

RAC #1401-1288

Phase I Study to Determine the Effects of Mesenchymal Stem Cells Secreting Interferon Beta in Patients with Advanced Ovarian Cancer

Shannon Westin, MD, MPH, Co-Principal Investigator

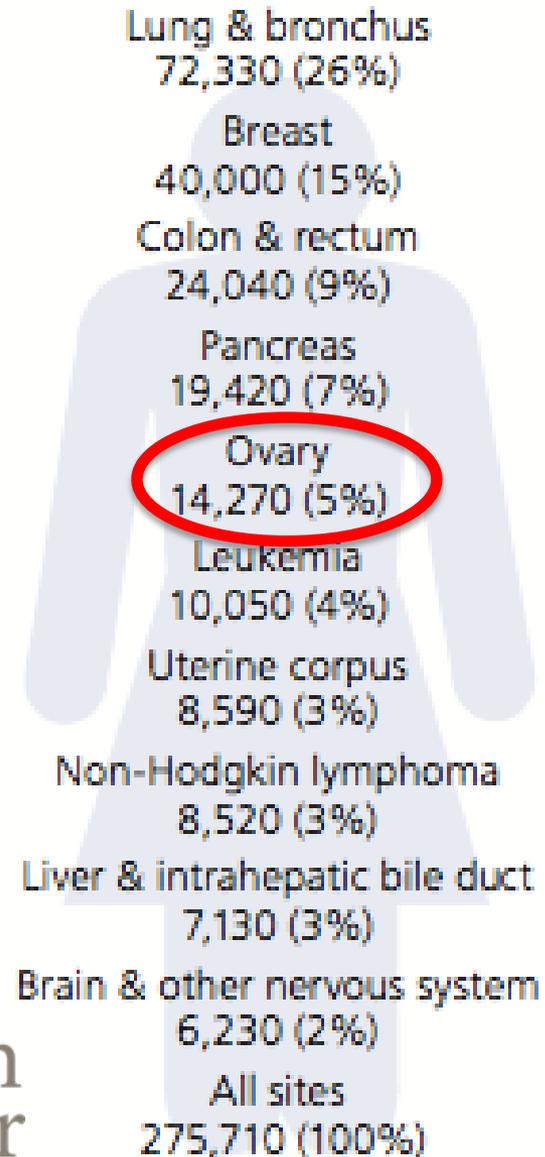
Amanda Olson, MD, Principal Investigator

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Ovarian Cancer Facts and Figures

- US
 - 21,980 new cases
 - 14,270 deaths
- Worldwide
 - 192,000 new cases
 - 114,000 deaths

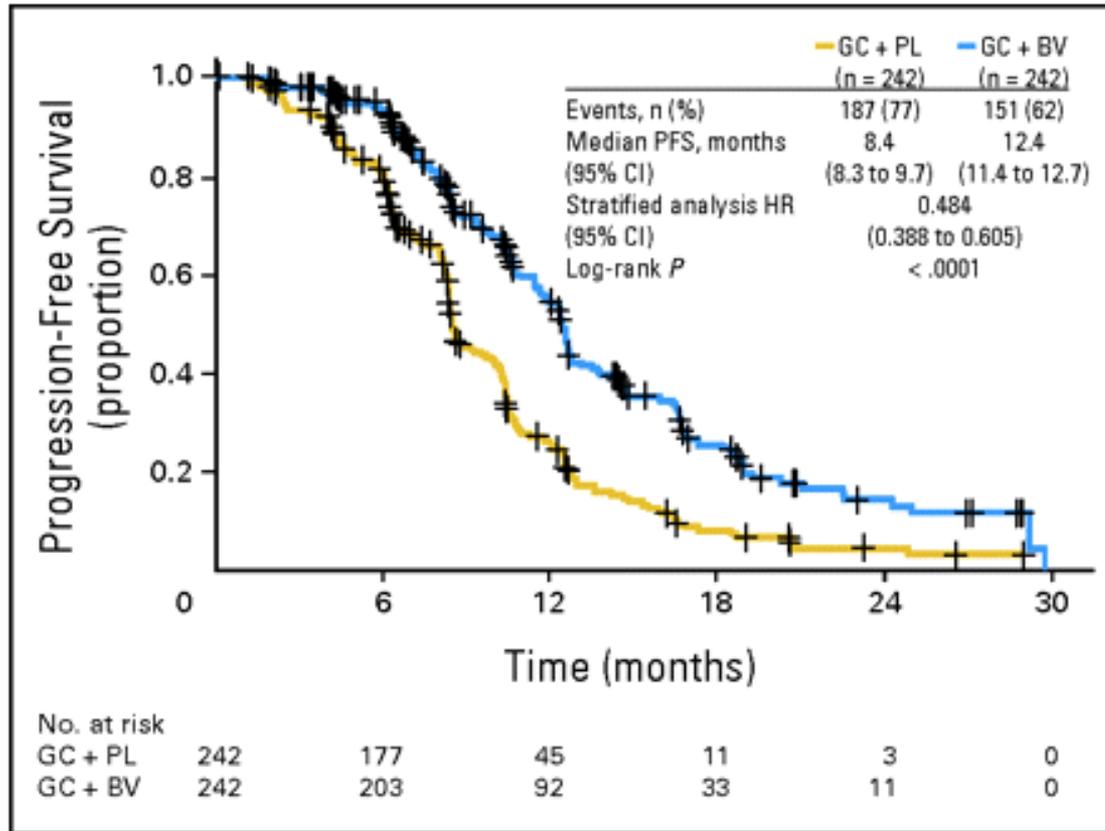
Siegel et al. CA J Clin 2014



Ovarian Cancer: Stage at Diagnosis

Stage	Description	Incidence	Survival
I	Confined to ovaries	20%	90%
II	Confined to pelvis	5%	66%
III	Confined to abdomen/ lymph nodes	58%	35%
IV	Distant metastases	17%	18%

Recurrent Ovarian Cancer



Current state-of-knowledge

• Front-line Therapy:

- Surgery
 - Comprehensive staging or cytoreduction for metastatic disease
 - Tumor residuum is the strongest prognostic factor
- Adjuvant therapy
 - Nearly all cases
 - Platinum-taxane based

Some are cured!

• Recurrence Therapy:

- Chemo-sensitive disease
 - Approach like frontline therapy
 - Surgery (+/-)
 - Platinum based
- Chemo-resistant disease
 - Emergence is cause of death
 - Multiple agents of poor response

None are cured!

*Roles of biologic and maintenance therapy
are undetermined*

Active Agents in Ovarian Cancer

FDA approved		
Altretamine	Carboplatin	Cisplatin
Cyclophosphamide	Paclitaxel	Pegylated liposomal doxorubicin
Topotecan	Gemcitabine/ Carboplatin	
Not FDA approved, compendium listed		
Aromatase Inhibitors	Bevacizumab	Capcitabine
Docetaxel	Doxorubicin	Etoposide
Ifosfamide	Irinotecan	Leuprolide acetate
Megestrol acetate	Melphalan	nab-paclitaxel
Pemetrexed	Tamoxifen	Vinorelbine

Alternative Therapies

Agents	Response
Cytotoxics	2 – 20%
Angiogenesis/Signalling	
Bevacizumab	10-25% RR alone/combo
Aflibercept	5% RR
PARP inhibition	15-40% RR

NCCN recommends clinical trial participation

MDACC – Phase I options

Clinical trials.gov

Consent Changes

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.”

Why Interferon?

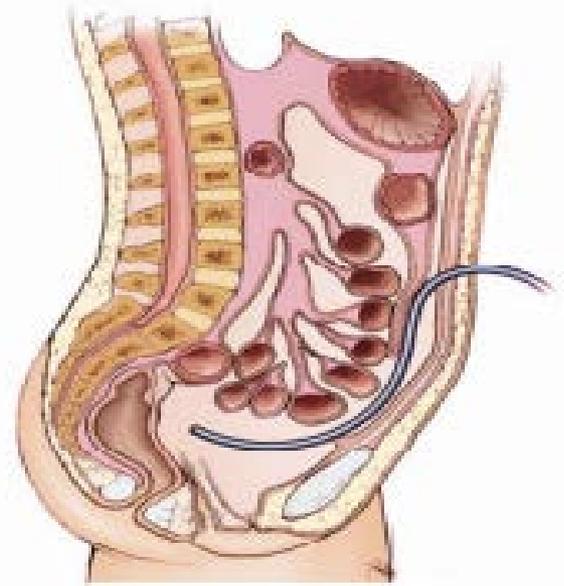
- Activity in ovarian cancer cell lines
 - Alone and in combination with cytotoxics
- Short $\frac{1}{2}$ life parenterally
- Small IP study: 1987
 - 45% response rate

Why Intraperitoneal?

Clinical Trial	IP OS	IV OS	P
GOG 104 (Alberts, 1996)	49	41	0.02
GOG 114 (Markman, 2001)	62	53	0.05
GOG 172 (Armstrong, 2006)	65	49	0.03

Why Intraperitoneal?

- 70% of MDs offer in upfront
 - 25% of patients treated
- Safety
 - Real-Time Imaging Guidance
 - +/- Fluoroscopy
 - Mitigate organ injury
- Minimal toxicity (10-20%)
 - Placement: bleeding, infection, organ damage
 - Presence: Infection, peritonitis, bowel dysfunction
 - Serious adverse events: < 5%



Patient Information

Overview of IP Therapy

What is IP therapy?

IP therapy is the delivery of anti-cancer drugs directly into the peritoneal space (abdominal cavity). This space lies between the abdominal muscles and the abdominal organs. The therapy is infused into the peritoneal space to provide direct contact with the cancer to coat and cover it.

How is the chemotherapy put in the peritoneal space?

