Oncocidex Presentation

- Clinical Overview—Tom Mikkelsen, Henry Ford Hospital
- Cell characterization and manufacturing—Alan Smith, Oncocidex
- Safety and efficacy—Ric Slauter, Oncocidex
- Additional responses to reviewers comments—Alan Smith

BMSC-12

- **Tom Mikkelsen, M.D.**
  - **Protocol PI**
- **Disclosures**
- **Background & experience**

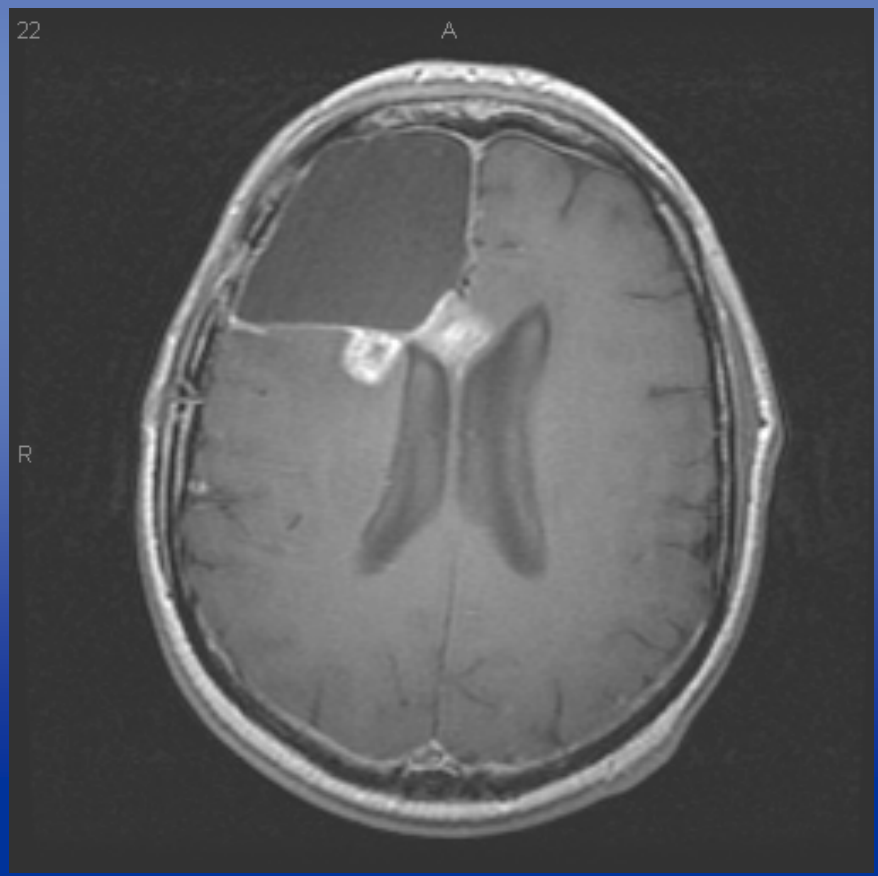
BMSC-12

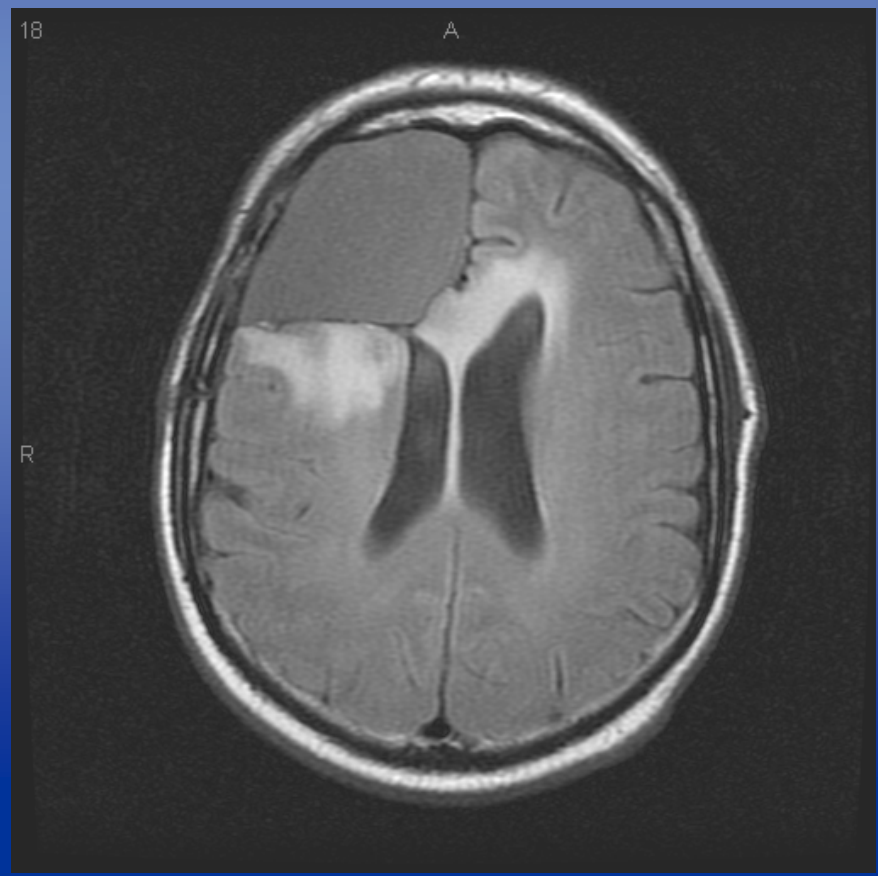
- Indication
- Background
  - Pre-clinical / Models - survival / utility
- Rationale
  - Vehicle - homing
  - Therapeutic gene electroporation
  - Implant
- Operations of protocol

Malignant Glioma

- *Glioblastoma multiforme*, WHO grade IV
- Recurrence - tumor cells found in opposite hemisphere 80%.
- Recurrence ~ extension of the primary lesion.
- Median survival after the 1st recurrence is 36-37 wks. with re-operation
- Repeat surgery is usually an option for discreet unilateral lesions
- Initial therapeutic RT is dose-limited and re-RT at recurrence is not usually an option.
- Conventional chemotherapy is ineffective

