE AGREEMENTS

A Clinical Trials Agreement (CTA #1042-17) was executed between the National Cancer Institute (NCI)/National Institutes of Health (NIH) and Horizon Pharma, Inc. to receive Actimmune.

A Clinical Trials Agreement (CTA #01047) was executed between the National Cancer Institute (NCI)/National Institutes of Health (NIH) and Merck Sharp & Dohme Corp. to receive Sylatron®.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

This study will be open to all women with recurrent epithelial ovarian, fallopian, and peritoneal cancer regardless of ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. This study will be recruited through internal referral, our local physician referral base, and through various websites such as clinicaltrials.gov. All individuals with epithelial ovarian, fallopian, and peritoneal cancer that is refractory to standard care are eligible according to the eligibility criteria within section **2.1.**

Female patients all racial /ethnic groups are eligible for this study if they meet the eligibility criteria outlined in section **2.1.** To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

12.2 PARTICIPATION OF CHILDREN

Epithelial cancer of the ovary, fallopian tubes, or peritoneum in children is an exceedingly rare, reportable disease. Patients under the age of 18 will be excluded from study.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **12.5**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

12.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The potential benefit to a patient who enters study is a reduction in the bulk of her tumor, which may or may not have a favorable impact on symptoms and/or survival. Potential risks include the possible occurrence of any of a range of side effects that are listed in the pharmaceutical section and the consent document. The procedure for protecting against or minimizing risks will be to medically evaluate patients on a regular basis as described earlier, including initial eligibility screening prior to enrollment on the protocol.

12.5 RISKS/BENEFITS ANALYSIS

This risk/benefits analysis applies to all adult subjects on study (i.e., including those who may become decisionally impaired/unable to consent during participation).

12.5.1 Potential Risks

12.5.1.1 Risk of Treatment: Details of the risk of drug therapy are detailed in section **14.**

12.5.1.2 Blood draws: Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

12.5.1.3 Apheresis: Side effects of apheresis include pain and bruising, lightheadedness, and rarely, fainting.

12.5.1.4 Ascites collection: The risks associated with colleting ascites are pain and bleeding at the catheter site. In order to minimize pain, local anesthesia will be used. Rarely, there is a risk of infection at the sampling site. Ultrasound guidance may be used in obtaining ascites.

12.5.1.5 CT scans: In addition to the radiation risks discussed below, risks associated with CT scans are allergic reaction to and kidney damage from the contrast dye, nausea, vomiting, and anxiety.

12.5.1.6 IP catheter/port placement: Risks associated with IP catheter/port placement include intestinal perforation, abdominal fluid collection, and death. With port placement, there is an added risk from general anesthesia, if done by a surgeon. The risks of general anesthesia could include an allergic reaction to the drugs used, pneumonia, stroke and heart attack. The risk associated with local anesthesia is an allergic reaction to the agent.

12.5.1.7 Biopsies: The risks associated with the biopsy procedure include pain, swelling and/or bleeding at the biopsy site; infection; and allergic reaction to the local anesthetic used.

All care will be taken to minimize risks that may be incurred by catheter placement or tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent. If patients suffer any physical injury as a result of the procedures, immediate medical treatment is available at the NIH's Clinical Center in Bethesda, Maryland. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

12.5.1.8 Risks of Radiation

The study will involve radiation from the following sources:

- Up to 3 CT scans as referenced above for the collection of optional biopsies
- Up to 7 CT scans per year for disease assessment

Subjects in this study may be exposed to approximately 10.1 rem. This amount is more than would be expected from everyday background radiation. Being exposed to excess radiation can increase the risk of cancer. The risk of getting cancer from the radiation exposure in this study is 1 out of 100 (1%) and of getting a fatal cancer is 0.5 out of 100 (0.5%).

12.5.2 Benefits

The benefits include a possible decrease in the size of the tumor and the scientific knowledge that could be acquired through this trial.

12.6 CONSENT PROCESS AND DOCUMENTATION

An associate or principal investigator on the trial will inform patients of the purpose, alternatives, treatment plan, research objectives and follow-up of this trial. The patient will be provided an IRB-approved consent for review and signature and his/her questions will be answered. After a decision is made to enroll into the study, a signature will be obtained from the patient at a subsequent visit. A copy of the signed informed consent will be placed in the patient's medical record.

All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on study.

For the optional biopsies performed on the protocol, the patient will consent at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

12.6.1 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in section **2.2.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether are or not they are eligible to sign a consent for additional screening.

12.6.2 Telephone reconsent:

Telephone reconsent will be permitted for amendments requiring reconsent when it isn't feasible for the participant to return to the Clinical Center for that purpose. Telephone consent will be obtained and documented per OHSRP/IRBO and CCR policies and procedures.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination

or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 Quality Assurance and Qualtiy Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 Confidentiality And Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be

held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 PEGINTERFERON ALFA-2B (SYLATRON)

Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Whitehouse Station, NJ

Source: The agent will be obtained from Merck through Clinical Trials Agreement (CTA) #01047.

For the commercial product given subcutaneously, product labeling is prefaced by a boxed warning that identifies adverse reactions, including: depression and other neuropsychiatric disorders. The most common adverse reactions described in product labeling (>60%) include: fatigue, increased AST, increased ALT, pyrexia, anorexia, myalgia, nausea, chills and injection site reactions.

Contraindications include known hypersensitivity reactions to peginterferon alfa-2b or interferon alfa-2b, autoimmune hepatitis, and hepatic decompensation with Child-Pugh scores >6 (classes B and C).

Drug interactions: Drug metabolized by cytochrome P-450 (CYP) enzymes: Monitor closely when used in combination with drugs metabolized by CYP1A2 and/or CYP2D6.

Formulation and Preparation: commercially marketed SYLATRON™ vials nominally labeled to contain 200-mcg and 300-mcg vials are overfilled to contain 296 mcg and 444 mcg lyophilized peginterferon alfa-2b, respectively. When each vial is reconstituted with 1 mL Sterile Water for Injection, USP, the resulting concentrations are approximately 296 mcg/mL and 444 mcg/mL, respectively.

Refer to the package insert for additional information**.**

14.1.1 DTM preparation (dose levels 2, 3, 4)

The process is performed aseptically, inside a biological safety cabinet, using a sterile pipette tip in a control, monitored environment. The detailed process for reconstitution and adding of SYLATRON™ into an infusion bag is in detail in the DTM SOP developed for this protocol and submitted as part of the IND package. A syringe will not be used to transfer calculated volume of peginterferon alfa-2b.

