



## Plasmid IFNB-MSC has Bioequivalence to Adv transfected MSC-IFNB in modulating tumors & cell lines in vitro or in vivo

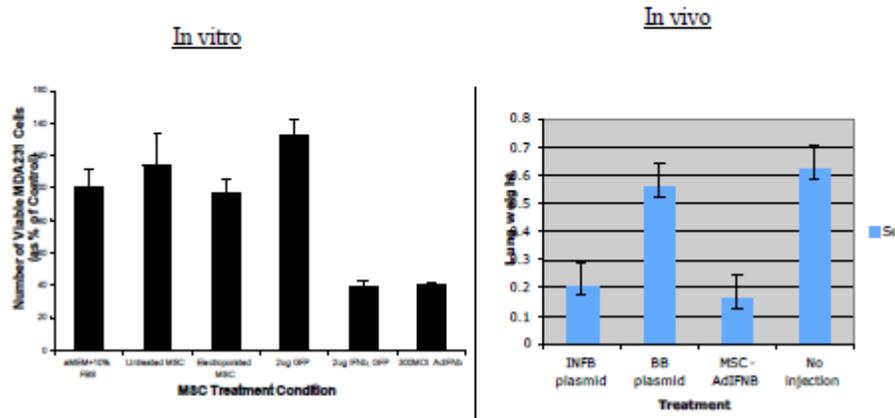


Figure 2

Distribution: we find no differences in the systemic or IP distribution of MSC transfected with either adenoviral delivery or electroporation with a plasmid. The cell still home to tumors, although in ovarian cancers, IP injection was superior to systemic injection. Figure 3 shows homing of plasmid electroporated MSC-IFN $\beta$  to ovarian cancer in vivo and the pronounced effect on tumor growth, alone and in combination with Taxol.

## Plasmid electroporated MSC-IFNB control ovarian tumors

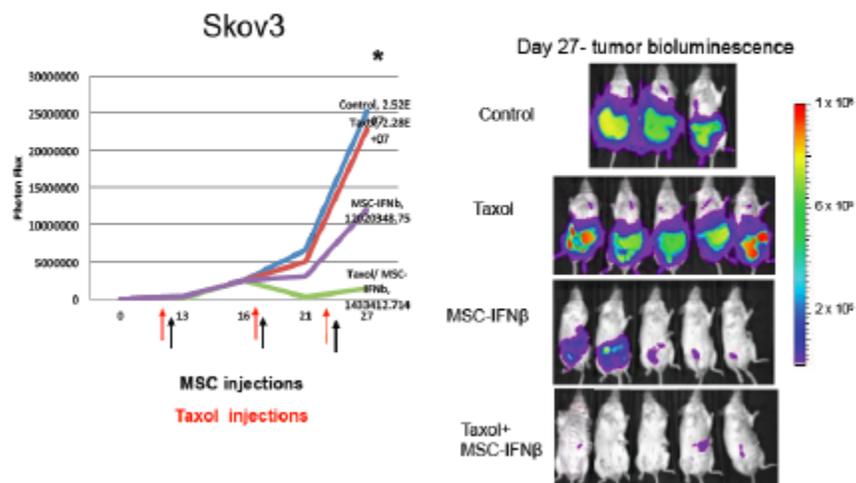


Figure 3

**2. Most of the other pre-clinical and clinical trials listed in support of this protocol used intravenous (i.v.) injection of MSC. What is the rationale behind using the intraperitoneal (i.p.) route for the proposed Protocol, other than the mice studies reported?**

Our thinking is one of safety and efficacy. Safety: The delivery of MSC systemically results in a first pass thru the lungs, that could potentially introduce IFNB into lung tissue and while human MSC have been demonstrated by Koc, Gershon etc all to remain in lungs for up to 3 hrs, we did not want any cell loss due to trapping. Efficacy: As this is an Ovarian cancer trial, our thinking is that by performing IP injections that the MSC-IFNB would have the greatest amount of successful engraftment by having the highest concentration of cells in the peritoneum where the tumor is, a loco-regional delivery.

Our preclinical animal data in which MSC were injected IP further demonstrate the above two points. 1: Minimal to no distribution of MSC outside of the peritoneum, and 2: increased numbers of engrafted MSC in the tumor tissue, as compared to systemic delivery in mouse models in which less than 10% of the injected cell population was detectable at the tumor site.

**3. No information is given on how the MSC cells are derived, cryopreserved etc., or on the health screening tests that will be done on the donor of MSC.**

The allogeneic MSC will be derived and cryopreserved as follows per Protocol PA13-0025 under which the MSCs will be generated. Information regarding how the MSCs are derived, cryopreserved (section 4.1) and the screening done on the donor (section 3.1) is copied from the protocol below.

Bone marrow will be obtained from normal volunteers with up to 60 ml aspirated from the posterior iliac crest. The marrow will be aspirated into heparinized syringes. Under GMP conditions, the mononuclear (MNC) fraction will be isolated using a density gradient with Lymphocyte Separation Media (specific gravity 1.077). The low-density cells will be collected and washed with Plasma-Lyte A containing 1% HSA. The washed cells will be samples and viable cell numbers determined. The BM MNC will be seeded into 225 cm<sup>2</sup> tissue culture flasks in alpha MEM containing 5% HPL (Human Platelet Lysate). After 14 days of culture, passage zero (P0) cells will be harvested by trypsin treatment and expanded into 60 flasks. These flasks are incubated for a further 7 to 10 days and the MSC harvested by trypsin treatment (P1 cells). The cells will then be frozen using a control rate freezer.