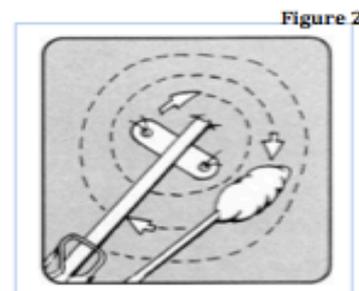
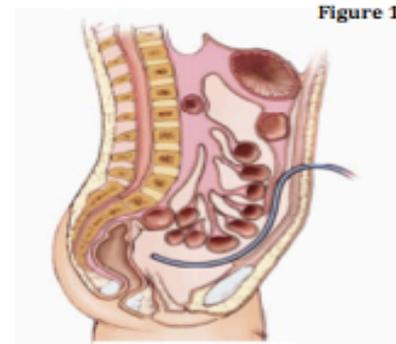
The therapy is mixed in fluid and then slowly infused into the peritoneal space through an external IP tunneled catheter. This device is inserted in the Department of Interventional Radiology. You will be taken to an imaging suite, where you will have either a fluoroscopy, CT scan or ultrasound. These tests are done to gain safe entry into the abdomen in order to place the IP catheter.

This flexible catheter is placed in the peritoneal space. The external IP catheter is placed in the peritoneal cavity and exits the body through a small opening in the abdomen. (Figure 1.) The exit site is covered with a transparent or alternate dressing. (Figure 2.)

Where will I receive the IP therapy?

You may receive IP therapy in the outpatient setting.

Who will give the IP therapy?



Amanda Olson, MD

March 12, 2014

THE UNIVERSITY OF TEXAS
MD Anderson
~~Cancer Center~~

NIH-OBA 1401-1288

Rationale

- Genetically modified Mesenchymal Stem Cells (MSC) have been shown to engraft and secrete gene products at tumor sites in animal models.
- By gaining entry to tumor stroma, the genetically modified MSC are believed to regulate tumor cell growth by modulating the microenvironment making it inhospitable for tumor growth.

Trial Overview

- MSCs derived from healthy male donors (generated in MDACC Clinical MSC banking protocol PA13-0025) will be transfected via electroporation with plasmid vector containing the IFN-beta (β) gene.
- MSC-IFN β will be administered intraperitoneally to patients with relapsed ovarian cancer with measurable disease to modulate the growth of epithelial ovarian cancer.

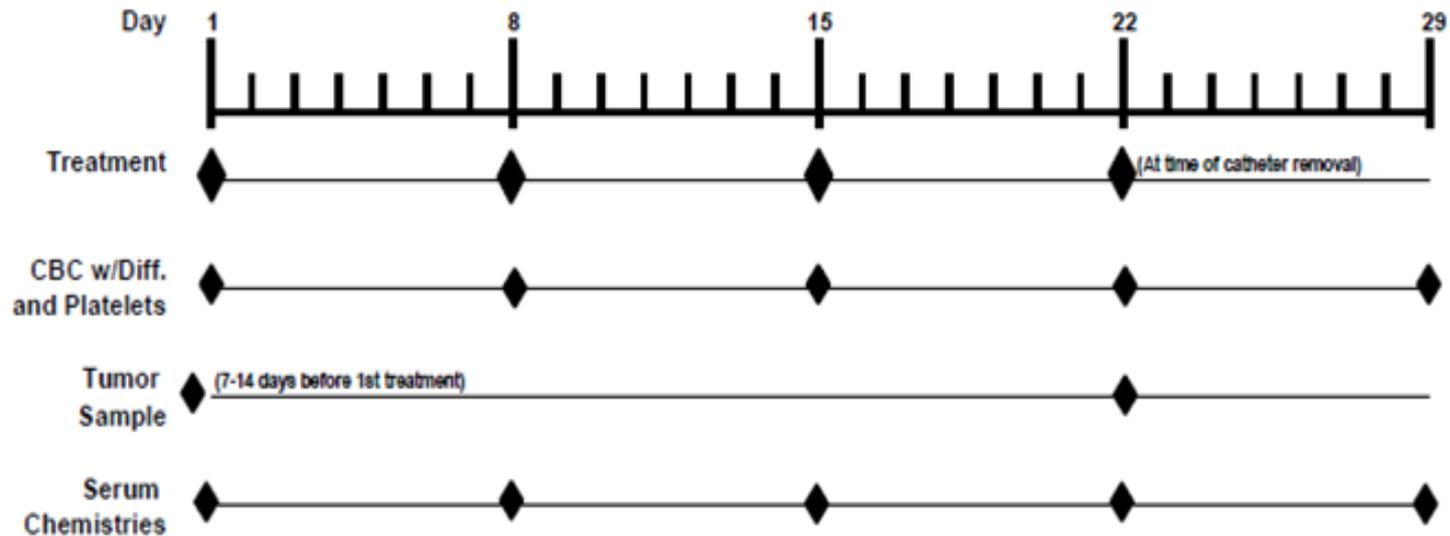
Protocol Objectives

- Primary
 - Safety and feasibility of allogeneic MSC-INF β in patients with relapsed, measurable, and biopsiable ovarian carcinoma.
 - Determine if there is a correlation between the number of MSC-INF β infused, the number of MSC-INF β detected at the tumor sites, and the production of INF β .
- Secondary
 - Determine the effect of pro-apoptotic and anti-proliferative effects of IP MSC-INF β in epithelial ovarian cancer lesions.

Treatment Schema

- Baseline tumor sample obtained by invasive radiology (IR) at catheter placement (7 to 14 days before treatment)
- MSC-IFN β cells infused IP weekly X 4 doses (+/- 2 days).
- At catheter removal additional tumor sample obtained by IR.

Treatment Schema



*CT within 21 days prior and within 21 days post treatment, then every 8 weeks for CR/CR.

Dose Escalation

Level	Dose
-1	10^4 MSC-IFN β /kg 4 treatments
+1	10^5 MSC-IFN β /kg 4 treatments
+2	10^6 MSC-IFN β /kg 4 treatments
+3	10^7 MSC-IFN β /kg 4 treatments

- 3 patients enrolled at Dose Level +1, and we will enroll patients in cohorts of size 3.
- If Grade 3 or 4 toxicity at dose level 1, decrease dose to level -1 for following cohort.

Response Assessment

- Pre and post-treatment biopsy specimens evaluated for:
 - Plasmid-IFN β using Taqman PCR
 - Presence/frequency of MSC-IFN β in tumor
 - FISH for Y chromosome using quantitative automated spectral imaging of >1,000 cells
 - Apoptosis/proliferation assessment by TUNEL/Ki67
- The effect of MSC-IFN β on measurable disease (i.e., efficacy) determined by imaging.

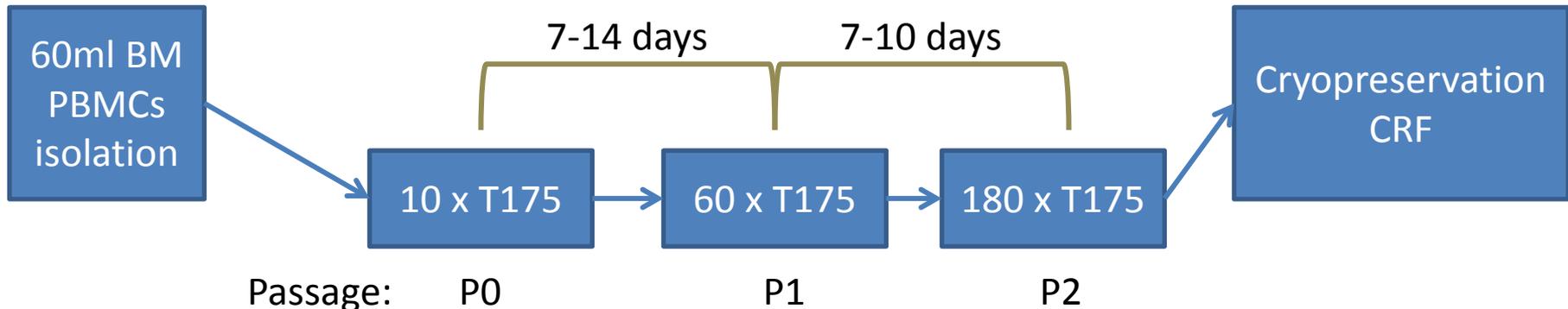
Management of Potential Toxicity

- Management of adverse event (AE) attributable to INF β (SQ or IV)
 - Close monitoring for common symptoms associated with systemic treatment with INF β :
 - Flu-like symptoms
 - Cough
 - Gastroenteritis
 - Conjunctivitis
 - Cystitis
 - Pneumonia
 - LFT abnormalities
 - Erythema/hives
 - Depression
 - Treatment with systemic steroids/additional immunosuppression as indicated.

MSC Derivation/Cryopreservation

- Allogeneic MSC derived and cryopreserved and donors screened per Protocol PA13-0025 under which the MSCs will be generated.
 - Donor screening involves extensive history/physical/laboratory workup to ensure no evidence of infection, immune disease or hematologic disorder and outlined in detail in protocol.