Recurrent GBM
- infiltrative disease

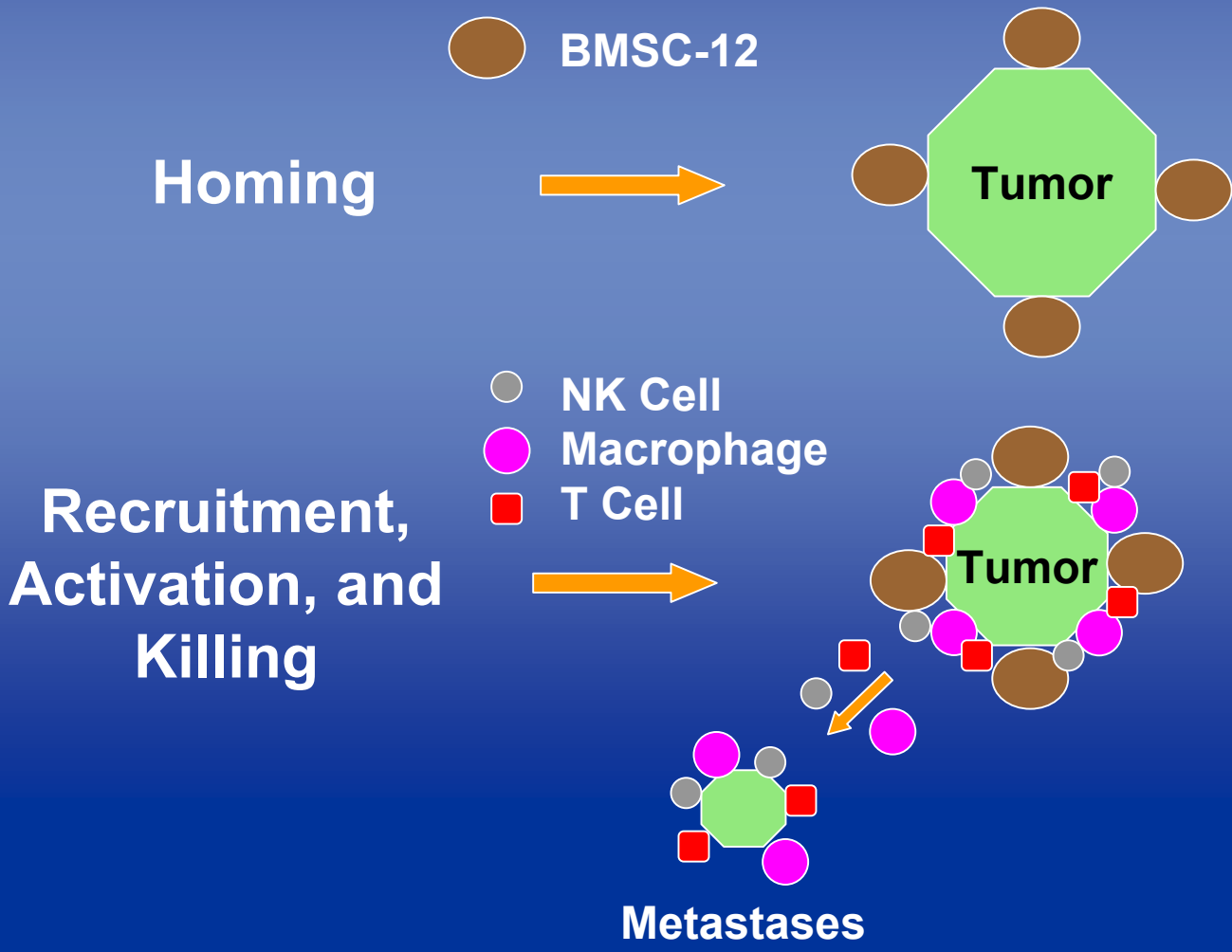




BMSC-12

- **Indication - recurrent GBM**
  - **Survival after 1st recurrence ~16-24 weeks (36-37 with re-operation)**
- **Options for therapy at recurrence**
  - **Chemotherapy**
    - **Phase 1/2 chemotherapy**
    - **2nd-line chemotherapy**
  - **Repeat RT**
    - **Radiosurgery Phase 1/2 trial**
    - **Brachytherapy**
  - **Re-operation - diagnosis/decompression/cytoreduction**
    - **Chemotherapy polymers**
    - **Brachytherapy balloon catheter**

Scientific Rationale



BMSC-12 logistics

- Clinically suspected recurrence
- Indication for re-operation
- Informed consent
- BM harvest & *ex vivo* IL-12 electroporation & expansion (at-risk cohort harvested after initial dx)
- Clinically indicated biopsy / resection
- Post-op cell implant via Rickham reservoir
- Repeat injection monthly
- f/u by clinical exam and MRI

BMSC-12

- Logistics
  - BM harvest - prior RT/CTX / steroids
  - Implant -
- Cell tracking
  - Distribution - FeO_3 MRI (FDA) phagocytosis
- Immune monitoring
  - Lumbar CSF for IL-12, IFN
  - Biopsy
  - Autopsy

BMSC-12

- **BMSC vehicle**
  - **Deliver payload**
  - **Dose escalation by multiple dosing**
  - **Homing & migration**
  - **T-cell recruitment may not require 100% distribution/delivery --?recruit/activate intrinsic inflammatory cells**

BMSC-12

- Safety
  - Autologous cells
  - Local implant - needle tract ?trauma/inflammatory chg
  - Transient IL-12 expression
  - Limited local inflammatory change
    - BMSC alone - no NK cells
    - BMSC IL-12 + NK cells, T cells, macrophage
  - BMSC ?less likely to be affected by local μenvironment

Inclusion Criteria

- **Confirmed recurrent malignant glioma on MRI, *or* High Risk primary malignant glioma after therapeutic RT.**
- **≥ 18 yrs, KPS ≥ 60**
- **> 100,000 plts/mm^3, ANC >1500/mm^3 , HGB >10 g/dl, bilirubin < 2.0 mg/dl, transaminases < 3x ULN, creatinine < 2 mg/dl and PT and PTT < ULN.**
- **For women of childbearing potential, a negative serum βHCG**
- **Willingness to practice contraception for a period of one year after the last BMSC administration**
- **Willingness to consider autopsy or brain autopsy**
- **Informed consent**

Exclusion Criteria

- Infratentorial tumor
- Clinically significant mass effect
- Treatment with chemotherapeutic agent ≤ 3 weeks before the initial BMSC-12 administration
- Subjects at high medical risk due to significant concurrent illness or those in need of permanent systemic anticoagulation
- Uncontrolled seizures within 14 days prior to enrollment
- Evidence of other malignancies
- Contraindication to surgery
- Positive Serum: HIV, HBV, HCV, RPR, FTA, or PPD
- Participation in another investigational drug, device, or biologics trial within 30 days of Screening Visit
- Participation in a prior gene and/or cell therapy trial any time in the past
- Current abuse of alcohol or drugs

Cell Fate

- Autopsy
  - H&E morphology, IL-12 PCR
  - Immunophenotype inflammatory infiltrate
- ?local differentiation into other cells
- Distribution

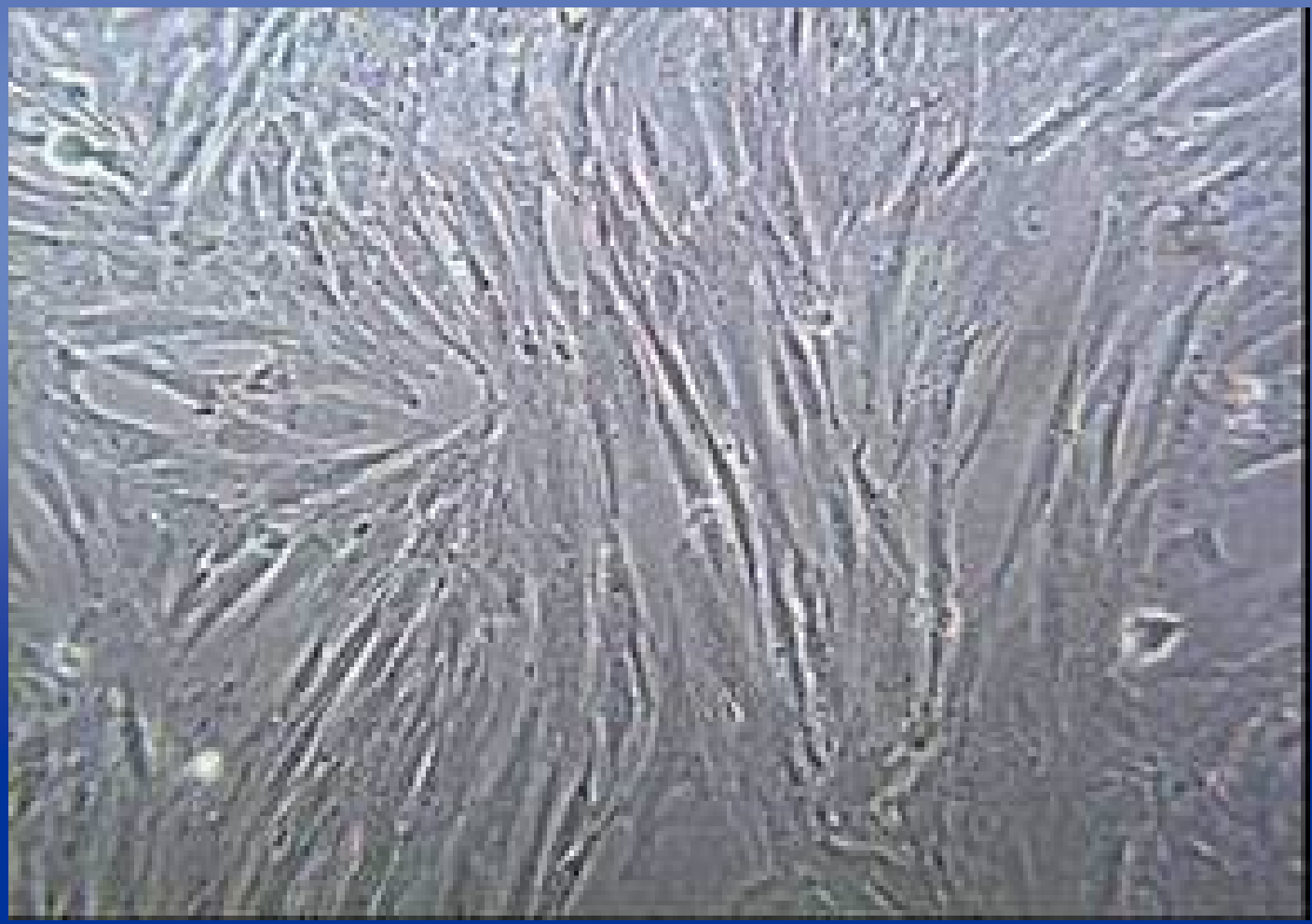
Schedule of events table: Biopsy-positive Recurrent Subjects