The donor eligibility:

Male donors between the ages of 20 to 35 years will be screened as potential bone marrow donors (maximum of 20) and shall be evaluated by history and physical examination. The history shall include the following:

- History of malignancy
- Bleeding abnormalities

- Deep venous thrombosis
- Cardio/pulmonary conditions
- Blood transfusions
- Vaccinations
- Questions to identify persons at risks of infectious disease transmission
- Questions to identify persons at risk of transmitting hematological or immunological disease

The physical examination shall be completed within 30 days of the bone marrow procedure and include evaluation for potential risk of the bone marrow aspiration procedure.

Prospective donors shall have the following blood tests on the first visit:

- Hepatitis B surface antigen (HBsAg)
- Anti-Hepatitis B core antibody (HBcAb)
- Anti-Hepatitis C virus antibody (HCV Ab)
- Anti-Human Immunodeficiency Virus (HIV) antibody (HIV 1/2 )
- Cytomegalovirus antibody (CMV)
- HCV/HIV Nucleic Acid test
- West Nile Virus Nucleic Acid test
- Rapid Plasma Reagin (RPR)
- Human T-lymphotropic Virus I/II (HTLV I/II)
- CBC, differential, platelet count
- Creatinine, ALT, bilirubin, alkaline phosphatase, glucose
- Na, K, Cl, Mg, calcium

Prospective donors shall have the following blood tests within 24 hours before the bone marrow aspiration procedure:

- CBC, differential, platelet count

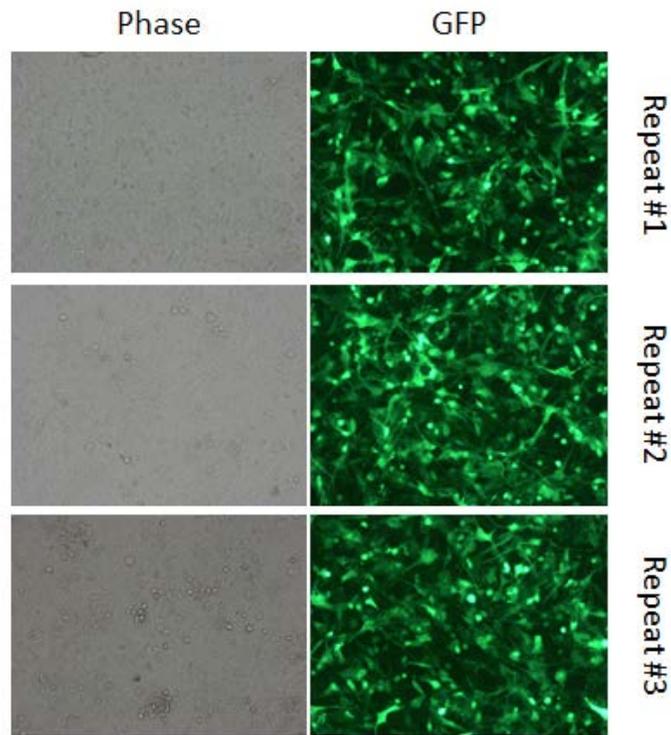
#### Eligibility Criteria for Normal Donors

- No history of malignancy
- No active coagulopathy and/or hypocoagulable state
- No history of cardio/pulmonary conditions
- Negative tests for Hepatitis B, Hepatitis C, RPR, HIV ½, HTLV I/II and NAT for HCV, HIV and WNV.
- Hemoglobin  $\geq$  13.0 g/dL
- Platelet count 140,000 to 440,000/ul
- WBC 3.0 to 11.0 K/ul

- No anomalies on the CBC and differential suggestive of a hematopoietic disorder
- Creatinine  $\leq 1.5$  mg/dL
- ALT  $\leq 112$  IU/L
- Bilirubin  $< 1.5$  mg/dL
- No diabetes
- Systolic blood pressure  $\leq 170$
- Diastolic blood pressure  $\leq 90$
- No history of autoimmune disorders

**4. How is the transfection efficiency controlled for consistency before patients' infusion of the MSC cells? The investigators mention 50% efficiency: is this value confirmed before each infusion and how?**

The Amaxa system is a system of simplicity. One places the same number of cells in the same size cuvette and then presses the same program button. That said we provide data which demonstrates reproducibility of the transfection efficiency and production of GFP. In experiments conducted 4 years ago (Drs. Marini/Andreeff) and repeated now in the cell production lab (Dr. Yvon), the high reproducibility of the method is evident (Fig. 4).



	<u>Ctle</u>	#1	#2	#3
TNC recovery (%)	100	100	100	100
Viability (%)	100	81	78	78
GFP expression (%)	0	71.9	69.2	61.4

Figure 4

Also when initially working out the electroporation parameters, we utilized a GFP marker system, this is where the 50% of transfection came from. The clinical protocol has NO such markers, and therefore this will not be a releases criteria.