For dose level 4, two vials will be needed if the 296-mcg/mL concentration is used and only one vial if the 444-mcg/mL concentration is used. Therefore, the procedure was written to specify use of one vial of the 444-mcg/mL product for the entire protocol. The experiments outlined in the validation process were performed using vials containing 444 mcg peginterferon alfa-2b (vials nominally labeled to contain 300 mcg).

Refer to **Appendix B** for additional information**.**

14.2 INTERFERON GAMMA-1B

ACTIMMUNE, Horizon Pharma, Inc.

Source: The agent will be obtained from Horizon Pharma through Clinical Trials Agreement (CTA) #1042-17.

Formulation: The commercially marketed product (ACTIMMUNE®) is available as a solution in single-use vials containing 100 mcg interferon gamma-1b (2 x10^6 International Units), formulated with 20 mg mannitol, 0.37 mg disodium succinate hexahydrate, 0.14 mg succinic acid, 0.05 mg polysorbate 20, and Sterile Water for Injection. Each vial permits extraction of up to 0.5 mL of solution containing interferon gamma-1b at a concentration of 200 mcg/mL.

Adverse reactions include AST/ALT elevation, exacerbation of a previous cardiac condition, reversible neutropenia and thrombocytopenia, gait disturbance, seizure. Other adverse effects include pyrexia, headache, rash, chills, injection site erythema or tenderness, fatigue, diarrhea, vomiting, nausea, myalgia and arthralgia.

Contraindications include patients who develop or have known hypersensitivity to interferon-gamma, E. coli derived products, or any component of the product.

Drug interactions: Cautiously monitor neutrophil and platelet counts when administering interferon gamma-1b concomitantly with other potentially myelosuppressive agents. Interferon gamma-1b use may decrease the concentrations of hepatic cytochrome P-450 enzymes.

Refer to the package insert for additional information**.**

14.2.1 DTM preparation (dose levels 2, 3, 4)
An automatic pipet capable of accurately measuring volumes as low as one microliter will be used to transfer the calculated volume of INTERFERON gamma-1b (shown in table below).

Dose levels	Cell suspension volume (*before sample testing removed)	Volume of 200 mcg/mL Interferon gamma-1b to add	Amount of Interferon gamma-1b 250 mL infusion volume
2	*383 mL	0.038 mL	5 mcg
3	*383 mL	0.038 mL	5 mcg
4	*383 mL	0.383 mL	50 mcg

Refer to **Appendix B** for additional information**.**

14.3 PREPARATION OF PEGINTERFERON ALFA-2B AND INTERFERON GAMMA-1B IN THE CLINICAL CENTER PHARMACY (FOR DOSE LEVELS 1 AND 3B)

In pharmacy practice, commercial hypodermic syringes will be used for transferring diluent between vials and for secondarily diluting peginterferon alfa-2b and for solution transfers and for secondarily diluting interferon gamma-1b. All product manipulations will be completed within the aseptic environment of a biological safety cabinet per standard pharmacy practices.

Vials nominally labeled to contain peginterferon alfa-2b 300 mcg will be reconstituted as described above (section **14.1**) to a concentration of 444 mcg/mL.

Dose Level		**PLASMA-LYTE-A Injection**	**Albumin (Human) 25%**	**Peginterferon alfa-2b (SYLATRON™)**	**Interferon gamma-1b (ACTIMMUNE®)**
1	25 mcg Peginterferon alfa-2b + 5 mcg Interferon gamma-1b in PLASMA-LYTE-A Injection + 4% Albumin (Human) Total Volume = 250 mL	209.3 mL	39.9 mL	0.56 mL of a **44.4**-mcg/mL* solution	0.25 mL of a **20**-mcg/mL* solution

3b	250 mcg Peginterferon alfa-2b + 50 mcg Interferon gamma-1b in PLASMA-LYTE-A Injection + 4% Albumin (Human) Total Volume = 250 mL	209.3 mL	39.9 mL	0.56 mL of a **444**-mcg/mL solution	0.25 mL of a **200**-mcg/mL solution

*** Dilution Instructions for Dose Level 1:**

To prepare a Peginterferon alfa-2b 44.4-mcg/mL solution from a 444-mcg/mL solution:

- Add 6 mL PLASMA-LYTE-A Injection *plus* 1.2 mL Albumin, (Human) 25% to a 10-mL empty sterile vial, and gently invert the vial to mix the solutions.
- To the same vial, add 0.8 mL Peginterferon alfa-2b (444 mcg/mL), and gently invert the vial to mix the solutions.

To prepare an Interferon gamma-1b 20-mcg/mL solution from a 200-mcg/mL solution:

- Add 3.8 mL PLASMA-LYTE-A Injection *plus* 0.7 mL Albumin, (Human) 25% to a 10-mL empty sterile vial, and gently invert the vial to mix the solutions.
- To the same vial, add 0.5 mL Interferon gamma-1b (200 mcg/mL), and gently invert the vial to mix the solutions.

The final product containing Peginterferon alfa-2b + Interferon gamma-1b in PLASMA-LYTE-A Injection + 4% Albumin (Human) should be stored at room temperature (15-25° C) and infused within 4 hours after preparation.

14.4 AUTOLOGOUS MONOCYTES

Source: The agent will be prepared at and obtained from the Department of Transfusion Medicine (DTM)

Formulation and Preparation: Refer to **Appendix B** for detailed procedures**.**