STUDY VISITS	-8 wks to -6wks	Bone Marrow Harvest	-5 wks to -2wks	Day -3 to -1	Day 0	Treatment 2 through 10	Every 2 months post-final treatment for 12 months	Year 2 &3	As clinically indicated
Informed Consent	X								
Medical History	X								
Physical, Vital Signs, Neuro	X		X	X		X	X		
Karnofsky Performnce Scale	X		X	X		X	X		
MRI	X				X	X	X		
MR Spectroscopy									X
Tumor Biopsy			X						
Tumor Resection (if indicated)			X						
CBC, Platelet & Chemistry	X		X		X	X	X		
PT, PTT	X		X		X	X			
Flow Cytometry (Mo1-3, 6, 9, 12)			X			X			
Bone Marrow Harvest Consent		X							
Bone Marrow Harvest		X							
Confirmation Cells Available			X						
Catheter/Rickham Consent			X						
Catheter/Rickham Placement			X						
HIV, HBV, HCV, RPR, FTA	X								
Liver Profile	X				X				
TB Skin test w PPD	X								
UA with Microscopic	X								
EKG	X								
b HCG				X					
10Million BMSC-12 Administration					X	X			
Telephone Contacts								X	
Concomitant Medications	X	X	X	X	X	X	X	X	
Adverse Events		X	X	X	X	X	X	X	

Adult Bone Marrow Stromal Cells

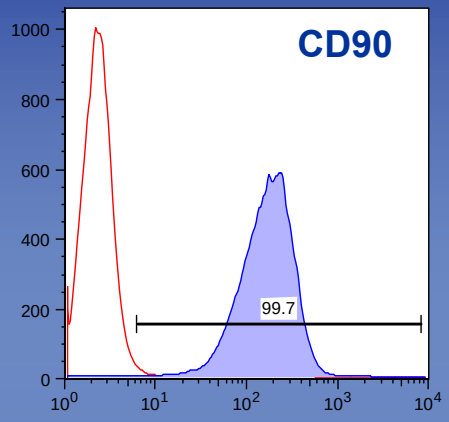
- Derived from adult bone marrow
- Can be readily grown in culture
- Large expansion potential
- Shown to be safe in over 100 patients—other indications

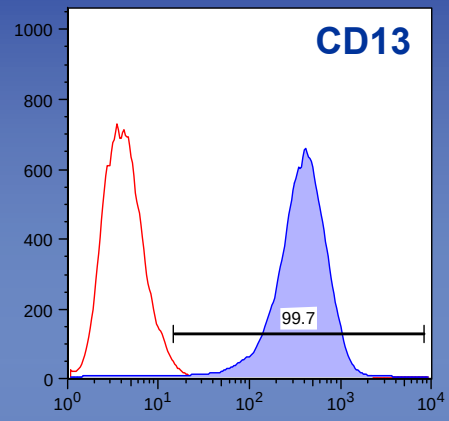
BMSC Morphology

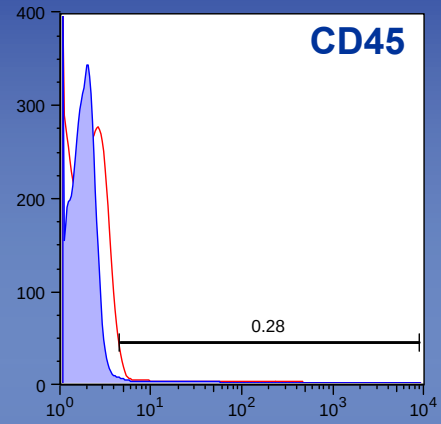


Phenotypic Characterization of hBMSCs

P1







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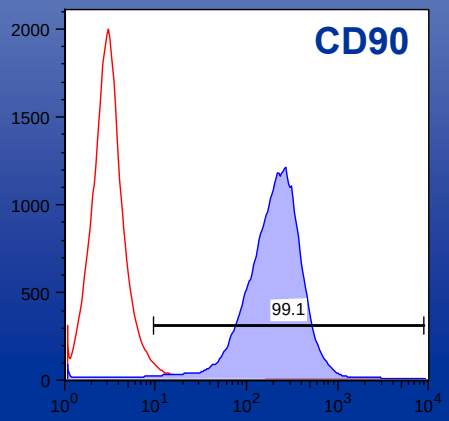
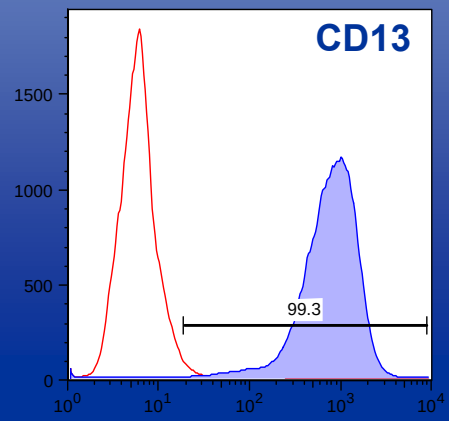
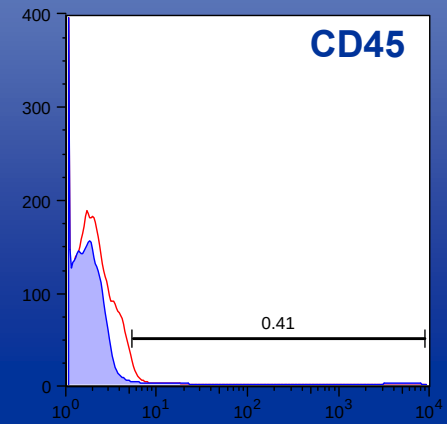






Phenotypic Characterization of hBMSCs: Five donors

Cluster Designation	Cluster Distribution or Common Name	BMSC				
		Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
		P3 [a]	P3	P4	P3	P3
CD3	(T Cell)	DIM [b]	DIM	DIM	DIM	DIM
CD11a	LFA-1 alpha	NEG	NEG	NEG	NEG	NEG
CD13	APN	BRIGHT	BRIGHT	BRIGHT	BRIGHT	BRIGHT
CD14	LPS-r	NEG	NEG	NEG	NEG	NEG
CD19	Pan B cell	NEG	NEG	NEG	NEG	NEG
CD29	β–1 Integrin	BRIGHT	BRIGHT	BRIGHT	BRIGHT	BRIGHT
CD31	PECAM-1	NEG	NEG	NEG	NEG	NEG
CD34	Hemat. Stem cell	NEG	NEG	NEG	NEG	NEG
CD40	Co-stimulation	NEG	NEG	NEG	NEG	NEG
CD44	H-CAM	BRIGHT	BRIGHT	BRIGHT	BRIGHT	BRIGHT
CD45	Pan Leukocyte	NEG	NEG	NEG	NEG	NEG
CD54	ICAM-1	DIM	DIM	DIM	DIM	DIM
CD80	B7-1	DIM	DIM	DIM	DIM	DIM
CD86	B7-2	NEG	NEG	NEG	NEG	NEG
CD90	Thy-1	BRIGHT	BRIGHT	BRIGHT	BRIGHT	BRIGHT
CD105	Endoglin	BRIGHT	BRIGHT	BRIGHT	BRIGHT	DIM
CD119	INF-γ-r	DIM	DIM	NEG	DIM	NEG
CD120a	TNFR1	NEG	NEG	NEG	NEG	NEG
CD123	IL-3-r	NEG	NEG	NEG	NEG	NEG
CD132	Common γ chain	NEG	NEG	NEG	NEG	NEG
CD133*	AC 133	DIM	DIM	DIM	DIM	DIM
CD212	IL-12-r	NEG	NEG	NEG	NEG	NEG
MHC Class I		BRIGHT	BRIGHT	BRIGHT	BRIGHT	BRIGHT
MHC Class II		NEG	NEG	NEG	NEG	NEG

[a] Passage number at time of characterization.