Using a reporter gene (GFP) we validated our procedure to achieve a >50% transfection rate.

**5. Taqman PCR for the detection of plasmid DNA for IFN-beta is listed briefly on 7.1 of the Protocol, but no other details are given on this assay, or of its limit of detection and applicability. Could this test be used to measure the transfection efficiency of the MSC?**

This is not ideal, because it cannot distinguish the expression levels per cell from the population average.

However, it could be established as an assay that at least provides information about the average IFN $\beta$  RNA levels found in a given sample. AN additional issue is the not necessarily linear relationship between cellular RNA and secreted protein.

#### **6. What is the limit of detection of the IFN-beta IHC test?**

We did not determine the sensitivity of this assay, however when detected in transfected cells, the levels determined by IHC correlated with clinical response in mouse models.

#### **7. Release criteria for the MSC post 14-day culture and Electroporation lists >50% cell viability. Since 50% of the electroporated MSC cells express the IFN-beta gene product, are the transfected cells all viable cells and when is the viability test done, compared to the transfection efficiency test?**

In pre-clinical studies, IFN $\beta$  can be detected in cell culture supernatant of IFN $\beta$ -transfected MSC up to day 5 after transfection indicating that live cells do contain the IFN $\beta$  plasmid. However, we cannot confirm that the transfected cells are all viable.

Because the electroporation method used to introduce the IFN $\beta$  plasmid into the MSCs results in membrane permeability which negates traditional methods for determination of viability, we will culture an aliquot of the transfected cells overnight and assess the viability of the cells at that time point. Therefore, MSC viability will not be part of the release criteria and C of A will be amended to reflect the change.

We will not control the transfection efficiency before patients' infusion as infusion will occur immediately after transfection as per protocol. However, a fraction of the transfected cells will be kept in culture and supernatant collected after 24 hrs for IFN $\beta$  evaluation using ELISA assay (retrospective analysis).

#### **8. What is the fate of the infused MSC in vivo? Will they last long-term in vivo since they appear to be resistant to T cell effector function? How will their persistence and potential toxicity be tested? Is there a way to eliminate these cells, should they produce unwarranted effects?**

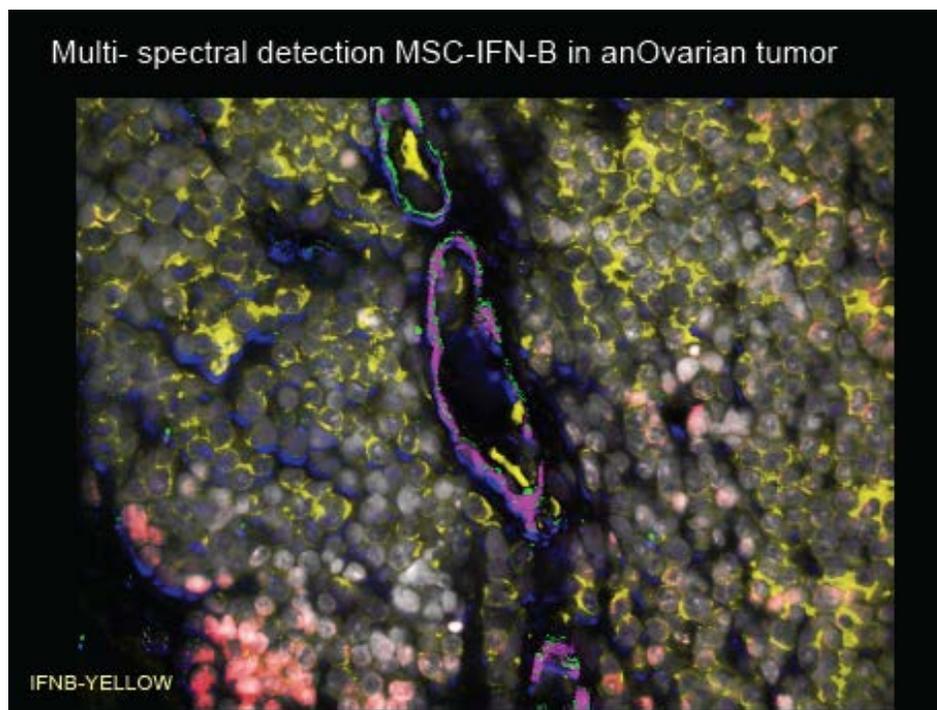
In all mice and animal models tested (rats and dogs) the cells are found exclusively in the tumor. Our data demonstrated that other peritoneal organs do not have detectable MSC. Whether MSC last long-term is unknown but biopsy will be done post-infusion and FISH for Y chromosome will be done to detect donor cells. As tumor size decreases the number of MSCs decreases in mouse models. There is not currently a way to eliminate these cells, the use of a suicide gene such as HSVtk is immunogenic and has been abandoned. We have caspase constructs in our center and will evaluate if needed. For any patient experiencing grade 3/4 toxicity attributable to the treatment, steroids and immunosuppression will be given.