15 REFERENCES

1. Ledermann, J.A., et al., *Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up.* Ann Oncol, 2013. **24 Suppl 6**: p. vi24-32.
2. Bast, R.C., Jr., B. Hennessy, and G.B. Mills, *The biology of ovarian cancer: new opportunities for translation.* Nat Rev Cancer, 2009. **9**(6): p. 415-28.
3. Jemal, A., et al., *Cancer statistics, 2010.* CA Cancer J Clin, 2010. **60**(5): p. 277-300.
4. Armstrong, D.K., et al., *Intraperitoneal Cisplatin and Paclitaxel in Ovarian Cancer.* New England Journal of Medicine, 2006. **354**(1): p. 34-43.
5. Hinrichs, C.S. and S.A. Rosenberg, *Exploiting the curative potential of adoptive T-cell therapy for cancer.* Immunol Rev, 2014. **257**(1): p. 56-71.
6. Bekisz, J., et al., *Immunomodulatory effects of interferons in malignancies.* J Interferon Cytokine Res, 2013. **33**(4): p. 154-61.
7. Griffith, T.S., et al., *Monocyte-mediated tumoricidal activity via the tumor necrosis factor-related cytokine, TRAIL.* J Exp Med, 1999. **189**(8): p. 1343-54.
8. Berek, J.S., *Intraperitoneal adoptive immunotherapy for peritoneal cancer.* J Clin Oncol, 1990. **8**(10): p. 1610-2.
9. Berek, J.S., et al., *Intraperitoneal recombinant alpha-interferon for "salvage" immunotherapy in stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study.* Cancer Res, 1985. **45**(9): p. 4447-53.
10. Iaffaioli, R.V., et al., *Alpha 2b interferon (IFN) by intraperitoneal administration via temporary catheter in ovarian cancer. Preliminary data.* Eur J Gynaecol Oncol, 1991. **12**(1): p. 69-75.
11. Lichtenstein, A., et al., *Intraperitoneal administration of human recombinant interferon-alpha in patients with ovarian cancer: effects on lymphocyte phenotype and cytotoxicity.* Cancer Res, 1988. **48**(20): p. 5853-9.
12. Willemse, P.H., et al., *Intraperitoneal human recombinant interferon alpha-2b in minimal residual ovarian cancer.* Eur J Cancer, 1990. **26**(3): p. 353-8.
13. Colombo, N., et al., *Anti-tumor and immunomodulatory activity of intraperitoneal IFN-gamma in ovarian carcinoma patients with minimal residual tumor after chemotherapy.* Int J Cancer, 1992. **51**(1): p. 42-6.
14. Allavena, P., et al., *Intraperitoneal recombinant gamma-interferon in patients with recurrent ascitic ovarian carcinoma: modulation of cytotoxicity and cytokine production in tumor-associated effectors and of major histocompatibility antigen expression on tumor cells.* Cancer Res, 1990. **50**(22): p. 7318-23.
15. D'Acquisto, R., et al., *A phase I trial of intraperitoneal recombinant gamma-interferon in advanced ovarian carcinoma.* J Clin Oncol, 1988. **6**(4): p. 689-95.
16. Pujade-Lauraine, E., et al., *Intraperitoneal recombinant interferon gamma in ovarian cancer patients with residual disease at second-look laparotomy.* J Clin Oncol, 1996. **14**(2): p. 343-50.

17. Faradji, A., et al., *Phase I study of liposomal MTP-PE-activated autologous monocytes administered intraperitoneally to patients with peritoneal carcinomatosis.* J Clin Oncol, 1991. **9**(7): p. 1251-60.

18. Andreesen, R., B. Hennemann, and S.W. Krause, *Adoptive immunotherapy of cancer using monocyte-derived macrophages: rationale, current status, and perspectives.* J Leukoc Biol, 1998. **64**(4): p. 419-26.

19. Stevenson, H.C., et al., *Fate of gamma-interferon-activated killer blood monocytes adoptively transferred into the abdominal cavity of patients with peritoneal carcinomatosis.* Cancer Res, 1987. **47**(22): p. 6100-3.

20. Schuller, J., et al., *Pharmacokinetic aspects of interferon alfa-2b after intrahepatic or intraperitoneal administration.* Semin Oncol, 1992. **19**(2 Suppl 3): p. 98-104.

21. Gordon, S., A. Pluddemann, and F. Martinez Estrada, *Macrophage heterogeneity in tissues: phenotypic diversity and functions.* Immunol Rev, 2014. **262**(1): p. 36-55.

22. Gordon, S. and P.R. Taylor, *Monocyte and macrophage heterogeneity.* Nat Rev Immunol, 2005. **5**(12): p. 953-64.

23. Soderquest, K., et al., *Monocytes control natural killer cell differentiation to effector phenotypes.* Blood, 2011. **117**(17): p. 4511-4518.

24. Pahl, J. and A. Cerwenka, *Tricking the balance: NK cells in anti-cancer immunity.* Immunobiology, 2015.

25. Artis, D. and H. Spits, *The biology of innate lymphoid cells.* Nature, 2015. **517**(7534): p. 293-301.

26. Andreesen, R., *Macrophage-directed tumour immunotherapy revisited — past and future of an old doctor's dilemma.* Research in Immunology, 1993. **144**(4): p. 291-298.

27. Wang, N., H. Liang, and K. Zen, *Molecular mechanisms that influence the macrophage m1-m2 polarization balance.* Front Immunol, 2014. **5**: p. 614.

28. Martinez, F.O. and S. Gordon, *The M1 and M2 paradigm of macrophage activation: time for reassessment.* F1000Prime Rep, 2014. **6**: p. 13.

29. Mantovani, A., et al., *Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes.* Trends in Immunology, 2002. **23**(11): p. 549-555.

30. Lan, C., et al., *Expression of M2-Polarized Macrophages is Associated with Poor Prognosis for Advanced Epithelial Ovarian Cancer.* Technology in Cancer Research & Treatment, 2013. **12**(3): p. 259-267.

31. Faradji, A., et al., *Apheresis-elutriation program for adoptive immunotherapy with autologous activated monocytes in cancer patients.* Int J Artif Organs, 1991. **14**(5): p. 304-12.

32. Faradji, A., et al., *Large scale isolation of human blood monocytes by continuous flow centrifugation leukapheresis and counterflow centrifugation elutriation for adoptive cellular immunotherapy in cancer patients.* J Immunol Methods, 1994. **174**(1-2): p. 297-309.

33. Baron, S., et al., *Clinical model: interferons activate human monocytes to an eradicative tumor cell level in vitro.* J Interferon Cytokine Res, 2007. **27**(2): p. 157-63.

34. Baron, S., et al., *Near eradication of clinically relevant concentrations of human tumor cells by interferon-activated monocytes in vitro.* J Interferon Cytokine Res, 2011. **31**(7): p. 569-73.

35. Johnson, C.L., D.S. Green, and K.C. Zoon, *Human monocytes in the presence of interferons alpha2a and gamma are potent killers of serous ovarian cancer cell lines in combination with paclitaxel and carboplatin.* J Interferon Cytokine Res, 2015. **35**(1): p. 55-62.

36. Nakashima, H., et al., *Potent antitumor effects of combination therapy with IFNs and monocytes in mouse models of established human ovarian and melanoma tumors.* Cancer Immunol Immunother, 2012. **61**(7): p. 1081-92.

37. Bell, J.B., et al., *Neutralising antibodies in patients with multiple myeloma receiving maintenance therapy with interferon alpha 2b.* Br J Cancer, 1994. **70**(4): p. 646-51.