[b] Qualitative designation based on median fluorescence intensity increase above respective isotype control, as follows

IL-12 Does Not Alter the Cell Phenotype

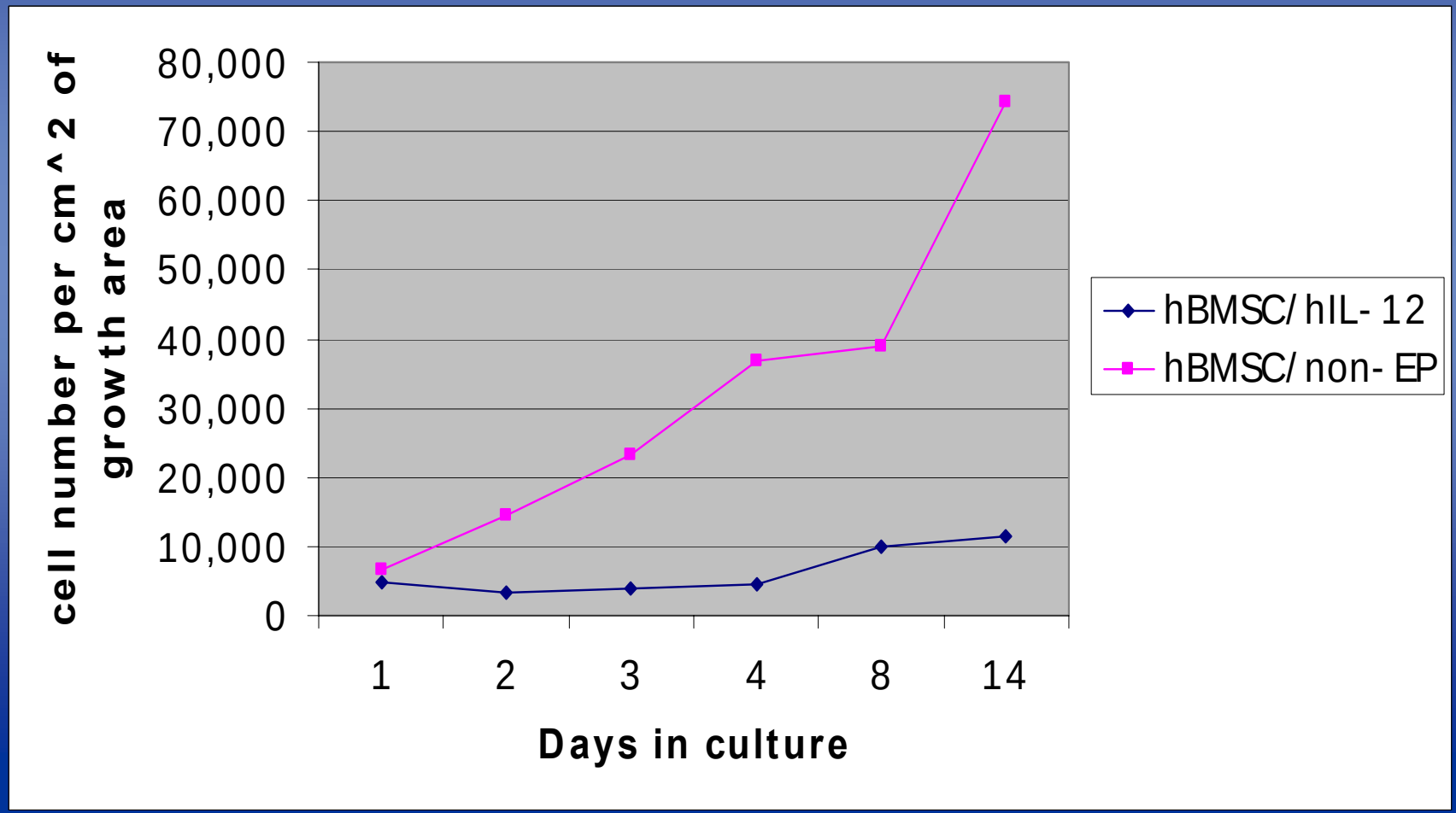
Marker	Donor H-BM-03-006		Donor H-BM-03-011		Donor H-BM-03-016		
	No EP	IL-12 EP	No EP	IL-12 EP	No EP	pVAX EP	IL-12 EP
CD13	99.3	98.4	98.3	98.3	98.4	98.4	98.3
CD34	0.5	0.7	3.3	3.9	3.8	3.7	2.7
CD44	98.5	98.4	98.5	98.3	98.3	98.4	98.4
CD45	neg	neg	neg	neg	neg	neg	neg
CD90	99.3	98.2	98.3	98.1	98.4	98	98.2
CD105	95.6	86.6	80.4	66.2	91.2	93.8	93.9
CD133	15.2	15.3	34.8	26.8	12.9	15.1	13.8
MHC I	98	98.3	98.2	98.2	98.4	98.1	98.2
MHC II	neg	neg	neg	neg	neg	neg	neg

IL-12 Does Not Alter BMSC Function

Transfection of BMSCs With IL-12 Does Not Change Their Cytokine Secretion Profile

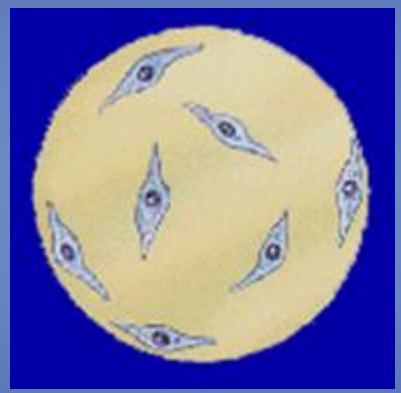
- Non-transfected and IL-12 Transfected BMSCs were cultured in vitro
- The conditioned media was tested for the presence of 96 different cytokines
- There was no appreciable change in either the type of cytokines secreted or in the quantity of individual cytokines secreted

Growth characteristics of IL-12 electroporated and Non-electroporated hBMSC

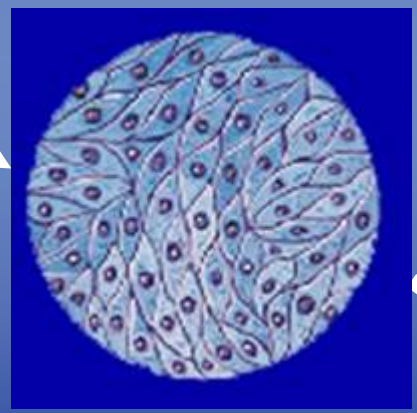


The Manufacturing Process

Collect tissue from donor or patient



A few million adhere initially



Electroporation

Cryopreserved

BMSC-12 Product

QC Release Testing

Released BMSC-12 Product

ONCOCIDEX, INC.

The Effects of Normal and Gene-modified Bone Marrow Stromal Cells (BMSCs) in Vivo

Results of Preclinical Development

December 3, 2003

Summary of In Vivo Safety Studies With BMSCs and IL-12

- Homing to tumor by BMSCs
- Local administration of IL-12 and BMSC12
- Systemic administration of IL-12 and BMSC12
- Single high dose safety of BMSC12
- Multiple high and low dose safety of BMSC12

Animal Model Used for Preclinical Studies

- Nude rat with human U87 tumor using human BMSCs for homing studies and some cytokine production studies.
- Fischer rats bearing F98 rat tumors for safety and efficacy studies.
  - Murine IL-12 gene (human IL-12 ineffective in the rodent).
  - F98 Fischer rat glioblastoma (ATCC) (human tumor in immunodeficient animal no good).
  - Fischer-344 rat BMSCs (human BMSCs immunogenic in xenotransplant model).
  - In a tumor bearing Fischer rat (nudes rat doesn't work).

What Is the Safe Dose?