**9. Appendix M-II-A-1-d: What alternative therapies exist? The answer simply says that there is no other study in the Gynecology Oncology group at MD Anderson Cancer Center. This answer is misleading, as it should include other therapies available in general at other Cancer Centers around the US or the world, not just in-house**

For the patient population, the NCCN recommends participation in clinical trials. Over the last decade, the average new agent has achieved response rates of only 5-10%, and progression free survival averages 2 months. Thus, the need for novel therapies and mechanisms for therapy administration is paramount. Alternative therapies for this patient population include early phase trials that do not place a limit on number of prior therapies. The number of these trials that are relevant to the ovarian cancer population typically range between 0 – 10. Indeed, relevant trials may be available at other institutions. Patients will be made aware of other resources, including the NCI Clinical Trials Search Engine, to review these options.

**10. The investigators provide evidence that in the adeno-vector treated MSC-IFNbeta cell mice infusion studies presented in support of this Protocol, the cells home to tumor sites and are undetectable in normal sites. How/when will the investigators demonstrate in the present Protocol, that the plasmid DNA transfected, infused MSC will also home to the ovarian cancer sites and not to other organs, or normal cell sites with the potential to cause toxicity?**

In preliminary experiments we have seen no evidence of differences in engraftment to normal organs. There are pre and post MSC infusion biopsies in which we will harvest tumor cells and subject these cells to IHC to detect FNB and MSC markers using FISH.



**11. In response to Appendix M-II-B-2-d Q1 (If a non-retroviral delivery system is used...) the investigators refer to a Mayo Clinic study on oncolytic adenovirus delivered by MSC into ovarian cancer. However, the investigators should provide evidence that the plasmid DNA-transiently transfected MSC do not give pathological or other undesirable consequences.**

As this is a safety study, in extensive testing in murine models, we have not detected or seen any pathological consequences even in our fully sygneic system (mouse MSC, mouse IFNB, immunocompetent BL6 mouse).

**12. Pregnancy test for females of child-bearing potential is listed in Table #1 (Table of Assessments) as being performed within 7-days of initiating MSC-IFN-beta therapy, while in the protocol (5.0 - Evaluation during Study) is states: "Pregnancy test for females of childbearing potential will be completed within days PRIOR to treatment", which would be a lot more reasonable. This discrepancy needs to be reconciled and Table #1 corrected.**

Table 1 has been corrected to state "prior."

**13. Under 7.3 of the Protocol (Assessment of Systemic Immunomodulation) the only test listed is ELISA for anti-FBS antibodies. No further explanation is given for this test. Is it performed because the MSC are grown in vitro in the presence of FBS? If so, is there a risk of carry-over of FBS into the infused patient? Is the FBS screened before its use, for adventitious agents/viruses? Why is not serum free medium used of MSC cell cultures?**

We will be using human platelet lysate (PLTMax®) in place of FBS for the expansion of our allogeneic MSC. Platelet lysate will also be used during the generation of MSC –expressing IFNb (see attached a copy of certificate of analysis of the PLTMax® from MILL CREEK Life Sciences).

The protocol 2013-0032 has been amended to reflect the fact that human platelet lysate will be used in place of FBS in the generation of MSC. Because we are using PLTMax and not FBS, there will be no need for the ELISA for anti-FBS antibodies.

**14. Serum cytokine levels being tested after the first two doses of MSC are listed under 6.0 (Evaluation During Treatment), but no details are provided on the type of cytokines tested, or on the laboratory performing such studies.**

This has been removed in the most recent version. Cytokines will not be tested in this protocol.

**15. In the Rationale it is stated that infused MSC differentiate into tumor-associated fibroblasts. As these fibroblast cells are transfected to secrete IFN-beta, have the investigators addressed the possibility that IFN will increase the expression of Class-I MHC molecules on these fibroblasts making them a more visible (allogeneic) target for T cell-mediated responses? Is there any plan to test for this potential response?**

This is an excellent question. We do not have the information but plan to evaluate the expression of Class I-MHC molecules on our MSCs following electroporation with the IFN-beta construct moving forward.

### **Minor Comments**

**1. Although the investigators in the Rationale refer to much published work in support of this Protocol, a whole crucial page from the protocol document 2013-0032, December 9, 2013 (page 20) is missing in the document W:\Quality\INDs\APPENDIX Ms\2013-0032 APPENDIX M – Final 011014.docx (please check pages 24 and 25 of 255 and you will see that it goes from page 19 to 21). The reviewer was unable to check those references.**

We apologize for the oversight and sent new references.

**2. In 4.6 (Long-Term Follow-up) there is twice the mention of infused T cells, which should be corrected to infused MSC cells.**

This has been corrected in the most recent version.

**3. At Appendix M-II-B-3-f Q7: How long will follow-up continue? Is the correct answer 15 years? If so, it's easier to state it explicitly.**

Yearly safety evaluations will be conducted for 15 years for patients who receive treatment. Patients will be simultaneously enrolled on MD Anderson Long Term Follow up for Gene Therapy IRB # 2006-0676 at time of enrollment per FDA Biologic Response Modifiers Advisory Committee (BRMAC) guidelines.