Overview of MSC expansion Protocol PA13-0025

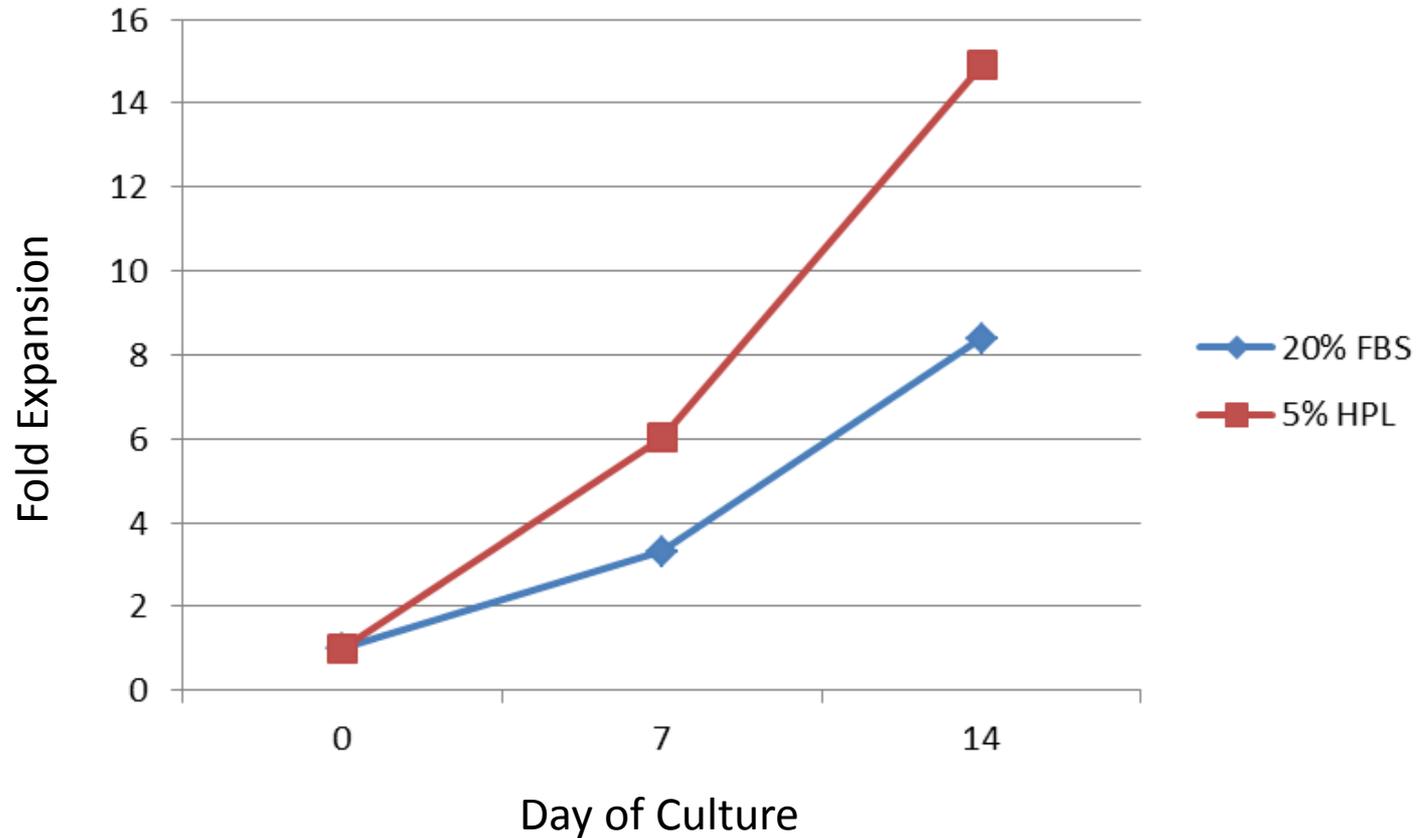


- Under GMP conditions, the MSC fraction isolated from 60ml of marrow and seeded into 10 tissue culture flasks in alpha MEM containing 5% Human Platelet Lysate. After 10-14 days of culture, passage zero (P0) cells harvested, expanded in 60 tissue culture flasks (P1), cultured for 7-10 days before second MSC expansion in 180 (P2) treatment (P1 cells). Cells frozen with control rate freezer.
- Using this approach we generate 1.5×10^9 MSCs per 60 ml of donor marrow.

Fetal Bovine Serum (FBS) vs. Human Platelet Lysate (HPL)

- For MSC ex vivo culture, HPL has been used increasingly in clinical MSC trials.
- Since submission, we have evaluated HPL for expansion of MSC in comparison with FBS.
- The HPL produced superior expansion results and we have amended our protocol for the expansion of MSC to reflect the use of HPL rather than FBS.

MSCs Fold-Expansion



Capability of Amaxa Nucleofector Device to Produce MSC Doses



We have completed production of 10^7 MSCs/cuvette and will use 70 cuvettes, utilizing several devices for the higher dose levels.

Allogeneic MSC Release Criteria

Test at time of cryopreservation of MSCs	Laboratory	Specifications
Visual Inspection	MDACC GMP QC	No evidence of contamination
Viability (7AAD)	MDACC GMP QC	≥ 70%
Endotoxin (LAL)	MDACC GMP QC	< 1 EU/ml
Mycoplasma (PCR)	BIORELIANCE	Negative
Sterility - BACTEC	MDACC Microbiology	No growth in 14 days
Immunophenotyping	MDACC Flow Cytometry Laboratory	CD3+ <5% - CD45+ <5% CD73+ >70% - CD90+ >70% HLA-ABC+ >70% - CD105+ >70%

MSC-IFN β Release Criteria

Test at time of infusion of MSCs	Laboratory	Specifications
Visual Inspection	MDACC GMP QC	No evidence of contamination
Endotoxin (LAL)	MDACC GMP QC	< 5 EU/kg
Sterility – Gram Stain	MDACC Microbiology	No organisms seen

Changes to Protocol

- Pregnancy test for females previously “within 7-days of initiating MSC-IFN-beta therapy” now “within 7 days PRIOR to treatment”
- Question re cytokine levels without specification: cytokine evaluation removed from protocol
- Correction from T cells to MSC cells has been made in Long-Term Follow up

Changes to Informed Consent (IC)

- Potential benefit: previously stated “There may be no benefit to you” changed to “there is no benefit to you in this study.”
- Autopsy request added to IC.
- Clarification of hypothesis now in IC: “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.”
- Donor cell source and explanation in IC: “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.”

Changes to Informed Consent (IC)

- Regarding the recommendation to include explanation of scientific terms we have included the following:

“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.”

Consent Changes/Explanations

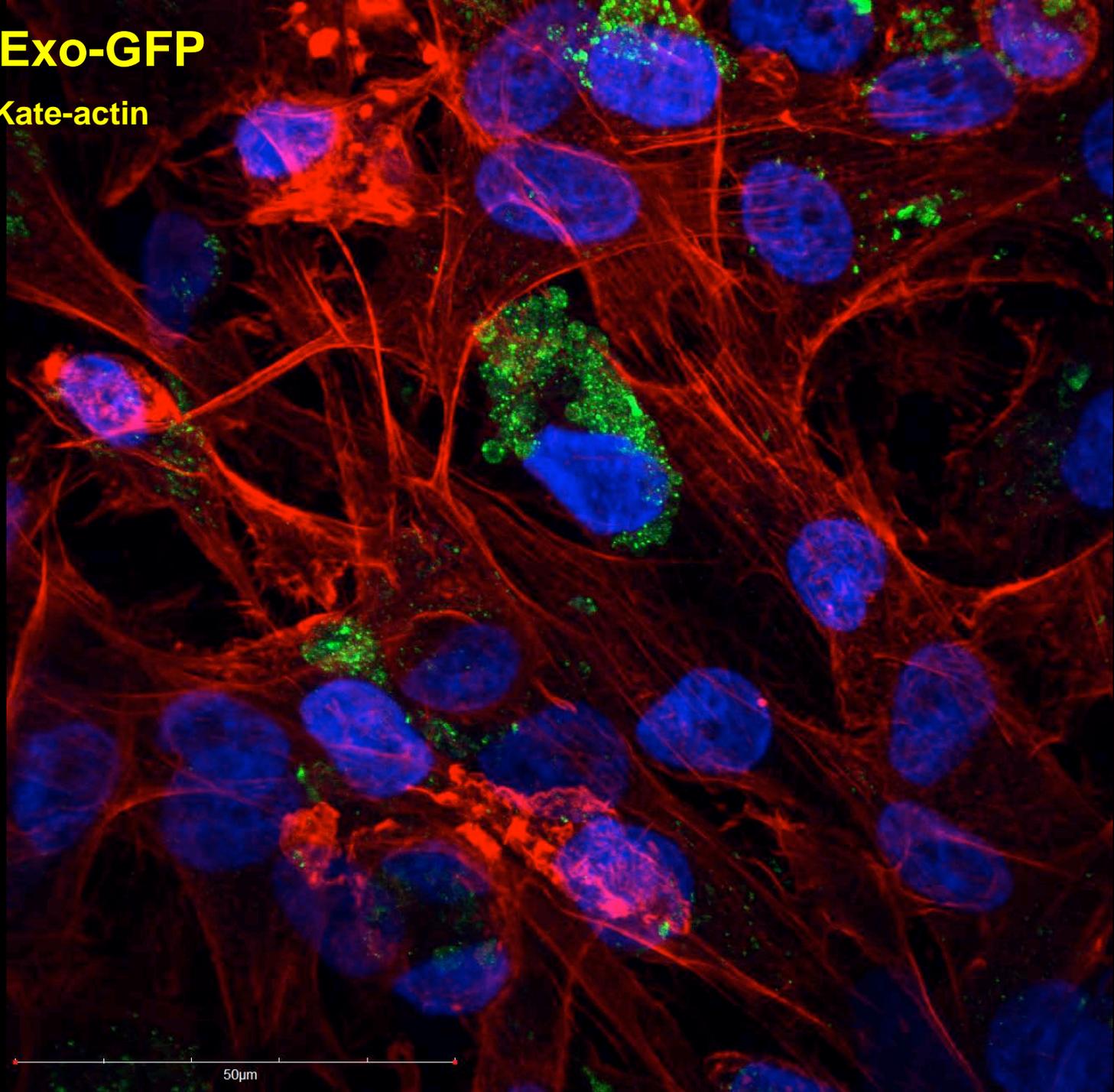
- Contact for advocacy:
 - The instructions to contact the study chair and or IRB chair are standard language in our Informed consent documents and standard practice in our institution. The phone number for the IRB office/PI is provided in the ICD.
 - Patient advocates are available to patients and can 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.”*

Consent Explanations

- Regarding 15 year follow up reference: The reference to 2006-0676 (LTFU study) is required for gene therapy studies by the FDA Biologic Response Modifiers Advisory Committee (BRMAC) guidelines.
- Admonitions to avoid pregnancy/contraception statements are automatically populated in the template when drugs have potential to be teratogenic are used and are required by our IRB.