Assume that BMSC-12 are expressing IL-12 at 400 ng/10^6 cells/day and this expression decreases in first-order fashion with a half-life of one day for five consecutive days.

- In humans at a dose of 10^7 cells IL-12 dose is 4000 ng/70 kg BW/day = 57 ng/kg/day
- Therefore, the maximum total dose of IL-12 achievable with 10^7 cells over five days would be 115.75 ng/kg.

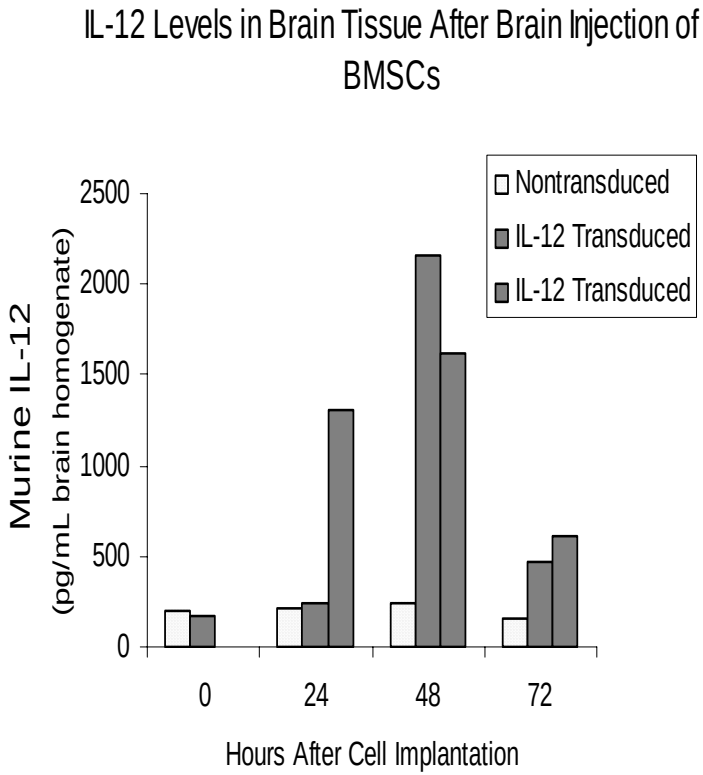
- The reported I.V. MTD in humans is 500 ng/kg/day for five days every three weeks (Atkins, et al). Or more than 20 times the highest dose that we can achieve assuming the agency were to allow us to start at 10^7 cells per patient and 100% of the dose were systemically bioavailable.

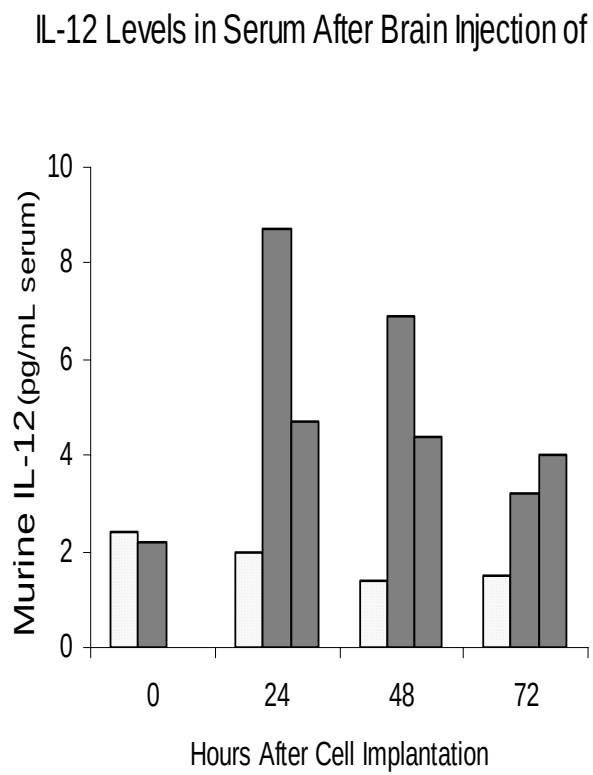
- We can conclude from these calculations that the proposed dose in humans should be well within the safe range for administration of IL-12.

What Is the Safe Dose

- In the rat a dose of 500,000 cells equals 2 million cells per kg of body weight.
- The proposed human dose is 10 million cells in a 70 kg human. This equals 150,000 cells per kg.
- Therefore, on a purely mass dose basis, the high dose in the rat studies equates to approximately 13 times the proposed human dose. We consider this to be a significant safety margin.

The Advantage of Local Delivery Via BMSCs in Avoiding Systemic Toxicity





Summary of In Vivo Safety Studies with Normal or Gene-Modified BMSCs

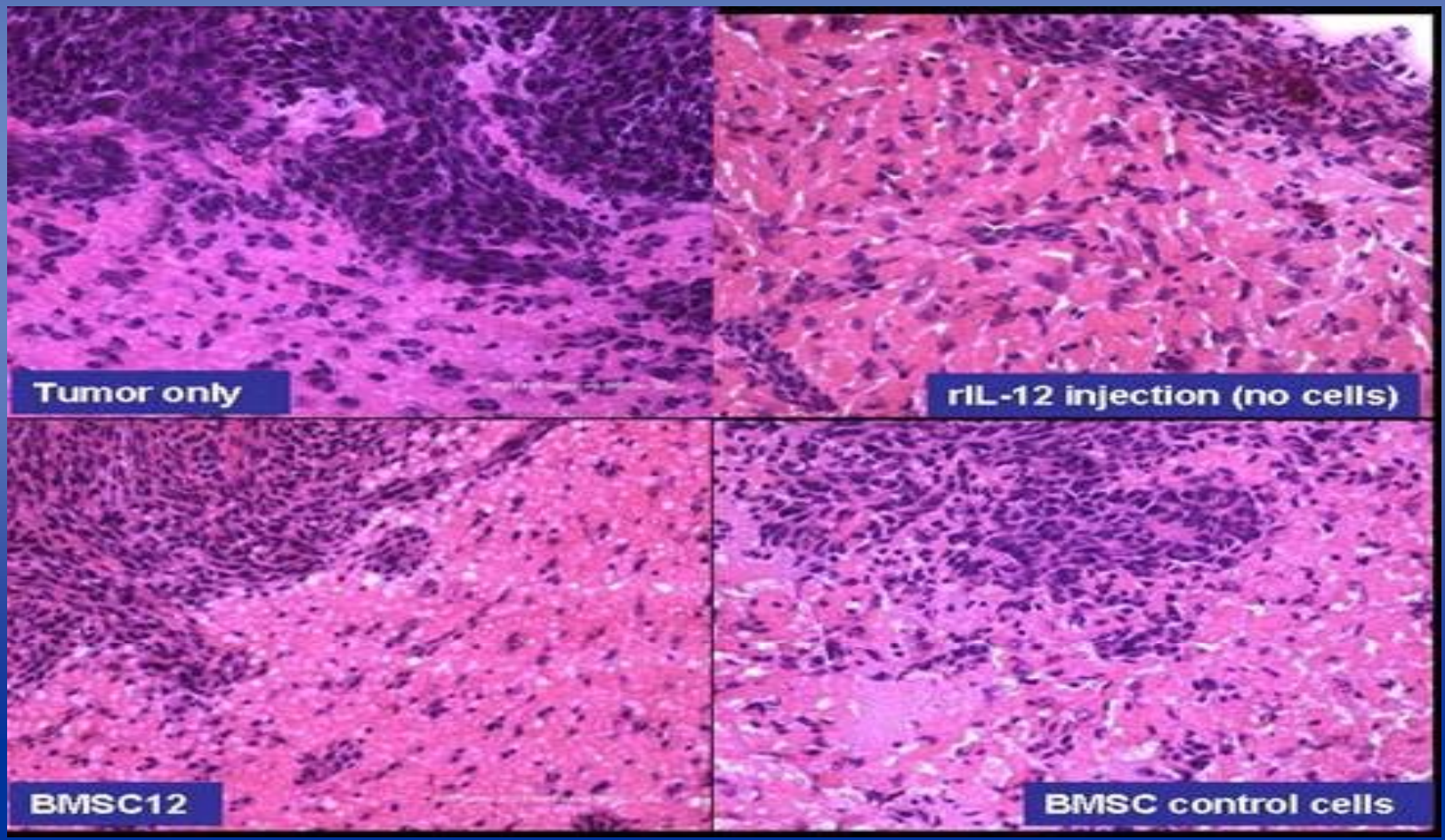
Study	Purpose	Animals	Groups	Route of Admin	Tumors	Safety Data	Efficacy Data	Tissues	Duration
2003-001	Tumor targeting	20 rats nude	Non-transduced huBMSC	Brain Implant	U87 human glioma	yes	Tumor Targeting	Brain	2 weeks
2003-002	High-Dose Safety Study	40 rats Fishers	Sham, plasmid, +/- Rat-AdVmuIL-12 Rat-BMSC	Brain implant, Tail Vein	F98 Fischer Rat Glioma	yes	Survival, tumor invasiveness, Micropath recruitment of NK, t-cells, macrophage,	Full list;	3 weeks
2003-003	Targeting/ Tissue Distribution	60 rats nude	Non-transduced huBMSC	Brain Implant, ICV, carotid, tail vein	U87 human glioma	yes	Tumor targeting and general systemic tissue distribution	Reduced List	3 weeks
2003-004	Basic Tumorigenicity	96 nude mice	Non-transduced HuBMSC and positive control	Subcutan	Pos. control	yes	no	Full List; H&E	13 weeks
2003-006	Initial GLP Autologous Safety Study; includes ACI allo groups	100 rats Fischers	Sham, Plasmid, Non-viral transduced, muIL-12, ratBMSC	Brain implant, Tail Vein	F98 Fischer Rat Glioma	yes	Survival, tumor invasiveness, local and systemic IL-12, IFN, Micropath recruitment of NK, T-cells, macrophage, flow data for recruitment	Full list	4 weeks