38. Doffinger, R., et al., *Autoantibodies to interferon-gamma in a patient with selective susceptibility to mycobacterial infection and organ-specific autoimmunity.* Clin Infect Dis, 2004. **38**(1): p. e10-4.

39. Ding, L., et al., *Determination of human anticytokine autoantibody profiles using a particle-based approach.* J Clin Immunol, 2012. **32**(2): p. 238-45.

40. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Cancer, 2009. **45**(2): p. 228-47.

41. Rustin, G.J., et al., *Re: New guidelines to evaluate the response to treatment in solid tumors (ovarian cancer).* J Natl Cancer Inst, 2004. **96**(6): p. 487-8.

42. Scher, H.I., et al., *Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group.* J Clin Oncol, 2008. **26**(7): p. 1148-59.

43. Bubley, G.J., et al., *Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: recommendations from the Prostate-Specific Antigen Working Group.* J Clin Oncol, 1999. **17**(11): p. 3461-7.

44. Vergote, I., et al., *Re: new guidelines to evaluate the response to treatment in solid tumors [ovarian cancer]. Gynecologic Cancer Intergroup.* J Natl Cancer Inst, 2000. **92**(18): p. 1534-5.

16 APPENDICES

16.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. 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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

16.2 APPENDIX B: DTM STANDARD OPERATING PROCEDURES

A. PRODUCT MANUFACTURING AND CHARACTERIZATION INFORMATION

1. PRODUCT MANUFACTURING – COMPONENTS AND MATERIALS

1. Cells

Patients will undergo up to 15 liter PBMNC apheresis (autologous), as estimated by weight and target cell dose in the Dowling Apheresis Clinic of the DTM, per standard operating procedure. Bilateral peripheral venous access will be used whenever possible. Alternatively, a temporary central venous catheter (CVL) may be placed for collection. The CVL will be inserted by Critical Care or Interventional Radiology staff with the assistance of anesthesiology as indicated.

Cells will be processed for further manufacturing in CPS/DTM according to SOPs (see Product Manufacturing section **2**: Procedures).

A. Donor Screening and Testing

Cells manufactured in the current protocol are of AUTOLOGOUS nature and donor eligibility determinations are not required (21 CFR 1271.90(a)(1)). However, eligibility requirements for product handling in CPS manufacturing facility require that subjects be HIV seronegative, hepatitis C seronegative and negative for Hepatitis B surface antigen. In addition, on the day of product collection, peripheral blood is submitted for testing: HIV 1 and 2 (antibody and NAT), HTLV I and II (antibody), Hepatitis C (antibody and NAT), Hepatitis B (surface antigen), West Nile Virus (NAT), T cruzi (antibody), and Treponema pallidum (antibody). Should any of these results be positive, DTM isolation procedures as defined in BB-DMF 11054 will be followed.

B. Cell Bank System

No feeder cells or cell lines were used in this protocol.

2. Reagents

1. Tabulation of Reagents Used in Manufacture: (see **Table 5**)

3. Excipients

a. Final product is infused freshly and contains monocytes and Peginterferon alfa-2b (Sylatron) and Interferon gamma-1b (Actimmune) suspended in Plasmalyte-A, 4% human serum albumin.
b. The concentration, source and qualifying documents of all excipients are listed in Table 1 and Appendix 3.

4. Additional Considerations (none)

Table 5: Reagents used for the manufacture of autologous monocytes with Interferon (IFN)

Product Name	Trade Name	Vendor	NDC	Qualification	Use	Regulatory Status	Product Manufacture
Human Serum Albumin	Flexbumin 25%	Baxter Healthcare Corp.	0944-0493-02	Package insert	Buffer additive	FDA approved	Final product preparation
Hanks Buffered Salt Solution	HBSS	American Pharmaceutical partners, Schaumbur, IL	NA	Certificate of Analysis	Buffer additive, elutriation procedure	GMP grade ancillary reagent	Intermediate product, elutriation
Sodium Chloride Injections USP 0.9% sodium chloride	Saline	B.Braun Medical Inc. Irvine CA	0264-7800-00	Package insert	Buffer additive, elutriation procedure	FDA approved	Intermediate product, elutriation
Sodium Citrate Concentrate 46.7%	Tricitrasol	Citra Labs, LLC	23731-6030-3	Package insert	Prevent cell clumping, elutriation procedure	FDA approved	Intermediate product, elutriation
Dornase alpha (DNAse)	Pulmozyme	Genentech, Inc.	50242-100-40	Package insert	Prevent cell clumping, storage	FDA approved	Intermediate product, elutriation
PLASMA-LYTE-A	PLASMA-LYTE-A injection pH 7.4	Baxter Healthcare Corp.	0338-0221-03	Package insert	Infusion Solution	FDA approved	Final product preparation
Peginterferon alfa-2b	SYLATRON™	Schering Corp.	0085-4348-01	Package insert	Activate monocytes	FDA approved	Final product preparation
Interferon gamma-1b	ACTIMMUNE®	Horizon Pharma, Inc.	42238-111-01	Package insert	Activate monocytes	FDA approved	Final product preparation

2. Product Manufacturing – Procedures

Figure 5. Manufacture of Autologous Monocytes in combination with Sylatron™ and Actimmune®