Michael Andreeff, M.D., Ph.D.
Frank Marini, Ph.D.

Skov3-Exo-GFP
MSC-mKate-actin

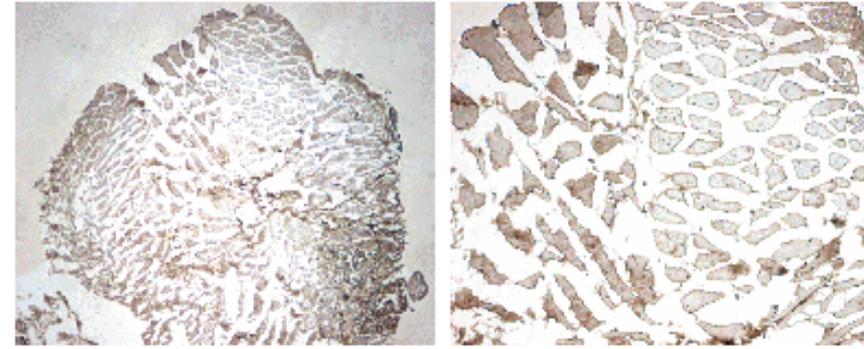
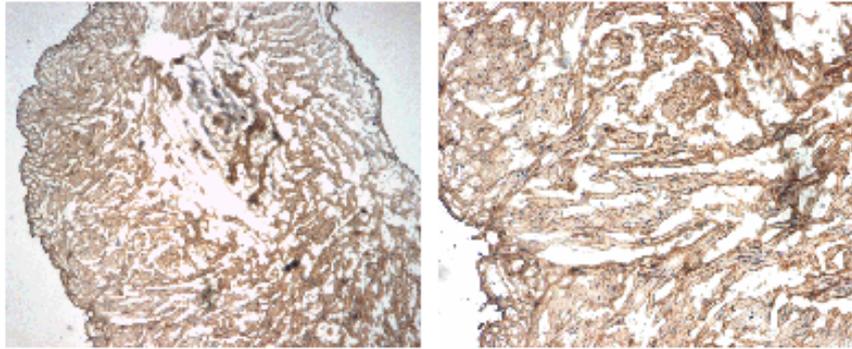


Intratumoral Distribution of IFN- β In Ovarian Cancer After I.P. Injection of MSC IFN- β

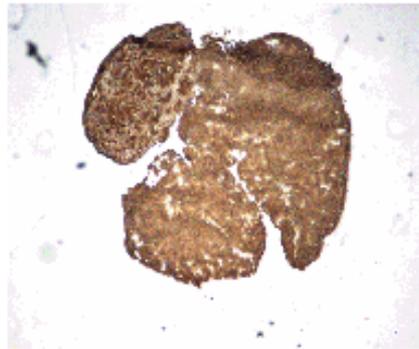
1 Day Post-Injection
of MSC-IFN β (I.P.)

OVCAR-3 Tumors

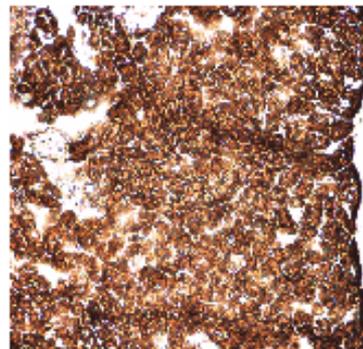
SKOV-3 Tumors



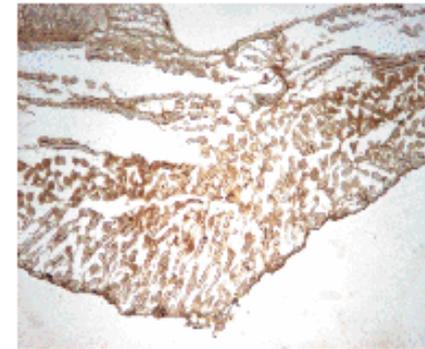
3 Days Post-Injection
of MSC-IFN β (I.P.)



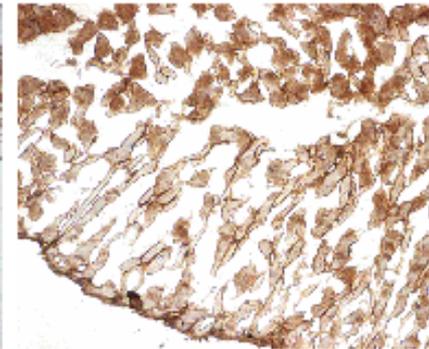
4x



10x



4x

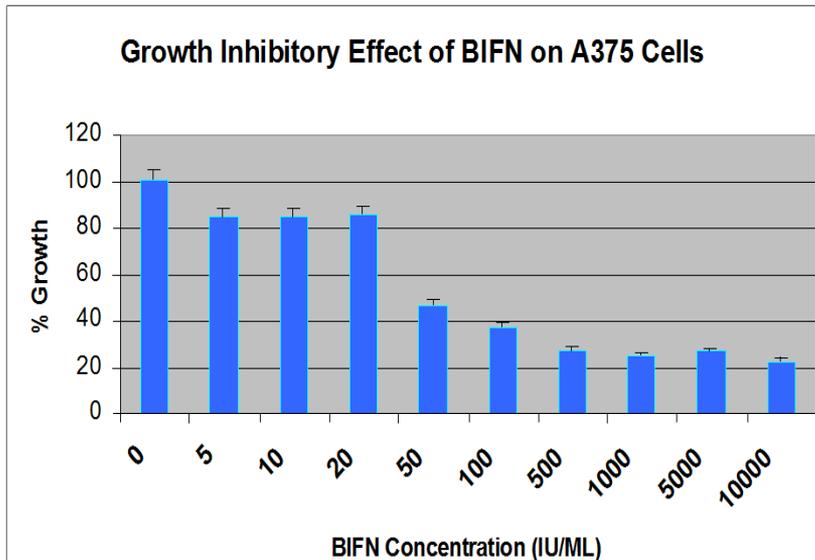


10x

Rationale of IFN- β For MSC Based Cancer Therapy

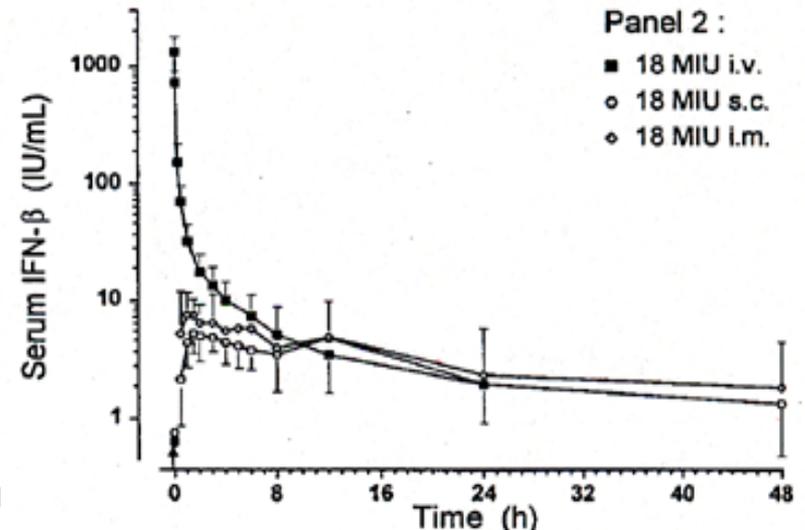
MSC will produce high local levels of IFN- β in tumors that can not be achieved by its systemic administration

High concentration is required for inhibition of malignant cells *in vitro*



I-OBA 1401

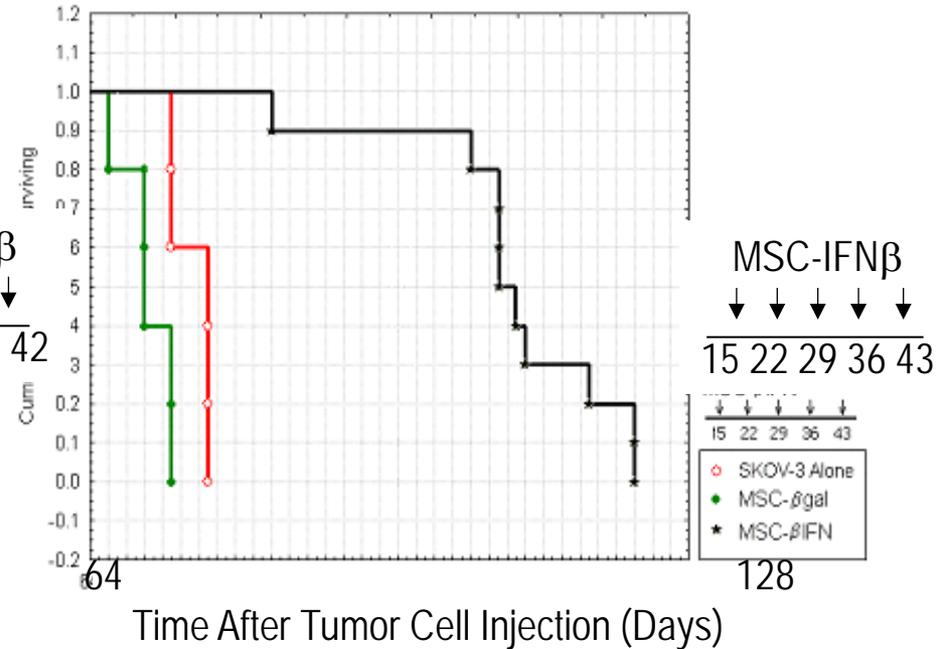
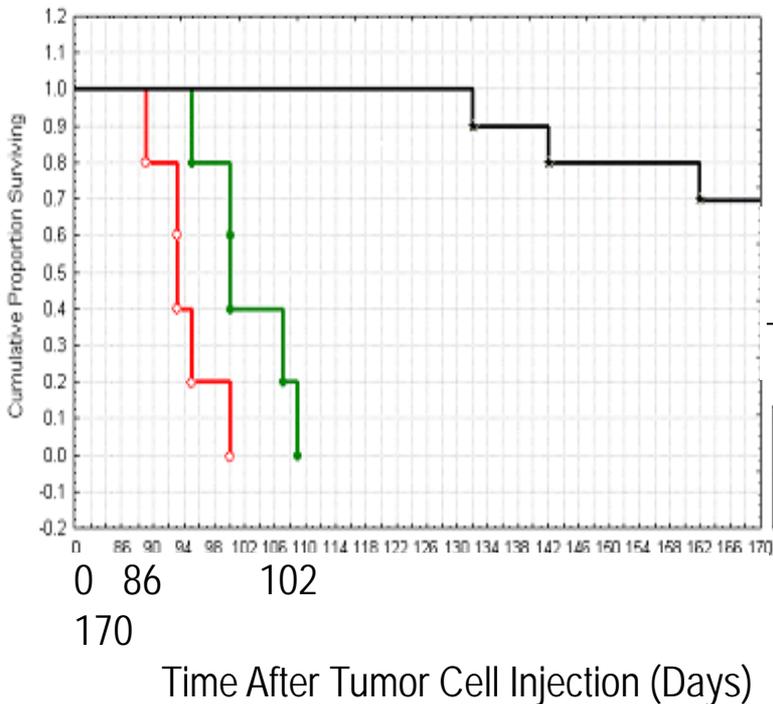
Low Serum concentration after MTD of IFN- β *in vivo*