**RAC Protocol 2014-1288 REVIEWER: KOHN**

Questions:

**1. The expression plasmid to produce interferon beta uses the “EF1-HTLV mini promoter.” Is there a reference that describes this composite element?**

We tested a number of promoters in MSC and found this composite promoter to be small, efficient, and constitutive in MSC

Enhancer: hCMV (human cytomegalovirus)

Core promoter: hEF1 (human elongation factor 1 alpha)

5'UTR: HTLV (human T lymphocyte virus)

(hCMV)EF-1  $\alpha$ /HTLV: The EF-1  $\alpha$ /HTLV promoter is a chimeric promoter that is comprised of the elongation factor- $\alpha$  (EF1- $\alpha$ ) promoter and the 5' untranslated region of the human T-cell virus (HTLV).

Bernadette Ferraro<sup>1</sup>, Yolmari L Cruz<sup>1</sup>, Domenico Coppola<sup>2</sup> and Richard Heller Intradermal Delivery of Plasmid VEGF<sub>165</sub> by Electroporation Promotes Wound Healing. *Molecular Therapy* (2009) 17 4, 651–657 doi:10.1038/mt.2009.12

This promoter construct is currently available as a cassette from INVIVO gen in their pDRIVE series

**2. Are the MSC grown with fetal calf serum or other xenobiotic reagents in the alpha MEM? This was not stated and if FCS is used, the cells may present those antigens and may induce immune response that limits cell persistence, especially with serial treatments.**

We will be using human platelet lysate (PLTMax<sup>®</sup>) in place of FBS for the expansion of our allogeneic MSC. Platelet lysate will also be used during the generation of MSC –expressing IFN $\beta$  (see attached a copy of certificate of analysis of the PLTMax<sup>®</sup> from MILL CREEK Life Sciences) Appendix 1.

**3. Minimal data were provided on the feasibility of producing cell dosages at the upper range. At the third dose level, a 70 kg subject would be receiving  $7 \times 10^8$  cells. Can the Amaxa electroporation device effectively treat this number of cells in a single or reasonable numbers of cycles to produce this cell dose?**

We certainly understand the concern of the reviewer and our plan is to use 70 cuvettes. We have completed production of  $10^7$  cells/cuvette.

**4. Also, minimal data were presented on the amounts and time-course of interferon beta expression that is produced from the specific MSC line to be used electroporated with the specific expression plasmid to be used (p. 54-255 Appendix M, presented the concentration of IFN-beta in cultures, but did not state the numbers of cells used). The murine data presented in the publication by Dembinski et al (Cytotherapy 15:20-32, 2013) were generated using an adenoviral vector to transduce the interferon beta gene, which may express at higher levels and/or longer time than the electroporation of an expression plasmid. Are more data available on this? The non-integrating nature of the interferon-beta expression plasmid will likely lead to dilutional loss of expression as the MSC divide over time. How do the amounts of interferon predicted to be produced given the cell dosages and the expression per  $10^6$  cells/24 hours compare to the amount given in prior systemic trials (e.g.  $12-18 \times 10^6$  units/m<sup>2</sup> daily or  $6-26 \times 10^6$  IU/day) that led to biomarker evidence of activity, but no clinical objective tumor responses or in the murine studies using adenoviral vector transduction (5 IU muIFN- $\beta$ /10<sup>6</sup> MSC/24hr)?**

This is an excellent question. For direct human relevance, we are referring to a paper by Daniel H. Serman et al. (Nature Clin. Practice, November 2006,3,633-639). The authors infused a single dose of an adenoviral vector encoding IFN $\beta$  ( $9 \times 10^{11}$  viral particles) via a tunneled pleural catheter. There was no detectable system IFN $\beta$ , but intrapleural IFN $\beta$  levels reached 11.5 ng/ml, which declined to undetectable within one week. Table 1 from that paper shows the levels over time. We have inserted our own levels which are in a similar range 9 ng/ml). The patient showed regression of tumor lesions in the abdomen and complete resolution of FDG-PET positive lesions in the abdomen. Since our IFN $\beta$  levels appear comparable, we hope to see tumor regressions.

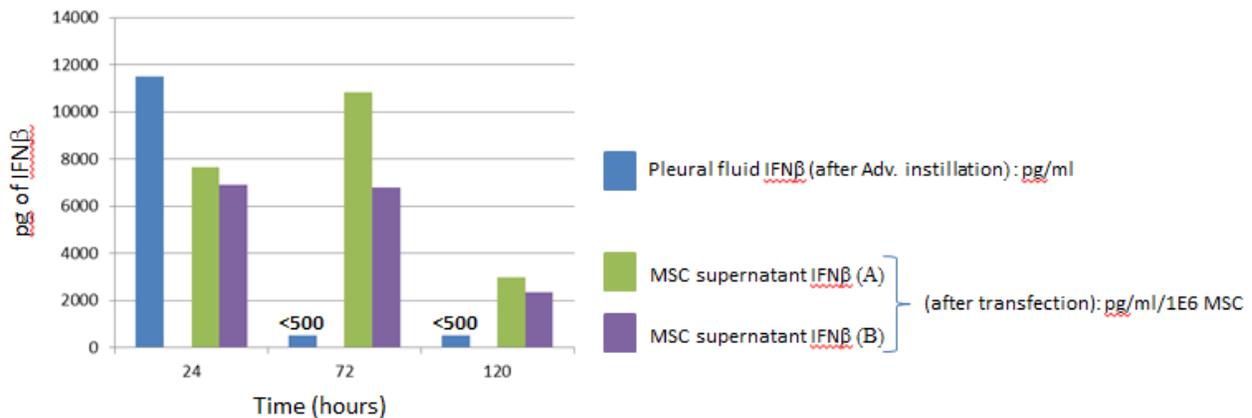
**Table 1** Evaluation of adenoviral shedding and interferon  $\beta$  gene transfer.