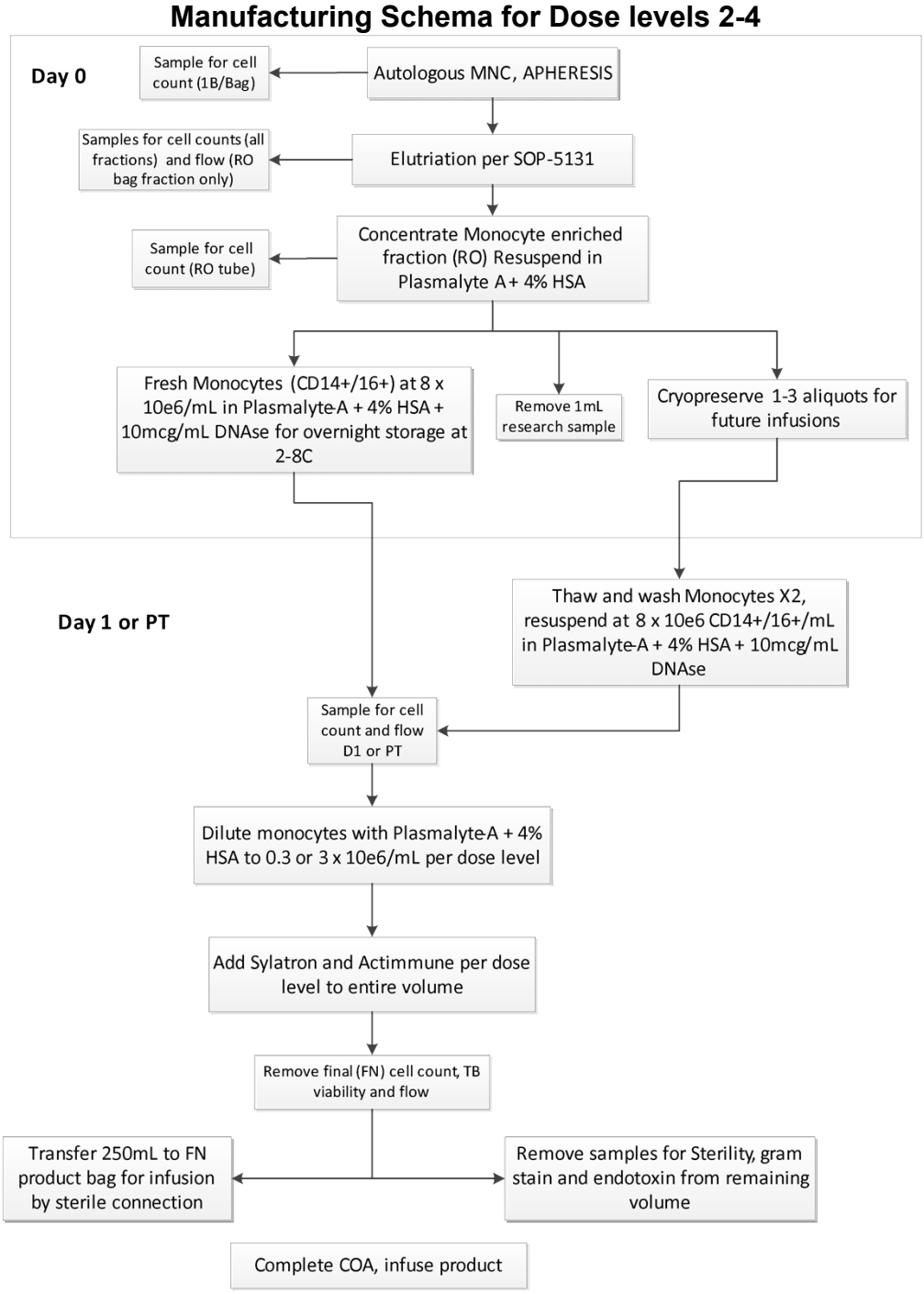


Figure 5. **Manufacturing process schema.** (Day 0) Autologous Peripheral Blood Mononuclear Cells (PBMNCs) will be collected by apheresis in DTM Dowling Clinic and transported to the Cell Processing Section (CPS) of DTM. The PBMNC product will undergo counter flow centrifugation elutriation (CCE) to enrich for monocytes in the product. After elutriation, all fractions except the monocyte fraction will be issued to PI laboratory. The monocytes will be washed and re-suspended at the appropriate concentration with 1-3 aliquots cryopreserved for future infusions. On the day of infusion, the monocytes will be washed, filtered, counted and re-suspended at dose level with Peginterferon alfa-2b (Sylatron™) and Interferon gamma-1b (Actimmune®) as described below. Assays for safety, purity, and identity will be performed before the product is issued for infusion.

- Preparation of AUTOLOGOUS cells

1. Method of cell collection/Processing
   1. Day 0 - Mononuclear cells will be collected by apheresis (up to 15 L) to reach a target cell number of monocytes. Apheresis will be performed in the Dowling Apheresis Clinic, Department of Transfusion Medicine (DTM) using a Cobe Spectra or an equivalent cell separator device using current standard operating procedures.
   2. Counter flow centrifugation elutriation (CCE) will be performed using the Elutra device.
   3. All products, intermediate and final, will be contained within a closed system using bags and sterile connection devices.
   4. The monocytes are contained in the rotor off (RO) fraction from elutriation. The monocytes will be stored overnight at refrigerated temperature before further processing on day of infusion.
   5. 1-3 aliquots will be cryopreserved for future infusions
   6. Day 1 – the monocytes are prepared for infusion, with final testing including FACS to assess percent of CD14 (cell purity), cell counts, viability, and sterility testing, including Gram stain.
2. Irradiation

   Not Applicable

3. Day of infusion
   7. On Day 1, day of infusion, cells are taken from refrigerated storage (4°C), mixed, and allowed to warm to room temperature for 30 minutes. Cells are filtered, washed, and re-suspended in infusion solution.
   8. For cryopreserved monocytes, cells will be washed twice and resuspended at 8 x10e6 CD14+/16+/mL in Plasmalyte A+ 4% Albumin (Human), USP (HSA) and 10mcg/mL DNAse
   9. Samples are taken for cell count, viability and FACS to determine subset components.
   10. Cells are filtered and diluted in Plasmalyte A+ 4% Albumin (Human), USP (HSA) at a concentration in accordance with Dose Level according to SOP:
   11. Dose level 1 and 3b: No cells will be given.
   12. Dose level 2: cells are diluted to $0.3x10^6$/mL (± 20%).
   13. Dose levels 3 and 4: cells are diluted to $3x10^6$ cells/mL (± 20%).

14. Peginterferon alfa-2b (Sylatron™) and Interferon gamma-1b (Actimmune®) are added in quantities according to SOP to achieve the doses specified in the treatment protocol.
15. Dose levels 1, 2, and 3 will contain 25 mcg Peginterferon alfa-2b (Sylatron) and 5 mcg Interferon gamma-1b (Actimmune);
16. Dose level 4 and 3b will contain 250 mcg Peginterferon alfa-2b (Sylatron) and 50 mcg Interferon gamma-1b **(**Actimmune).
17. Monocyte cell suspension, in Plasmalyte-A and 4% HSA, with Peginterferon alfa-2b and Interferon gamma-1b, is issued for intraperitoneal infusion.

- Process Timing and Intermediate Product Storage

(See **Table 6**)

- Final Formulation

Final product is autologous monocytes suspended in Plasmalyte-A + 4% HSA + Peginterferon alfa-2b (Sylatron) + Interferon gamma-1b (Actimmune).

Table 6. Timeline and Lot Release Criteria for the manufacture of autologous monocytes with Peginterferon alfa-2b (Sylatron™) and Interferon gamma-1b (Actimmune®).