Results of Single Administration Studies

- No adverse effects noted following stereotaxic implantation of up to $1x10^6$ cells up to 28 days
- No adverse effects noted following iv injection of up to $2x10^6$ cells up to 28 days
- In tumor-bearing nude animals, cells found surrounding tumor within 5 days, not in other tissues
- In non tumor-bearing nude animals, cells seem to be eliminated within 7 days

Lack of Observable Effect of Implantation of BMSCs on Local Neuropil

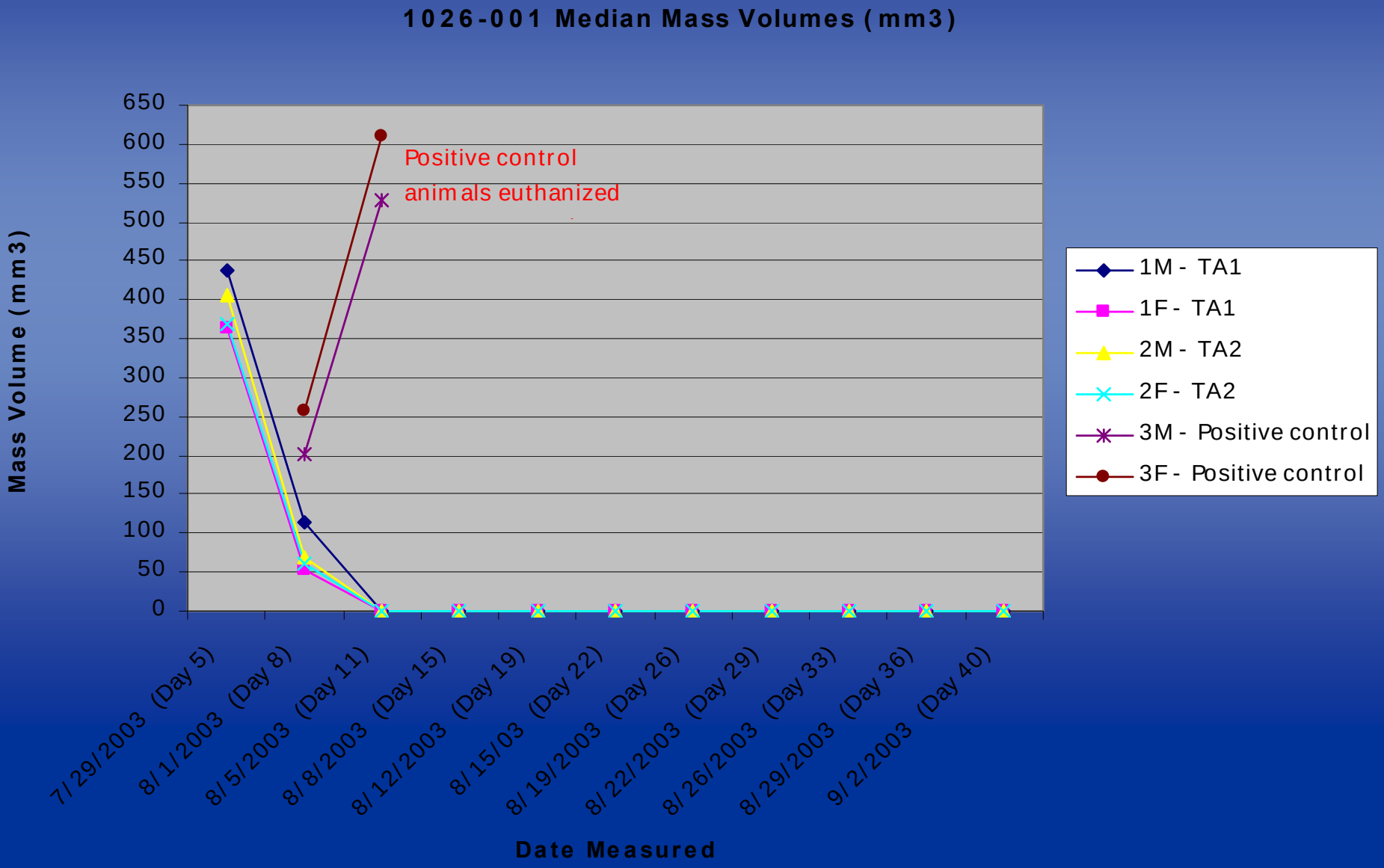
Figure (20X) F98 glioma model in rats: 4 treatment groups



Results of Repeated Administration Studies

- No adverse effects noted following stereotaxic implantation of up to $5x10^5$ cells once per week for three weeks with animals sacrificed 1 week following last dose.
- No adverse effects noted following iv injection of up to $2x10^6$ cells once per week for three weeks with animals sacrificed 1 week following last dose.

Results of Tumorigenicity Study



In Vivo Studies of the Physiological Effects Implantated BMSC-12s in the CNS

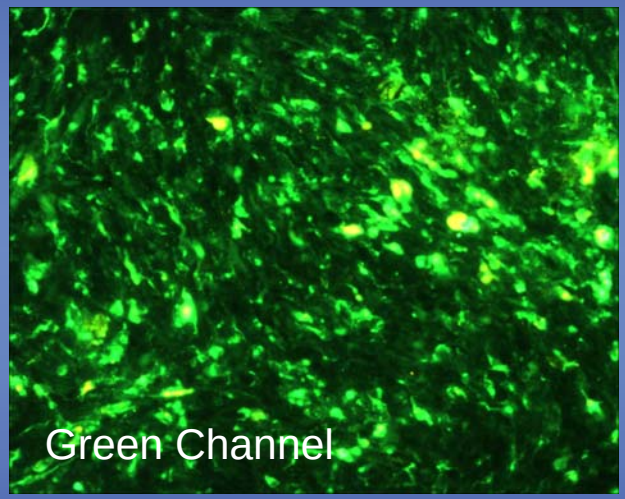
Study/Purpose	Animals	Groups	Route of Admin	Tumors	Safety Data	Efficacy Data	Tissues	Duration
IL-12 direct injection and continuous infusion	24 rats, Fischer,	Direct microinjection and continuous infusion of muIL-12,	Micro-injection continuous perfusion	+/-F98 rat glioma	yes	Measure local and systemic IL-12, IFN, TNF. Micropath recruitment of NK, t-cells, macrophage	Brain, plasma	7 days
In Vivo IL-12 secretion, duration of expression ratBMSC12	24 rats, Fischer ,	Non-viral transduced muIL-12, rat BMSC	Brain Implant	+/-F98 rat glioma	yes	Measure local and systemic IL-12, IFN, TNF. Micropath recruitment of NK, T-cells, macrophage	Brain, plasma	Up to 10 days
IL-12 direct injection	16 rats Fischer and nude	Non-viral transduced muIL-12, rat BMSC	Micro injection	+/-F98 rat glioma	Yes, , clinical effects	Measure local and systemic IL-12, IFN, TNF.	Brain, plasma	Up to 72 hr
In Vivo IL-12 secretion, duration of expression	18 rats fischer	Non-viral transduced muIL-12, rat BMSC	Brain Implant	+/-F98 rat glioma	Yes, , clinical effects	Measure local and systemic IL-12, IFN, TNF. Flow recruitment of NK, t-cells, macrophage	Brain, plasma	Up to 72 hr
In Vivo IL-12 secretion, duration of expression human BMSC12	Nude rats	Non-viral transduced huIL-12, huBMSC12	Brain Implant	none	Yes, clinical effects	Measure local and systemic IL-12, IFN, plasmid	Brain, plasma	Up to 21 days