Test	Day 1 (before vector installation)	Day 2	Day 3	Day 4	Week 1
<b>Adenovirus culture<sup>a</sup></b>					
Chest-wall swab	Neg	Neg	Neg	Neg	Neg
Plasma	Neg	Neg	Neg	Neg	Neg
Pleural fluid	Neg	Neg	Pos	Pos	Pos
<b>Adenovirus PCR</b>					
Chest-wall swab	Neg	Neg	Neg	Neg	Neg
Plasma	Neg	Neg	Neg	Neg	Neg
Pleural fluid	Neg	Pos	Pos	Pos	Pos
Pleural fluid interferon $\beta$ (ng/ml)	<0.5	11.5	3.1	<0.5	<0.5
Pleural fluid interferon $\alpha$ (ng/ml)	<3.0	<3.0	<3.0	<3.0	<3.0
Pleural fluid interferon $\gamma$ (ng/ml)	<0.2	<0.2	<0.2	<0.2	<0.2
Pleural fluid interleukin 10 (ng/ml)	<0.1	<0.1	<0.1	<0.1	<0.1

<sup>a</sup>Culture for replication-defective vector (as measured on 293 cells). Abbreviations: ND, not done; Pos, positive; Neg, negative.

Adapted from *Sterman et al. 2006*:  
**Interferon  $\beta$  adenoviral gene therapy in a patient with ovarian cancer**

Daniel H Sterman, Colin T Gillespie, Richard G Carroll, Christina M Coughlin, Elaine M Lord, Jing Sun, Andrew Haas, Adri Recio, Larry R Kaiser, George Coukos, Carl H June, Steven M Albelda and Robert H Vonderheide\*



**5. Even the highest cell dose level to be tested ( $1 \times 10^7$  cells/kg) is ~5-fold below the effective dose in the pre-clinical data, where mice were treated with  $1 \times 10^6$  cells which is approximately  $5 \times 10^7$  cells/kg.**

Responses vary and depend on many factors including molecular features of the targeted tumor, the biodistribution of injected MSC and their IFN $\beta$  production kinetics. Not all of these factors can be modeled in mice. This is also a factor why we are proposing IP and not systemic injection. If we see tumor homing of MSC and some evidence of anti-tumor activity, it will be possible to increase the dose of injected cells, hopefully without risking increased systemic toxicity. Depending on experimental conditions, we have also seen responses at lower dose levels.

**6. It appears that the data showing potential efficacy were either done using murine MSC that were syngeneic to the murine hosts or were done using human MSC in immune-deficient mice. Have the potential immune responses to allogeneic MSC, such as those will be used in the clinical trial, been assessed to determine if they further limit the net amounts of interferon beta delivered to the tumor (e.g. Balb/c MSC into C57bl6 mice IP weekly x 4)?**

As the reviewer stated we have no evidence of immune rejection with the human MSCs infused into our NSG mice. We know from our cardiac studies that unmanipulated allogeneic MSCS were as effective as autologous MSCs at improving cardiac function.

**Consent issues:**

**1. Potential Benefit states that “there may be no benefit to you” implying some likelihood of benefit, which is not known for this first-in human Phase I trial This should be stated more realistically, to the effect of “It is unlikely that you will receive benefits from this study”**

The consent has been changed to state “there is no benefit to you in this study.”

**2. The consent describes potential complications of the placement and presence of the intra-peritoneal catheter in very brief wording. Risks of intra-abdominal infection from the presence of the indwelling catheter over the course of 4 weeks, which is being done solely for the research investigation, should be stated more fully.**

We have modified an existing institutional patient education resource to provide trial participants. This resource may be found in Appendix 2 (attached). This document provides information on the definition of intraperitoneal therapy, placement of the catheter, and care of the catheter during the trial. In addition patients will have separate consent in person by our IR collaborators, who will discuss the procedure fully with patients.

The risks have been expanded and modified to include the long-term risk of the indwelling catheter. Previous wording: “Intraperitoneal catheter insertion may cause pain, redness, swelling, bruising, infection, and/or vein irritation may occur where the catheter enters the body.”

The new wording is as follows: “Intraperitoneal catheter insertion may cause pain, redness, swelling, bruising, infection, and /or vein irritation may occur where the catheter enters the body. The placement of the abdominal cavity catheter can result in a puncture of the bowel wall which may result in an infection and require further surgery. Therapy through the intraperitoneal catheter may cause adhesions, interference with bowel function, or obstruction of the bowel. Also, the presence of a catheter in the abdominal cavity can lead to infection (peritonitis). In some cases the catheter may not function well and may need to be replaced in an additional surgical procedure.”