Day	Time required (hours)	Process Step	Product (or Intermediate Fraction)	Sample Designation	Sample Type (Cells/Sup)	Assay	Lot release (Yes/No)
0	NA	Patient sampling		Patient Pre-Apheresis	Patient PB	CBC, TTD[1], ABO/Rh	Yes (TTD)
0	NA	Patient sampling		Patient Post-Apheresis	Patient PB	CBC	No
0	1	Apheresis product receipt	PBMNC product	Bag	Cells + Sup	Cell count, volume, Flow differenti	No
0	2-3	Elutriation	Post-Elutriation	RO Fraction	Cells+Sup	Cell count, Volume, Flow phenotype	No
0	1	Supplemented bag	Supplemented monocyte bag	D0 (RO tube)	Cells+Sup	Cell count, viability	No
1	2	Stored bag	Stored monocyte bag	D1 or PT (post thaw)	Cells+Sup	Cell count, viability Volume, FACs	Yes (FACs)
1	2	Final resuspension	Final product	FN	Cells+Sup	Cell count, viability, FACS, gram stain, sterility, endotoxi	Yes

[1]Transfusion Transmitted Disease Testing

B. PRODUCT TESTING

1. MICROBIOLOGICAL TESTING: BACTERIAL AND FUNGAL TESTING

1 Sterility Testing:

Samples for sterility testing will be taken from Final product (FN) (see **Figure 5**).

The sterility sample will be prepared per CPS-SOP-5008 for bacterial and fungal testing which is performed by the Department of Laboratory Medicine, Clinical Center, NIH (See Appendix 4 for applicable CPS SOPs). Sterility tests comply with 21 CFR 610.12.

In addition to samples inoculated into the Bactec bottles, a sample is submitted for fungal testing, consisting of plating onto solid media, for incubation for 14 days.

2. IDENTITY

Identity will be tested on Final product (FN) by flow phenotype and cell count. The final release criteria on the final product will include Trypan blue viability: ≥ 70%, $CD14^+$ of viable: ≥ 40%

3. POTENCY

CD14 will be used as a surrogate marker for potency since the addition of monocytes to IFN therapy correlates with a reduction in tumor size in animal models with xenografted human ovarian tumors (Nakashima et al. 2012).

4. OTHER

- **General Safety Testing**

  Action plans for positive results on safety testing

  General action plans for dealing with positive tests for sterility testing are presented in our Master File BB-MF-11054. See Cross reference letter for BB-MF-11054 contained in Section I.

- **Viability**

  Viability testing will be performed on the Final product (FN). Trypan blue viability on the final product must be ≥ 70%.

- **Cell Number/Dose**

  The cell number target will be based on the following dose levels.

  Dose level 1 and 3b: No cells will be given

  Dose level 2: 75×10^6 (± 20%) viable $CD14^+$ monocytes total

  Dose levels 3 and 4: 750×10^6 (± 20%) viable $CD14^+$ monocytes total

C. FINAL PRODUCT RELEASE CRITERIA TESTING

Product Testing: See CPS-SOP-[15-CPS-05]

Product Release Criteria: final product testing is performed on the day of infusion (Day 1). On day of infusion, samples for trypan blue viability, STAT gram stain, and FACS will be submitted and results used for release criteria. Sterility culture will also be

performed on the final product but results will not be available at the time of infusion. These results will be reported on the Certificate of Analysis listed below.

Test	Method	Acceptable Limit
Appearance	Visual check	Normal - milky; no aggregates
% Viable monocytes (CD14+)	FACs	≥ 40%
% Viability	Trypan Blue	≥ 70%
Sterility on final product	Bactec	*
Gram stain	Gram stain	No organism seen
Donor eligibility	NA	Auto

*sent on the day of infusion. Result available 14 days later.

D. PRODUCT STABILITY

The final product will be delivered to the patient care unit immediately after required assays for release. Expiration time is 4 hours post final product sampling.

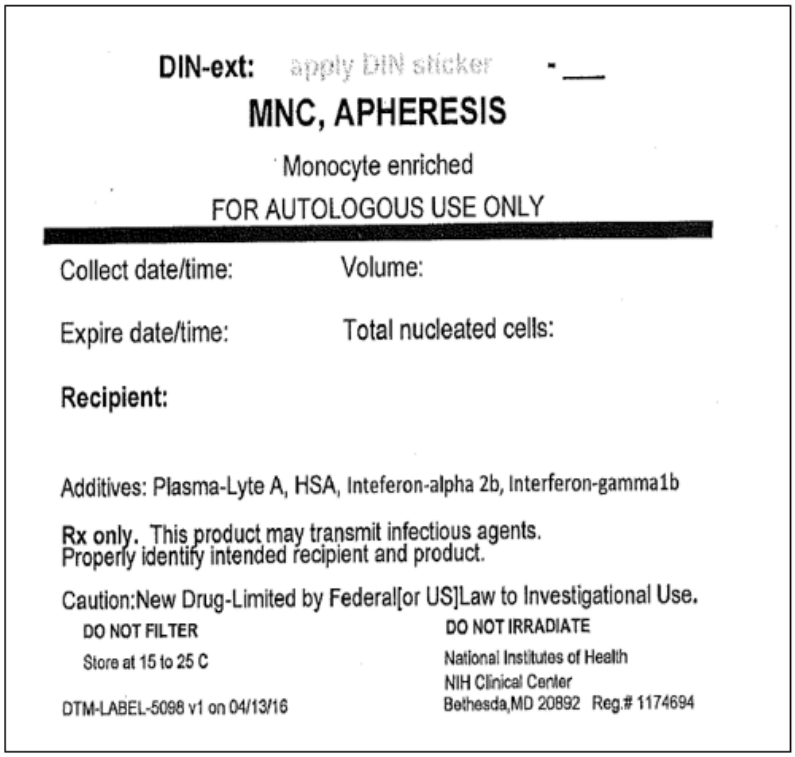
E. OTHER ISSUES

- **PRODUCT TRACKING**

Manufacturers of cellular therapy products are required to maintain traceability of the products received, processed and stored in the facility through the final disposition of the product. CPS-SOP-5107 describes the procedures for receipt of products in the Cell Processing Section (see Drug Master File: 11054). CPS-SOP-5105 outlines the requirements for distribution and documentation of products to administration sites, research or discard (see Drug Master File: 11054). At final disposition, the product is assigned a status of infused, shipped, allocated to research or discarded.