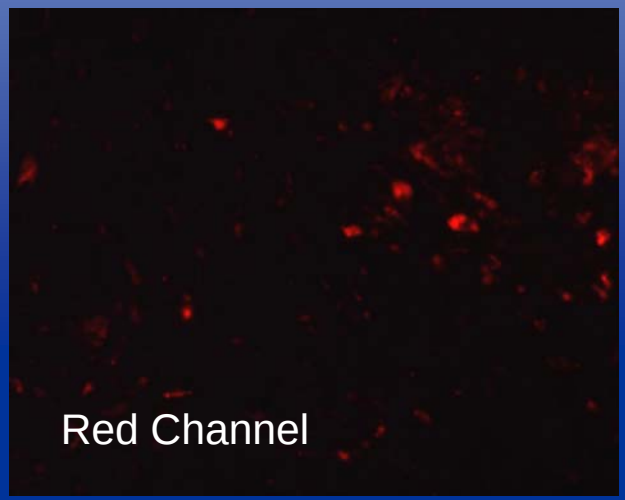
Results of BMSC Homing Studies

- MSCs or BMSCs stereotaxically implanted in the brain of nude rats
  - Ipsilateral
  - Contralateral
  - ICV
  - Local vascular
  - Global vascular
- Tumor-bearing or non tumor-bearing
- Homing observed only in tumor-bearing animals, following all but global vascular delivery

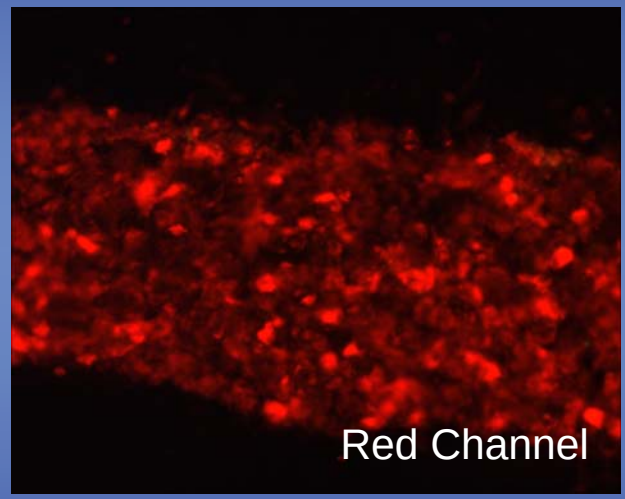
Migration of BMSCs to Contralateral Glioma One Day After Implantation