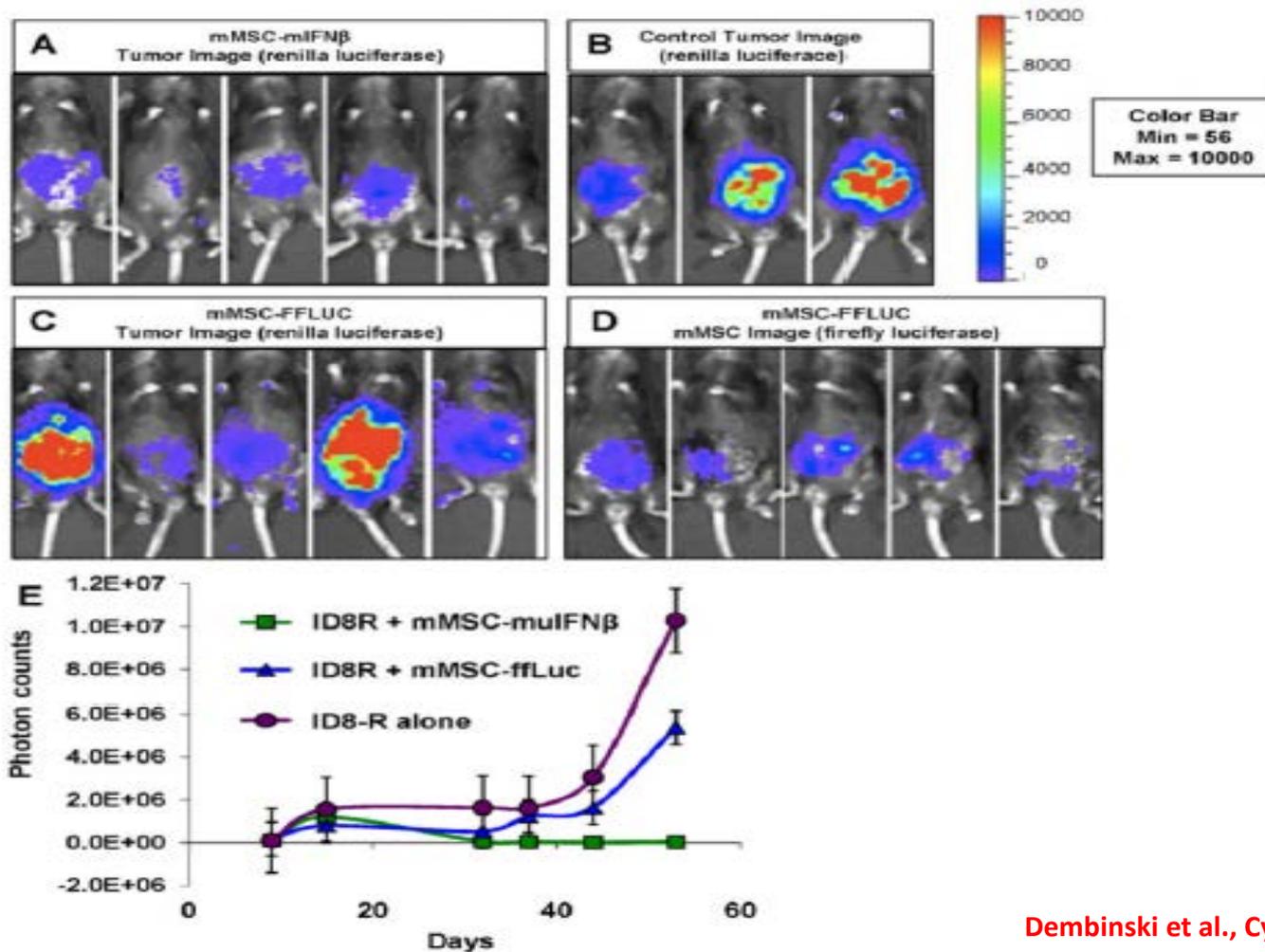
Effect of MSC-IFN β on Survival of Ovarian Cancer Xenografts in Mice

OVCAR-3

SKOV-3



Effect of MSC-IFN β on Syngeneic ID8-R Ovarian Carcinoma



Dembinski et al., *Cytotherapy* 2013

Adeno-Vector Expressing INF-beta in MSC vs. Transient Transfection of MSC with Plasmid DNA

Our decision to utilize plasmid-based delivery system:

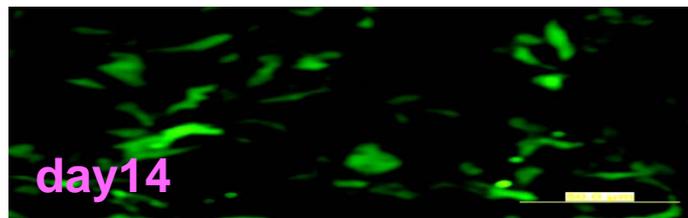
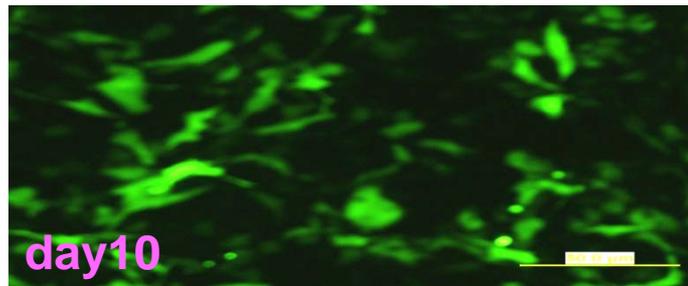
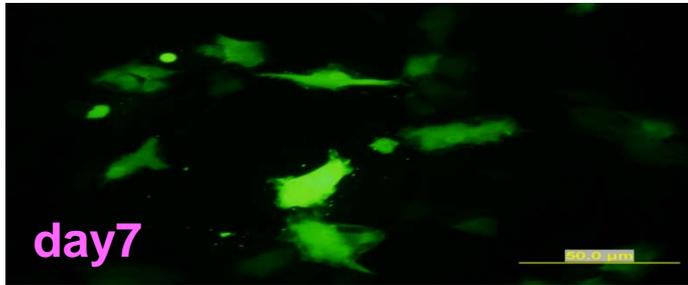
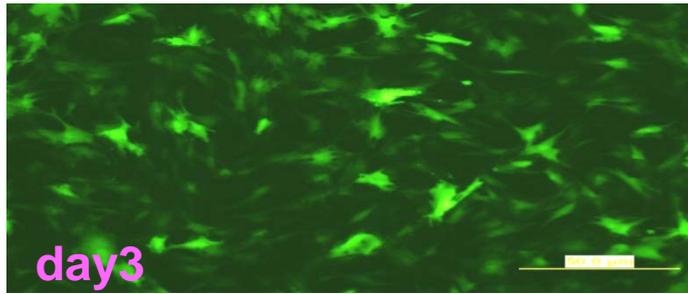
Safety

- Less immunogenicity
- No integration
- Higher level of IFN β expression with adenovirus (50,000 IU), could potentially produce toxicity

Simplicity

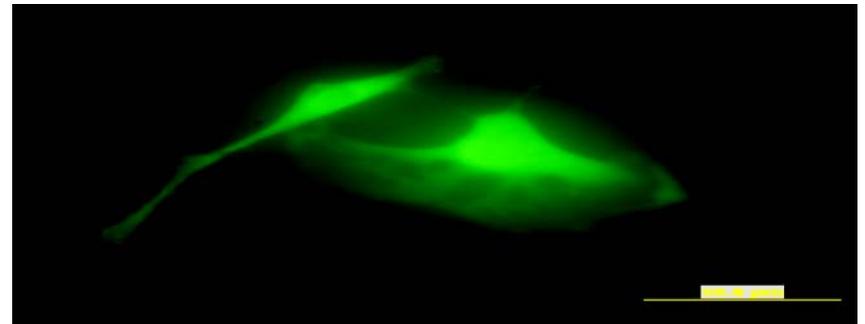
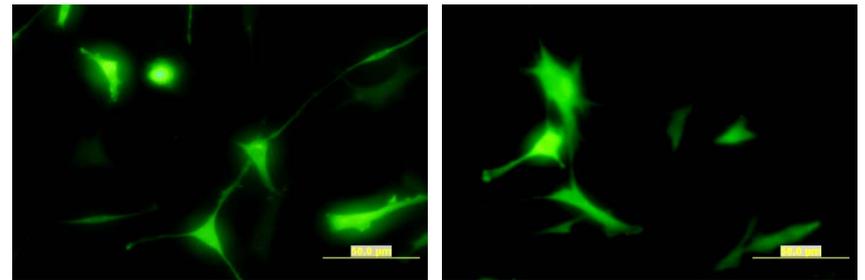
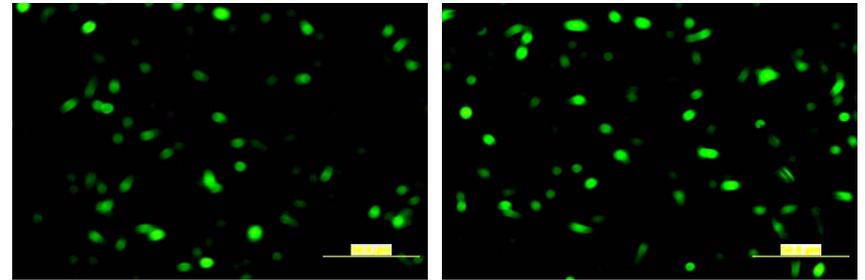
- Amaxa technically simple
- Reproducible