- **PRODUCT LABELING:**

The following is an example of the product label for final product, autologous monocyte cell suspension, in Plasmalyte-A and 4% HSA, with Peginterferon alfa-2b and Interferon gamma-1b



- **CONTAINER/CLOSURE:**

  The starting apheresis material, intermediate products, and final product will be contained within a closed system using sterile transfer pack containers. All connections will be made using a Sterile Tube Welder (TerumoBCT).

- **ENVIRONMENTAL IMPACT:**

  The Women's Malignancies Branch of the National Cancer Institute, National Institutes of Health requests a claim for categorical exclusion for this proposed clinical trial as provided for in 21 CFR 25.31(e), in that the agents studied under this IND are intended to be used in clinical trials in which the amount of waste expected to enter the environment may reasonably be nontoxic. Furthermore, it is understood that the clinical supplies of the investigational agents are to be used under the direction of qualified investigators (physicians, pharmacists and nurses) knowledgeable in the use and handling of investigational agents, including the professional staff in the Department of Transfusion, Cell Processing Section, NIH.

  All waste is disposed in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland.

- **QUALIFICATION OF THE MANUFACTURING PROCESS:**

Validation Summary:

Preparation of monocytes in combination with Peginterferon alfa-2b (SYLATRON™) and Interferon gamma-1b (ACTIMMUNE®)

The NCI protocol entitled '**Phase I study of intraperitoneal infusion of autologous monocytes with Sylatron (Interferon alpha 2b) and Actimmune (Interferon gamma) in women with recurrent or refractory ovarian cancer, fallopian tube cancer, or primary peritoneal cancer**' (17-C-0011), utilizes an elutriated apheresis product to obtain monocytes which are mixed with varying doses of interferons before being administered to patients. Here, patients undergo an apheresis procedure each time they are admitted, which can be up to every 4 weeks. In the following study, we designed and validated a new process that would allow us to prepare one initial fresh dose of monocytes and cryopreserve the remaining cells so that they may be thawed and infused at a later date. This new modification would reduce unnecessary stress to the patient due to having to undergo multiple apheresis and it will also relieve the burden of performing many of these aphereses altogether by Dowling Clinic. Three separate samples were tested independently, two samples from healthy donors and one sample taken from a patient with ovarian cancer. Each sample was processed as a fresh and as a cryopreserved specimen as they would be during clinical manufacturing. Results below compare multiple attributes of each of these specimens.

First, we examined cell recovery following cryopreservation to ensure the cells were capable of surviving the cryopreservation process. Cells from all three donors were cryopreserved in aliquots >330x10^6 TNC (or >260x10^6 CD14/CD16 monocytes) (**Table 7**). These aliquots represent the typical cell number that would be cryopreserved for dose level 2 and one-third of dose level 3 and 4. Upon thaw, cells were counted and recovery was calculated. TNC recovery from all three samples was >88% (>91% for CD14/CD16 monocytes). We conclude that there is no detrimental effect of the cryopreservation procedure on the TNC or monocyte recovery.

Table 7 Cryopreserved cell recovery

Sample	TNC			CD14+/CD16+		
	Pre Thaw	Post Thaw	Recovery	Pre Thaw	Post Thaw	Recovery
PD17060149 B	3.50E+08	3.50E+08	100.0%	2.85E+08	2.94E+08	103.3%
PD17060190 B	4.22E+08	3.97E+08	94.1%	3.16E+08	3.12E+08	98.6%
PD17060193 B	3.33E+08	2.93E+08	88.0%	2.60E+08	2.37E+08	91.3%

Next, we compared the frequency of CD14/CD16 cells that were in the starting product (bag), fresh sample, and cryopreserved sample. After elutriation, we observed a consistent increase in the frequency of monocytes that were within the RO fraction. The frequency ranged from 14-43% in the starting bag to >75% post elutriation (**Table 8**). There was <5% difference when comparing the frequency of monocytes between SM and day 1 of fresh sample and cryopreserved sample. Similarly, there was <9% difference when comparing the frequency of monocytes between FN samples of fresh vs. cryopreserved samples. Together, this data indicate that cryopreservation of the intermediate product did not significantly affect the monocyte frequency.

Table 8 Frequency of CD14/CD16 monocytes

Sample	Fresh sample (A)			Cryo sample (B)	
	Bag	SM	FN	Day 1	FN
PD17060149	15.30%	81.40%	79.10%	84.10%	87.60%
PD17060190	14.00%	74.80%	79.10%	78.40%	74.80%
PD17060193	43.50%	78.10%	76.80%	81.00%	80.60%

Loss of CD14/CD16 monocytes that occurred during processing from day 1 until the FN was packaged was minimal. Fresh sample recovery during this time point was >93% and cryopreserved sample recovery was >86% (**Table 9**).

Table 9 Absolute cell number and recovery of CD14/CD16 monocytes from day 1 through FN

Sample	Fresh sample (A)			Cryo sample (B)		
	Day 1	FN	Recovery	Day 1	FN	Recovery
PD17060149	7.50E+07	7.55E+07	100.67%	2.45E+08	2.25E+08	91.84%
PD17060190	2.16E+08	2.20E+08	101.85%	2.80E+08	2.43E+08	86.82%
PD17060193	7.50E+07	7.03E+07	93.73%	2.61E+08	2.36E+08	90.34%

Functional assays were performed in Dr. Annunziata's laboratory using the final product from both the fresh and cryopreserved samples. Monocytes plus IFN's (per protocol) were mixed with ovarian cancer cells as outlined in the validation plan and allowed to incubate together for 3 days. Monocyte and IFN cytotoxicity is measured as percent survival compared to untreated controls. Percent killing is calculated as (100-% survival). There were no significant differences in % killing between fresh and cryopreserved samples. All samples were highly efficient in their ability to lyse target tumor cells. The percent killing from all samples was >94%, and the difference of percent killing between individual fresh vs. cryopreserved samples was <2% (**Table 10**). This data indicate that there was no loss of function that occurred within the cryopreserved samples.

Table 10 Functional assay (% Killing)

Sample	Fresh sample (A)	Cryo sample (B)
PD17060149	94.1%	95.8%
PD17060190	96.1%	94.7%
PD17060193	95.7%	95.3%

Monocyte viability pre and post cryopreservation was examined next. Samples were taken at day 1 and in the final product after resuspension with cytokines and assessed for viability using AO/PI uptake on the cellometer instrument. The viability of all samples >92% (**Table 11**). In addition, we examined the stability of the monocytes post thaw at 2 hour and 4 hour time points after final packaging with cytokines to ensure that they remained stable while they are

transferred to the clinic for administration to the patient. We found that the viability at these time points was not significantly different than the viability at the 0 hour time point (all >93%) and conclude that the monocytes would remain stable for up to 4 hours (**Table 12**).