**3. I did not see that the consent states that an autopsy would be requested in the case of the subject’s demise.**

Consent has been changed to include the statement “If you die, autopsy will be requested but is not required.”

**RAC Reviewer: L. Zoloth**

**Review of Ethical Considerations of the Protocol:**

**Ethical Issues**

**1. The informed consent document:**

- A. The informed consent document does not clearly explain that the reason the subject is being asked to participate is that she is at the end of standard therapeutic efficacy. The team uses a standard form, with references to 15 year follow-up and admonitions to avoid pregnancy. Should an informed consent to critically ill women facing a grim prognosis include such references? It is important tell a consistent and factual narrative about the situation that the women are facing. This situation should be clearly stated.**

Section 1, paragraph 2 has been revised to state, "You are being asked to take part in this study because you have ovarian cancer that has not responded to treatment or has relapsed (returned) after standard of care treatment and no other standard treatments are available and there are no curative options for your cancer." Regarding the references to the 15-yr follow up, and admonitions to avoid pregnancy, the contraception statements are automatically populated in the template when drugs that are teratogenic are used in a study and is required by our IRB. The reference to 2006-0676 (LTFU study) is required for gene therapy studies by the FDA.

- B. There is reference to an ombudsman at the Cancer Center, someone who would act as advocate in clinical trials, in the documents for the RAC. But in the informed consent document, the subject is not told about this person, but instead is told to go to the study director, or the chair of the IRB. Neither of these options are reasonable, in my opinion. In the first instance it would be counter intuitive, and in the second wildly intimidating for a patient to find the name, contact information, etc, for a senior medical faculty member, who chair a committee that is never explained.**

The instructions to contact the study chair and or IRB chair are standard language in our Informed consent documents and standard practice in our institution. The phone number for the IRB office is provided in the ICD.

We do have patient advocates available to patients that generally serve as a liaison between the care team and the patient to bridge situations of concern. We have added into the consent document the following statement:

"You may contact the Patient Advocate that was assigned to you at registration if you have concerns or problems at 713-792-7776 (after hours and weekends, call 713-792-7090). The Patient Advocacy offices are located at MD Anderson in the Main Building,

Floor 1, near Elevator A, R1.1532 and at Mays Clinic, Floor 2, near the Tree Sculpture, ACB2.2135.

<http://www.mdanderson.org/patient-and-cancer-information/guide-to-md-anderson/patient-and-family-support/patient-advocacy.html>

**C. The information that INFb infusions only affected the blood chemistry and not the actual disease process when it was tried should be explained, and it should be explained what the new hypothesis is, in clear terms.**

The following statement has been included in the informed consent:

“We hypothesize that since MSCs will deliver the interferon beta directly to tumor that interferon may affect the tumor and we will be doing biopsies after treatment to determine the effect of treatment on tumor tissue.”

**D. The source of the cell, (male) and why they use male cells should be explained.**

The following has been added to the consent “Mesenchymal stem cells are obtained from healthy male donors. The use of male cells will help us to detect the cells that are infused versus your own female cells.”

**E. The process of plasmid infection should be described and the actual term “genetically modified” and “viral vector” used and explained. In short, everything that is in the scientific abstract should be made clear to the subject.**

We are not using a viral vector for the MSC transfection but an expression plasmid.

The following has been added to the consent document:

The mesenchymal stem cells will be transfected with an expression plasmid that produces interferon beta. This is accomplished by use of an electroporation device (Amaxa System), a tool commonly used to deliver genetic material into cells. The process will be carried out in cell culture (in vitro). Transport of the interferon beta genes into the mesenchymal stem cells is called transduction. This is the process by which the cells are genetically modified (have new gene material) to allow them to then produce interferon beta.”

**F. The term “may be of no benefit to you,” used to describe the intervention needs to be eliminated. The consent should say clearly that the only reason**

The consent has been changed to state “there is no benefit to you in this study.”

## 2. The process of home care.

- A. The infusion is delivered at the medical center, but the patient is sent home. There is some reference to housing for staff. The side effects described are impressive. What are the pros and cons of this informal monitoring system?**

Given the minimal and extremely limited side effects noted in millions of rheumatoid arthritis patients who receive interferon systemically and the thousands of patients who have been treated with MSCs for cardiac ischemia and graft versus host disease we do not expect the patients to require additional monitoring beyond our standard policies for Phase I trials.

In a case report in *The Annals of Pharmacotherapy*, Falcone et al, 2005 a MS patient attempted an overdose by self-administering 6-7 syringes containing 44 µg of interferon beta and only resulted in a modest rise in body temperature and mild skin redness, this was treated with aspirin and fluids and resolved in 24 hours. 1<sup>^</sup>MSCs roughly produce (in vitro) around 10 ng of IFNβ in the first 24 hrs after transfection and then the level of secreted IFNβ goes down with time therefore, one prefilled syringe with 44ug of IFNβ is 4,400 times higher than what 1E6 MSC can produce at maximum peak (within the first 24hrs) thus the dose this patient received was considerably higher than the dose levels we expect.