Ad-GFP-F/RGD



Comparison between Adenovirus and Plasmid Electroporation in MSC

AMAXA-cmv-GFP



IFN β Levels after Adenoviral Gene Therapy of an Ovarian Cancer Patient and Plasmid Electroporation of MSC (24 – 72 -120 hrs)

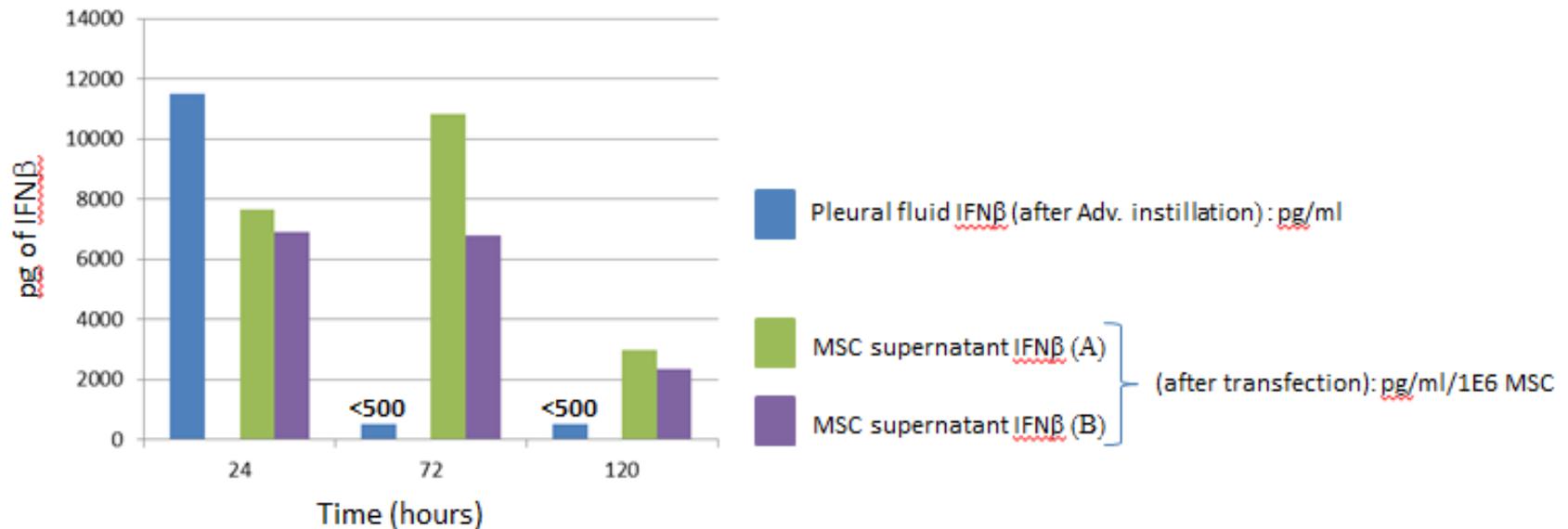
Table 1 Evaluation of adenoviral shedding and interferon β gene transfer.

Test	Day 1 (before vector installation)	Day 2	Day 3	Day 4	Week 1
Adenovirus culture*					
Chest-wall swab	Neg	Neg	Neg	Neg	Neg
Plasma	Neg	Neg	Neg	Neg	Neg
Pleural fluid	Neg	Neg	Pos	Pos	Pos
Adenovirus PCR					
Chest-wall swab	Neg	Neg	Neg	Neg	Neg
Plasma	Neg	Neg	Neg	Neg	Neg
Pleural fluid	Neg	Pos	Pos	Pos	Pos
Pleural fluid interferon β (ng/ml)	<0.5	11.5	3.1	<0.5	<0.5
Pleural fluid interferon α (ng/ml)	<3.0	<3.0	<3.0	<3.0	<3.0
Pleural fluid interferon γ (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

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

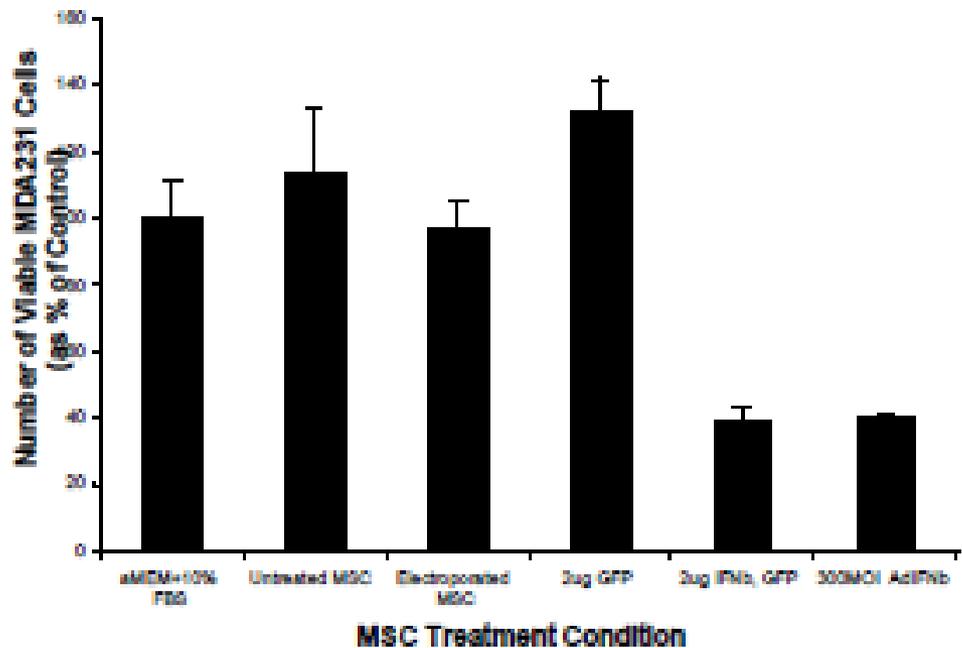
Adapted from *Sterman et al. 2006:*
Interferon β adenoviral gene therapy in a patient with ovarian cancer

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

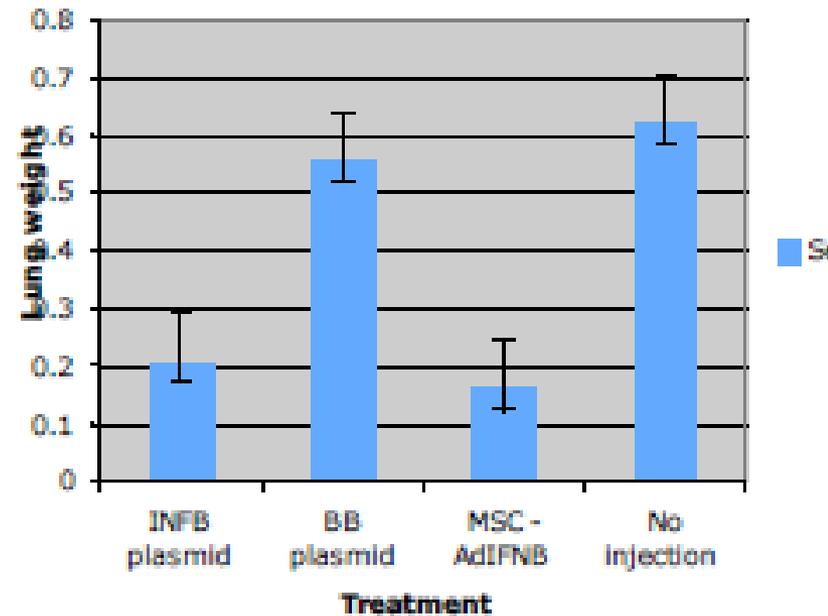


Plasmid MSC-IFN β are Bioequivalent to AdV MSC-IFN β in vitro and in vivo in suppressing Tumor Growth