Table 11 Cell Viability

Sample	Fresh sample (A)		Cryo sample (B)	
	Day 1	FN	Day 1	FN
PD17060149	99.0%	98.0%	99.6%	98.9%
PD17060190	97.0%	94.5%	92.8%	93.8%
PD17060193	99.0%	99.0%	96.4%	98.8%

Table 12 Stability of Cryopreserved Monocytes

Sample	FN (0hr)	FN (2hr)	FN (4hr)
PD17060149 B	98.9%	98.6%	99.0%
PD17060190 B	93.8%	94.8%	93.6%
PD17060193 B	98.8%	98.3%	98.2%

Conclusion: We validated a new manufacturing process for the preparation of cryopreserved monocytes and found that the cell product was extremely consistent between cryopreserved fraction vs. fresh fraction and also consistent among the three different donors tested here. All samples tested passed the validation criteria set forth in the validation plan, which also included product safety testing (sterility, endotoxin). We therefore approve the implementation of this process within DTM Cell Processing Section.

F. Quality Assurance Practices

For a complete description of Quality Assurance and Quality Control practices in CPS DTM refer to BB-MF 11054.

16.3 APPENDIX C: LIST OF DRUGS INTERACTING WITH CYP1A2OR CYP2D6

Patients receiving any medications or substances that are strong inhibitors or inducers of CYP1A2 and CYP2D6 are ineligible. Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated list. We will refer to http://medicine.iupui.edu/clinpharm/ddis/; medical reference texts such as the Physicians' Desk Reference may also be consulted. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

16.4 APPENDIX D: STANDARD OPERATING PROCEDURE FOR INTRAPERITONEAL INFUSION

If patient has existing intraperitoneal catheter, this will be used. If not, then a tunneled 6F or 9F catheter will be placed in interventional radiology.

1) Access Port-a-Cath or peritoneal infusion catheter

2) Place patient on complete bed rest in semi-Fowler's position throughout administration of IP infusion. Head of bed must be no higher than 30 degrees to prevent dislocation of port needle or kinking of infusion catheter, and to allow IP fluid to flow freely. A flat position during infusion may increase pressure on diaphragm, causing respiratory compromise/GI upset in patients receiving IP infusions. If any significant movement of patient occurs during IP infusion, the nurse must assure proper needle positioning is maintained.

3) In this protocol, 0.9% Sodium Chloride Injection is administered warmed or at room temperature per patient preference. If warming is preferred, place 500 mL 0.9% Sodium Chloride Injection in warm water bath (keeping outer manufacturer wrapping intact) for approximately 15 minutes. RATIONALE: Warmed fluid is more comfortable for patient during infusion and decreases the incidence of cramping associated with IP infusions.

4) Prime IV tubing with attached Y port with 0.9% Sodium Chloride Injection, either warmed or room temperature per patient preference. Attach to port or peritoneal infusion catheter.

5) Infuse 200-250 mL of 0.9% Sodium Chloride Injection, as rapidly as possible via gravity.

6) Observe catheter site for swelling, leakage, or redness. Observe entire abdominal surface for unusual local swelling. Observe patient for complaints of pain, SOB, dyspnea, respiratory distress, and cramping. Stop infusion and notify physician if any of above conditions occurs. RATIONALE: Migration of catheters or dislodging of right angled needle may occur.

7) If no adverse effects noted after completion of 0.9% Sodium Chloride infusion, attach primed Monocyte infusion agent to free Y connector. Clamp 0.9% Sodium Chloride infusion line and infuse monocytes as rapidly as possible, via gravity. Monocytes should be infused at room temperature. NOTE: IP monocyte infusion may take as little as 30 minutes or as long as 1 hour (+/- 10 min) to infuse. If infusion takes longer than 1 hour, 10 min (70 min), RN should notify physician for troubleshooting.

8) After infusion of monocytes is complete, clamp monocyte tubing and open 0.9% Sodium Chloride tubing. Infuse an additional 50-100 mL of 0.9% Sodium Chloride Injection as rapidly as possible by gravity.

9) Flush port needle or catheter with 10 mL of low strength heparinized (10 Units/mL) 0.9% Sodium Chloride. De-access Port-a-Cath or clamp catheter.

10) After de-accessing needle or clamping catheter, begin turning protocol: reposition patient every 15 minutes clockwise, (i.e.: from side, to back, to other side, if possible prone (abdomen), Fowler's, Trendelenburg and knee chest) for a total of two hours. RATIONALE: Repositioning disperses fluid throughout the peritoneal cavity.

11) Patient may ambulate after turning protocol is complete.

12) Document chemotherapy administration according to institutional policy

16.5 APPENDIX E: SAMPLE CHEMOTHERAPY/BIOTHERAPY TREATMENT NOTE

IF APPLICABLE:

Paracentesis will be done prior to treatment regimen and repeated after at least 24 hours following treatment have elapsed.

DOSE LEVEL 1 or 3B (Put in dose depending on dose level at asterisks):

- 200-250 mL warmed 0.9% Sodium Chloride Injection (or room temperature per patient preference) will be given intraperitoneally first followed by:

- Peginterferon alfa-2b ** mcg
  Interferon gamma-1b ** mcg
  The drug will be mixed with PLASMA-LYTE-A, HSA, and IFN in a total quantity of 248 ±2 mL
  Infuse by gravity over 30-60 min

- Infuse an additional 50-100 mL of 0.9% Sodium Chloride Injection, flush port with heparin flush of 10 Units/mL (10 mL)

- The patient will then be repositioned for a total of 2 hours to allow for maximal distribution of the treatment.

DOSE LEVEL 2, 3, or 4 (Put in dose depending on dose level at asterisks):

- 200-250 mL warmed 0.9% Sodium Chloride Injection (or room temperature per patient preference) will be given intraperitoneally first followed by:

- Monocyte cell suspension (**) in PLASMA-LYTE-A + 4% HSA and will be mixed with:
  Peginterferon alfa-2b **mcg
  Interferon gamma-1b **mcg
  The infusion will be in a total quantity of 248 ±2 mL
  Infuse by gravity over 30-60 min

- Infuse an additional 50-100 mL of 0.9% Sodium Chloride Injection, flush port with heparin flush of 10 Units/mL (10 mL)

- The patient will then be repositioned for a total of 2 hours to allow for maximal distribution of the treatment.