In randomized, double-blind, placebo-controlled study of MSCs in patients following acute MI adverse event rates were actually lower for MSC-treated versus the placebo-treated groups N = 53 and no AEs were considered to have probable relation to study treatment (*J Am Coll Cardiology*, Hare, 2009) the dose used in this study was a single infusion of 2.5 X 10<sup>^</sup>6 MSCs/ml.

It is possible (but unlikely) that with genetically modified MSCs secreting interferon beta there will be unexpected side effects but the patient instructions, which will be reiterated by the infusion center staff, are to report to the emergency center immediately if experiencing anything out of the ordinary.

- B. Is there an assumption that the subject will have a support system for close monitoring? If so, this needs to be a part of the consent process. What of women who do not have such a system? In the name of justice, no women should be excluded because she is alone.**

Please see above answer to 2A, we do not suspect the subject to require additional monitoring beyond our standard policies based on available toxicity data in humans.

- C. The project requires an indwelling abdominal catheter, but no picture is shown that describes this, how one lives with it, and what sort of care is needed to prevent**

**infections. Since the immune system is being altered with the trial itself, will this problem need special attention?**

We have modified an existing institutional patient education resource to provide trial participants. This resource may be found in Appendix 2 (attached). This document provides information on the definition of intraperitoneal therapy, placement of the catheter, and care of the catheter during the trial. The impact of this treatment on the risks of intraperitoneal therapy is unknown; however, this will be monitored closely. In addition patients will have separate consent in person by our IR collaborators, who will discuss the procedure fully with patients.

The risks listed in the consent form have been expanded and modified to include the long-term risk of the indwelling catheter. Previous wording: "Intraperitoneal catheter insertion may cause pain, redness, swelling, bruising, infection, and/or vein irritation may occur where the catheter enters the body."

The new wording is as follows: "Intraperitoneal catheter insertion may cause pain, redness, swelling, bruising, infection, and /or vein irritation may occur where the catheter enters the body. The placement of the abdominal cavity catheter can result in a puncture of the bowel wall which may result in an infection and require further surgery. Therapy through the intraperitoneal catheter may cause adhesions, interference with bowel function, or obstruction of the bowel. Also, the presence of a catheter in the abdominal cavity can lead to infection (peritonitis). In some cases the catheter may not function well and may need to be replaced in an additional surgical procedure."

### **3. The hypothesis**

- A. The plan is for the MSC to be administered into the peritoneum, and the cells to hone into the tumor. What are the reasons to prevent the cells from engrafting to other organs? If the effect of INFb is to disrupt cellular microenvironments, how is the risk contained in the animal model? This needs to be explained. Can the cells migrate and engraft in other places?**

The effects of IFN $\beta$  are threefold. 1) it has direct cytotoxic effects on cells as it activates caspases (Kidd paper) , 2) it disrupts the cellular milieu by recruiting a number of immune cells which now recognize the tumor microenvironment as hostile and 3) its modulates the local immune responses (John Ling paper).

In dissection of post mortem mouse specimens the MSCs were not found in other organs. As the tumor shrinks, we have shown that the number of MSCs also decreases.

Kidd S, Caldwell L, Dietrich M, Samudio I, Spaeth EL, Watson K, Shi Y, Abbruzzese J, Konopleva M, Andreeff M, Marini FC. [Mesenchymal stromal cells alone or expressing interferon-beta suppress pancreatic tumors in vivo, an effect countered by anti-inflammatory treatment.](#)

Cytotherapy. 2010 Sep;12(5):615-25. doi: 10.3109/14653241003631815. Erratum in: Cytotherapy. 2011 Apr;13(4):498

[Ling X, Marini F, Konopleva M, Schober W, Shi Y, Burks J, Clise-Dwyer K, Wang RY, Zhang W, Yuan X, Lu H, Caldwell L, Andreeff M.](#) Mesenchymal Stem Cells Overexpressing IFN- $\beta$  Inhibit Breast Cancer Growth and Metastases through Stat3 Signaling in a Syngeneic Tumor Model. Cancer Microenviron. 2010 Mar 19;3(1):83-95. doi: 10.1007/s12307-010-0041-8.

**B. The side effects range from hives to suicide in an undifferentiated list. Why is suicide on the list? Depression? This sort of thing needs to be explained.**

We have clarified this in the most current version of the protocol; these are all possible listed side effects of interferon beta. Depression and suicide have been reported and this is why we have included a collaborating psychiatrist and incorporated the PHQ-9 (Depression screening) into our protocol to be administered as a screening for new symptoms weekly while patients are receiving treatment.

**C. It is unclear what happens to the cells in vivo. How is this monitored?**

The MSCs have been shown to travel to sites of inflammation, we have documented this in published papers. This will be monitored by post-treatment biopsies.

[Tracking inflammation-induced mobilization of mesenchymal stem cells.](#)

Spaeth EL, Kidd S, Marini FC. Methods Mol Biol. 2012;904:173-90. doi: 10.1007/978-1-61779-943-3\_15.

[Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescent imaging.](#)

Kidd S, Spaeth E, Dembinski JL, Dietrich M, Watson K, Klopp A, Battula VL, Weil M, Andreeff M, Marini FC. Stem Cells. 2009 Oct;27(10):2614-23. doi: 10.1002/stem.187.