In vitro

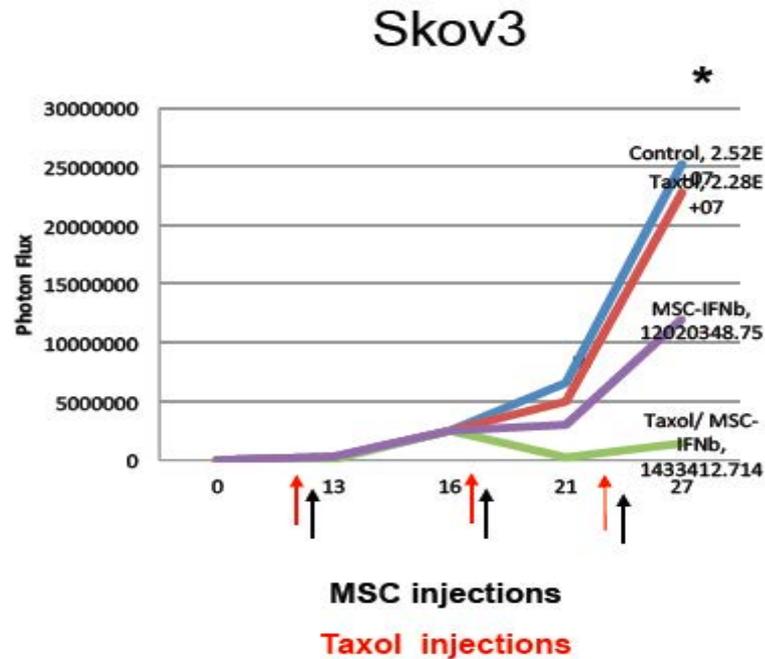


In vivo

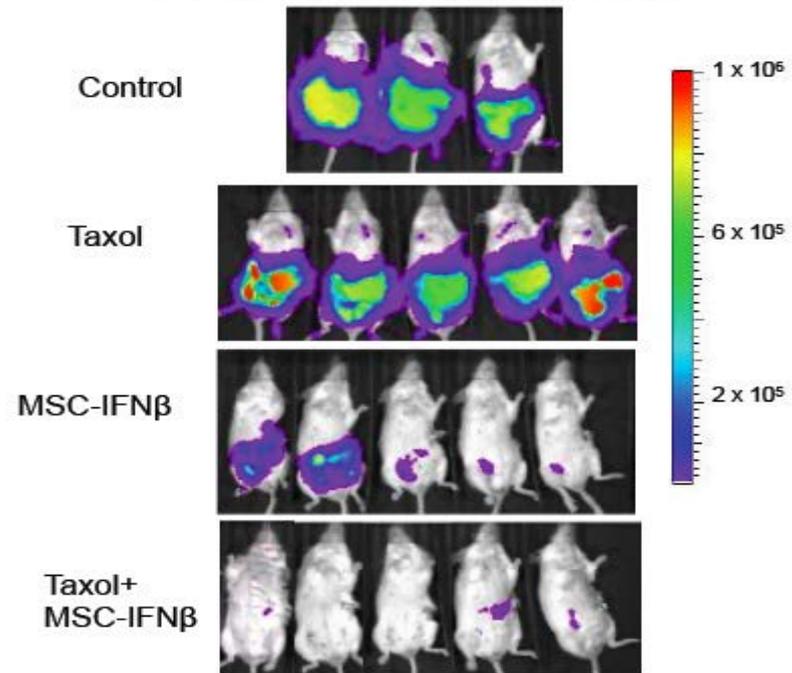


Effect of Plasmid Electroporated MSC-IFN β on Ovarian Cancer (SKOV3, 3 weekly injections only)

Plasmid electroporated MSC-IFN β control ovarian tumors



Day 27- tumor bioluminescence

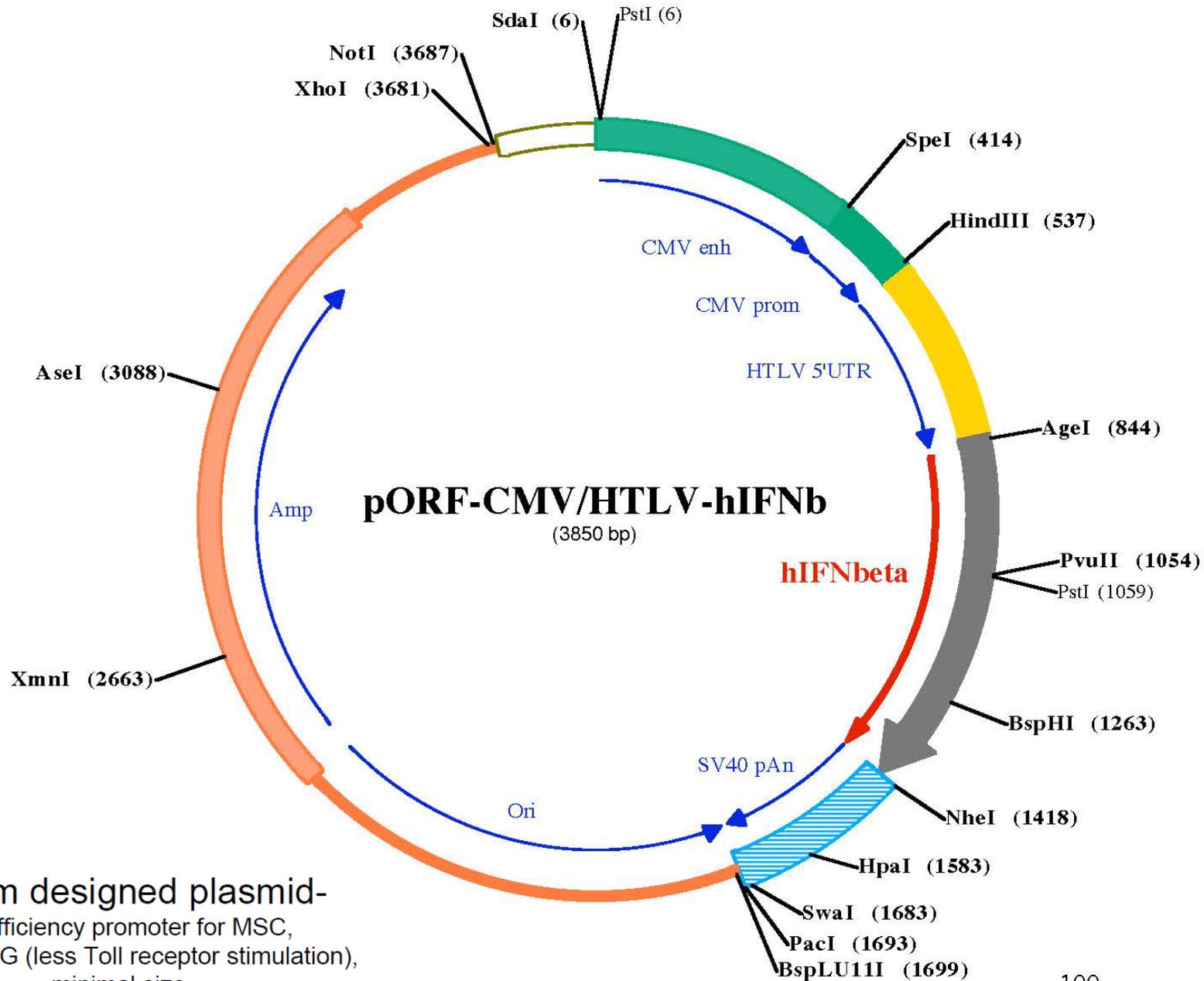


Biodistribution of Plasmid DNA-Transfected vs. Adeno-Vector Treated MSCs

- No differences in biodistribution after systemic or IP injection of MSC transfected with adenovirus or electroporated with plasmid
- MSCs home to tumors with both strategies

EF1-HTLV Mini-Promoter Composite Element Reference?

- Bernadette Ferraro, Yolmari et al. Intradermal Delivery of Plasmid VEGF₁₆₅ by Electroporation Promotes Wound Healing. *Molecular Therapy* (2009) 17 4, 651–657
- This promoter construct is currently available as a cassette from INVIVOGEN in their pDRIVE series



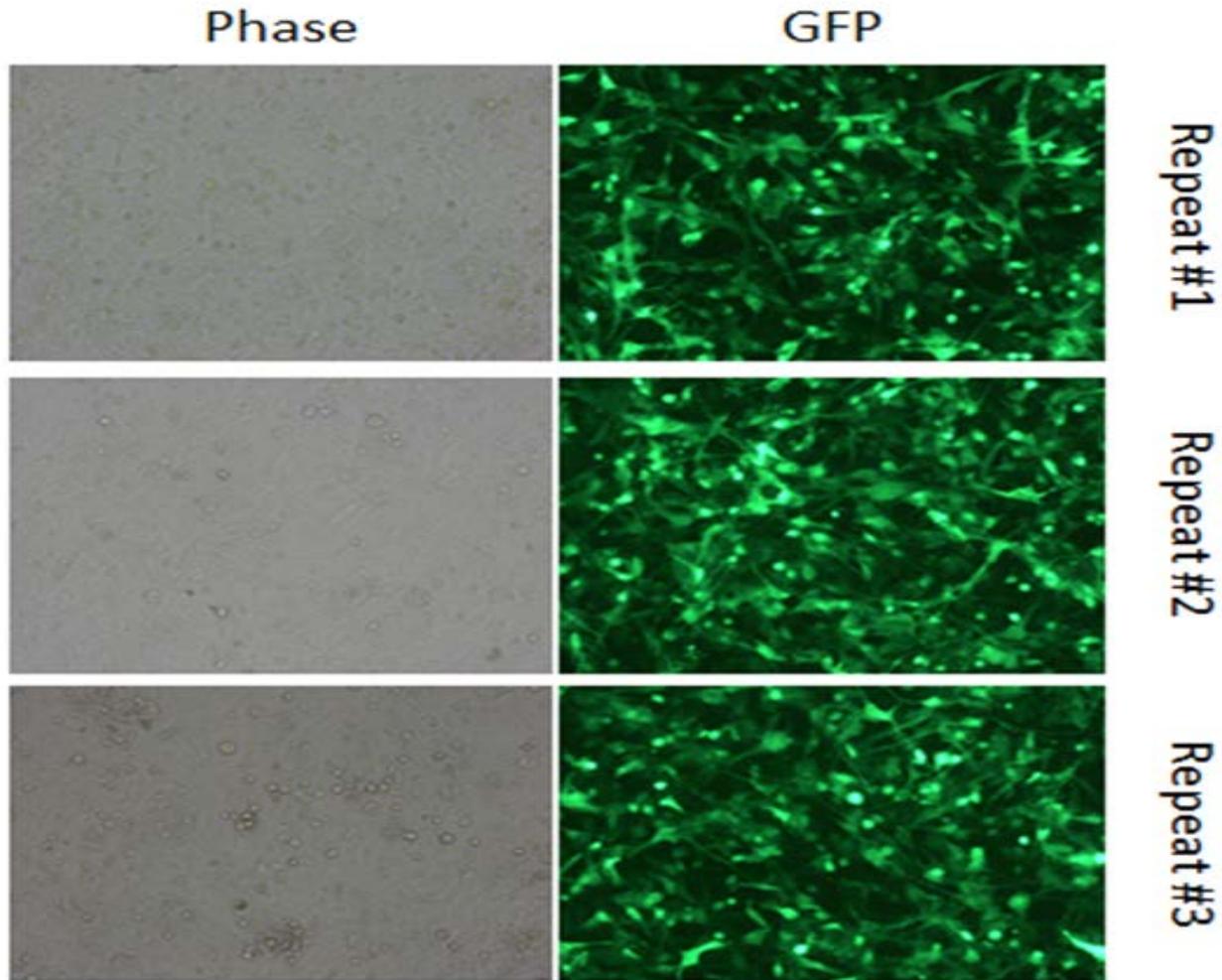
Custom designed plasmid-
 high efficiency promoter for MSC,
 reduced CpG (less Toll receptor stimulation),
 minimal size

100
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Transfection Consistency Control Before MSC Infusion

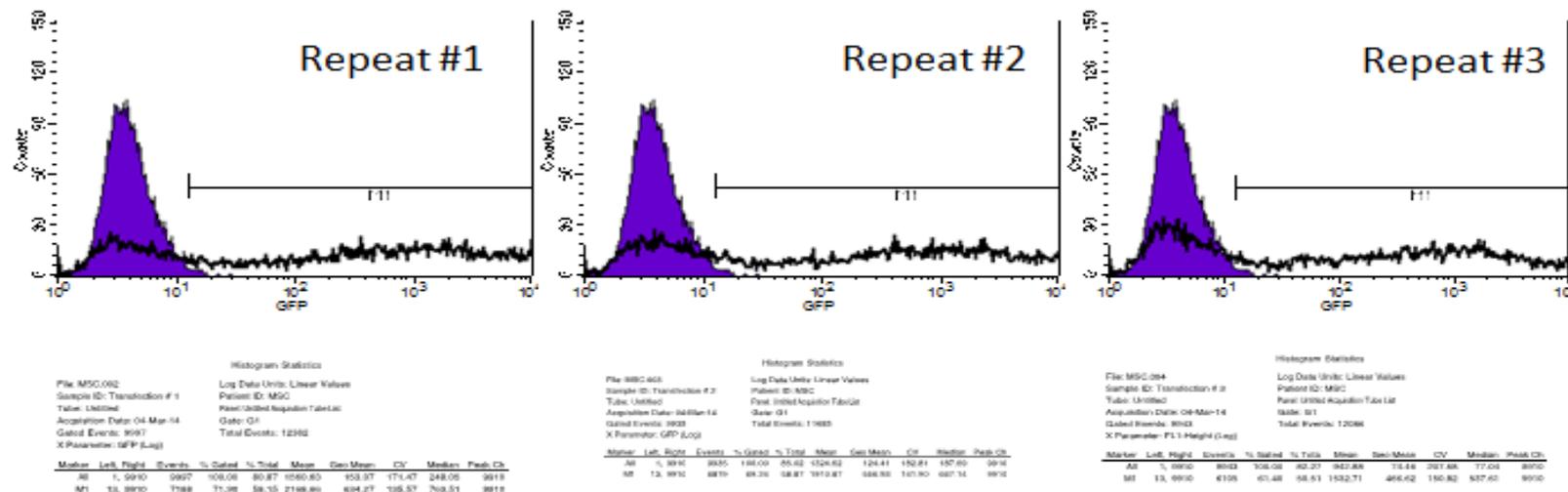
- In experiments conducted 4 years ago (Drs. Marini/Andreeff) and repeated now in the GMP laboratory (Drs. Yvon/McNiece/Shpall), high reproducibility using GFP plasmids electroporated into MSCs and evaluated 24 hours later demonstrate > 50% transfection frequency
- The clinical protocol has NO such GFP markers, and therefore this will not be a release criteria

Figure 4



Reproducibility of MSC Electroporation

Flow analysis for GFP expression



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

Rationale For Intraperitoneal (IP) Route

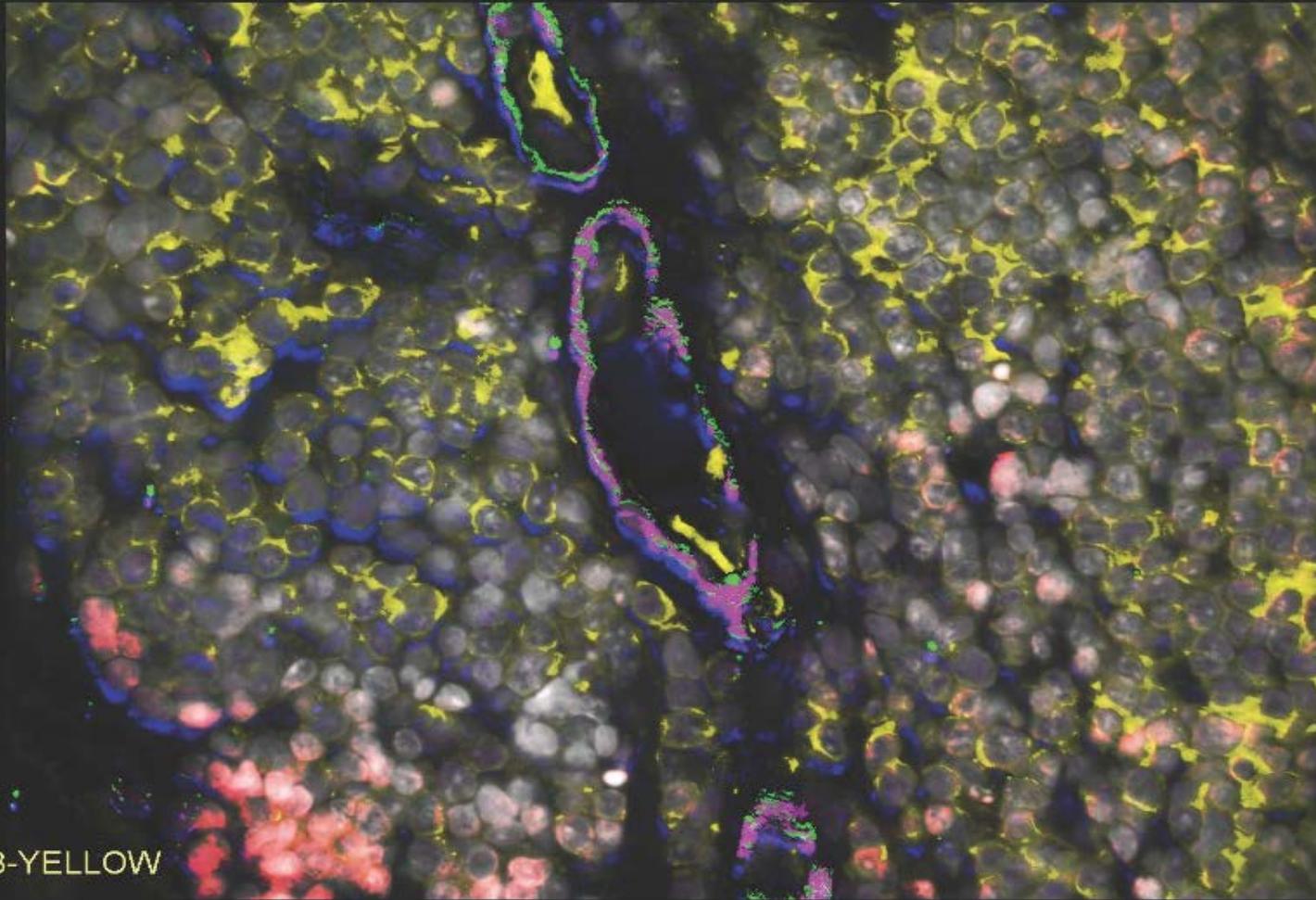
- **Safety:** Delivery of MSC systemically results in a first pass through the lungs, that could potentially introduce IFNB into lung tissue
- **Efficacy:** IP injection the MSC-IFNB would have the greatest concentration in the peritoneum, where the tumor is located
- **Preclinical Animal Data**
 - Minimal/ no detectable distribution of MSC outside of the peritoneum
 - Vastly increased numbers of engrafted MSC in the tumor tissue

Limit of Detection of IFN-beta IHC

- 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
- The method used is quantitative, without background (Spectral Imaging combined with automated cell analysis ; Vectra/InForm software, PerkinElmer)
- Sensitivity can be boosted, if necessary using the Tyramide Signaling Amplification system (TSA, PerkinElmer), which increases sensitivity 10 – 100 fold). (*Kidd et al., PLoS ONE, 2012; Spaeth et al., Cancer Res. 2013*)

Background-free Multi-spectral Imaging of IFNb in Ovarian Tumors

Multi- spectral detection MSC-IFN-B in anOvarian tumor



IFNB-YELLOW

Release Criteria for MSC Post 14-day Culture/Electroporation Viability

- Pre-clinically, IFN β is detected in cell culture supernatant of IFN β -MSC up to day 5+ post-transfection
- Electroporation method used increases membrane permeability; traditional methods for viability determination cannot be used
- We will not control the transfection efficiency before patients' infusion as infusion will occur immediately after transfection as per protocol.
- A fraction of the transfected cells will be kept in culture and supernatant collected after 24 hrs for IFN β evaluation using ELISA assay (**monitoring parameter**).

Highest Cell Dose 5X Below Effective Dose in Pre-Clinical Studies

- Responses vary and depend on many factors: molecular features of the targeted tumor, biodistribution of injected MSC and their IFN β production kinetics. Not all of these factors can be modeled in mice.
- IP rather than systemic injection will be utilized.
- 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.

MSC after IP Injection into Mice harboring Ovarian Tumors