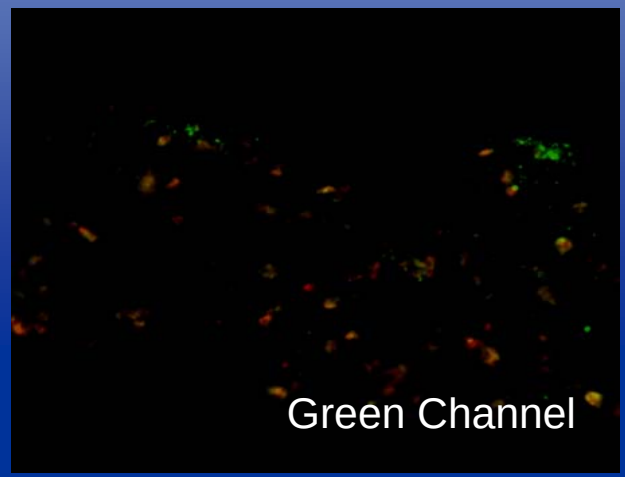
DiO-Green Tumor on the Left



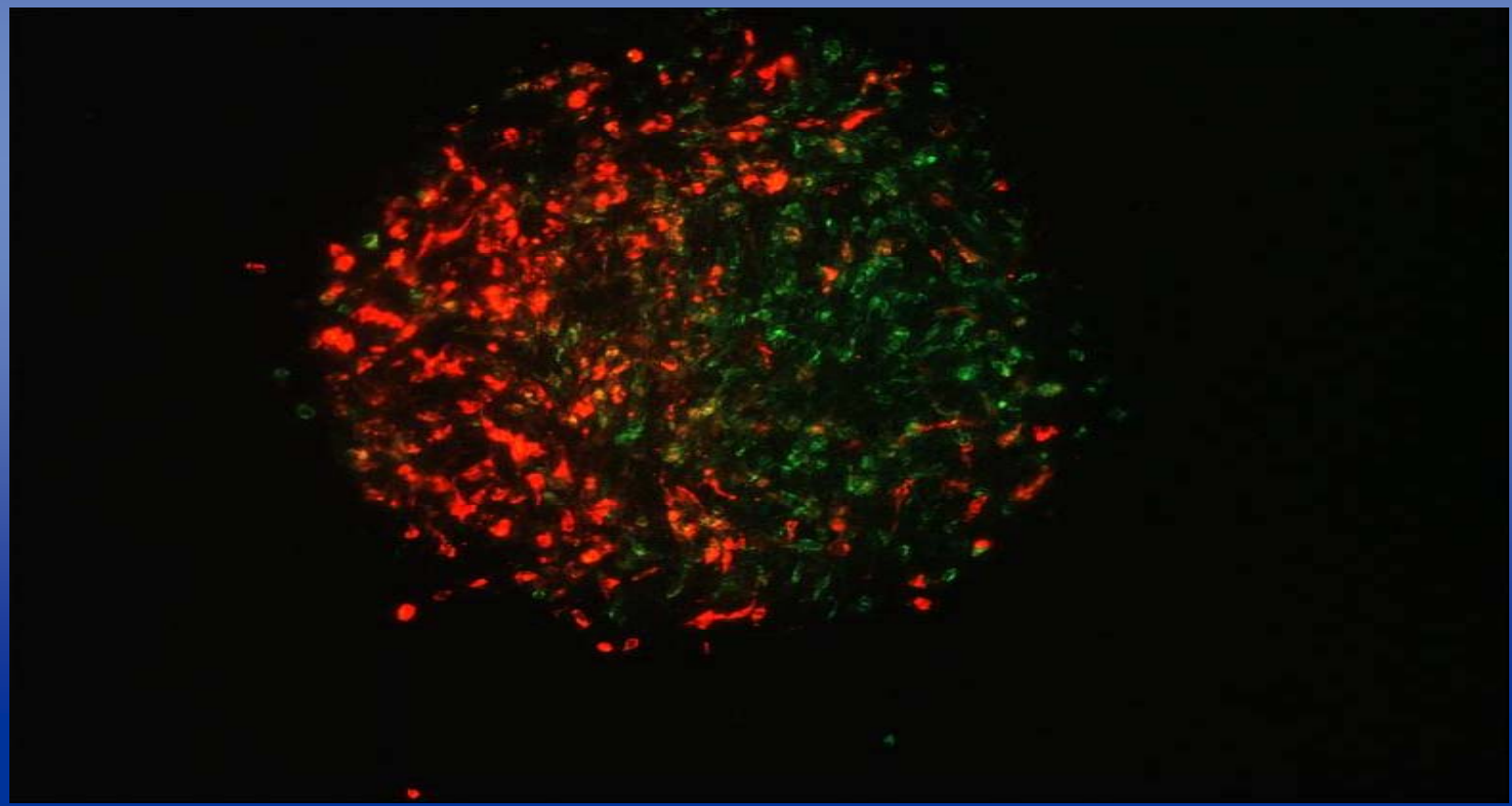


DiI-Red BMSC on the Right

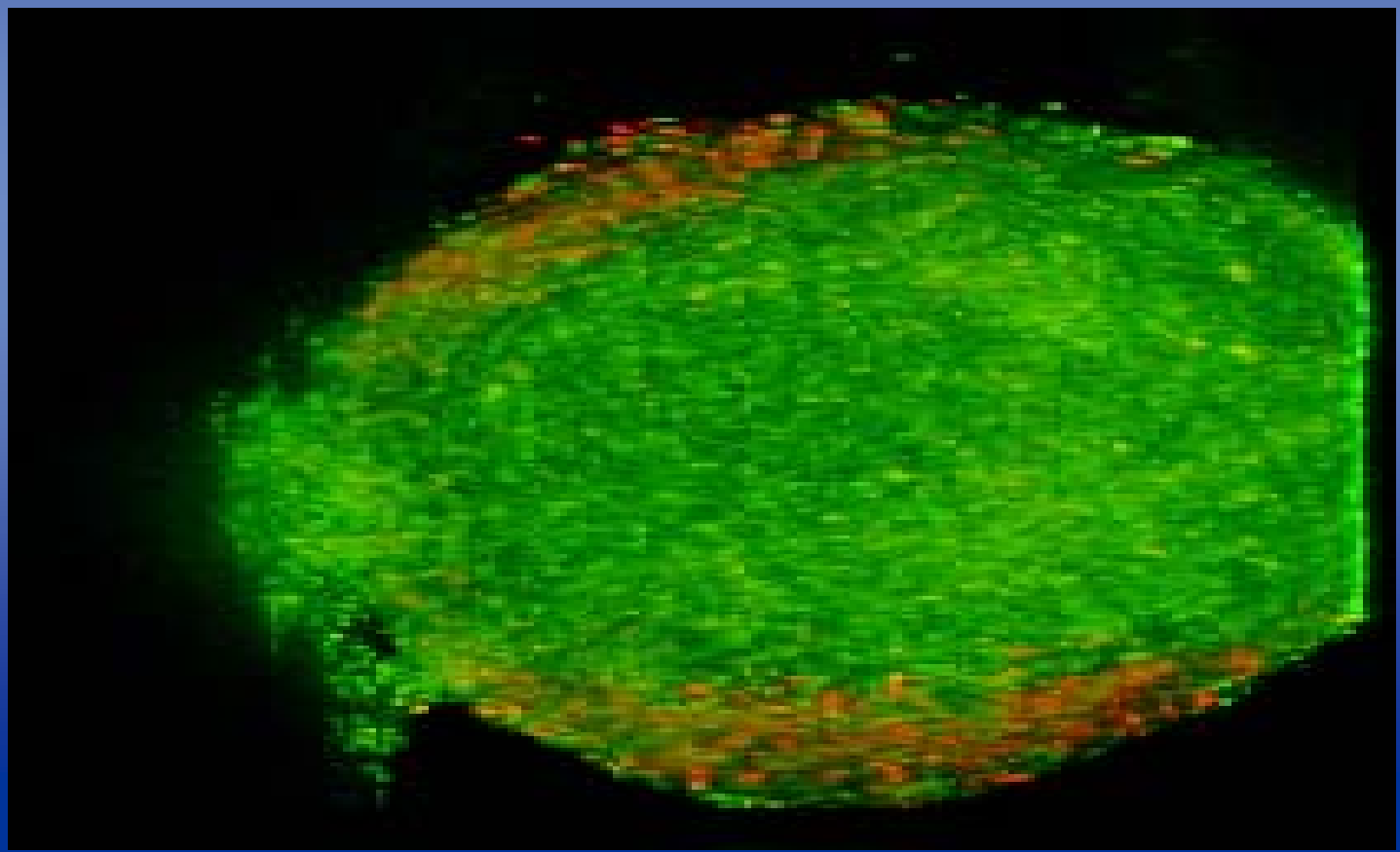




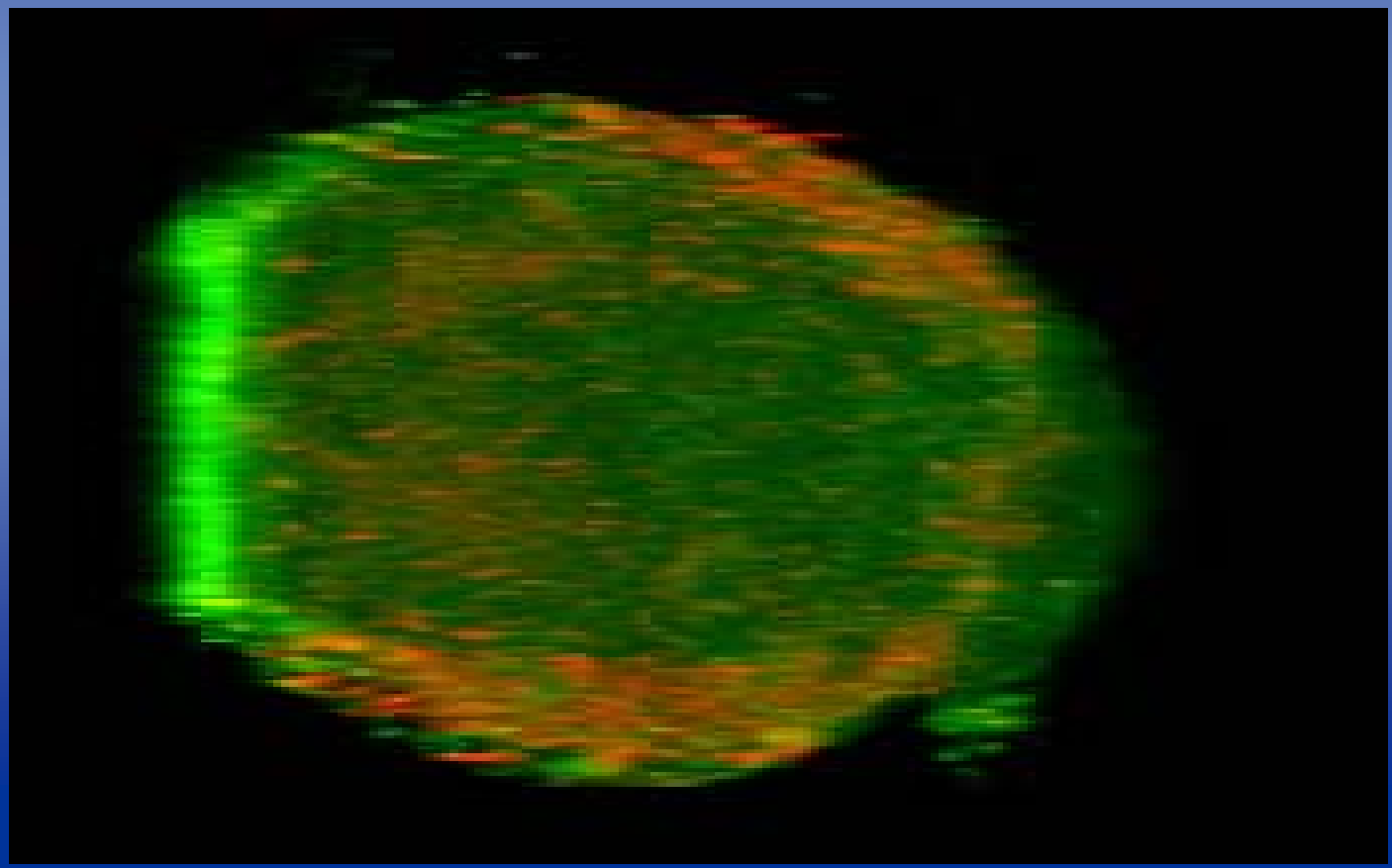
Homing of Contralateral MSCs to U87 Human Tumor in Nude Rat Brain 7 Days Following Implantation



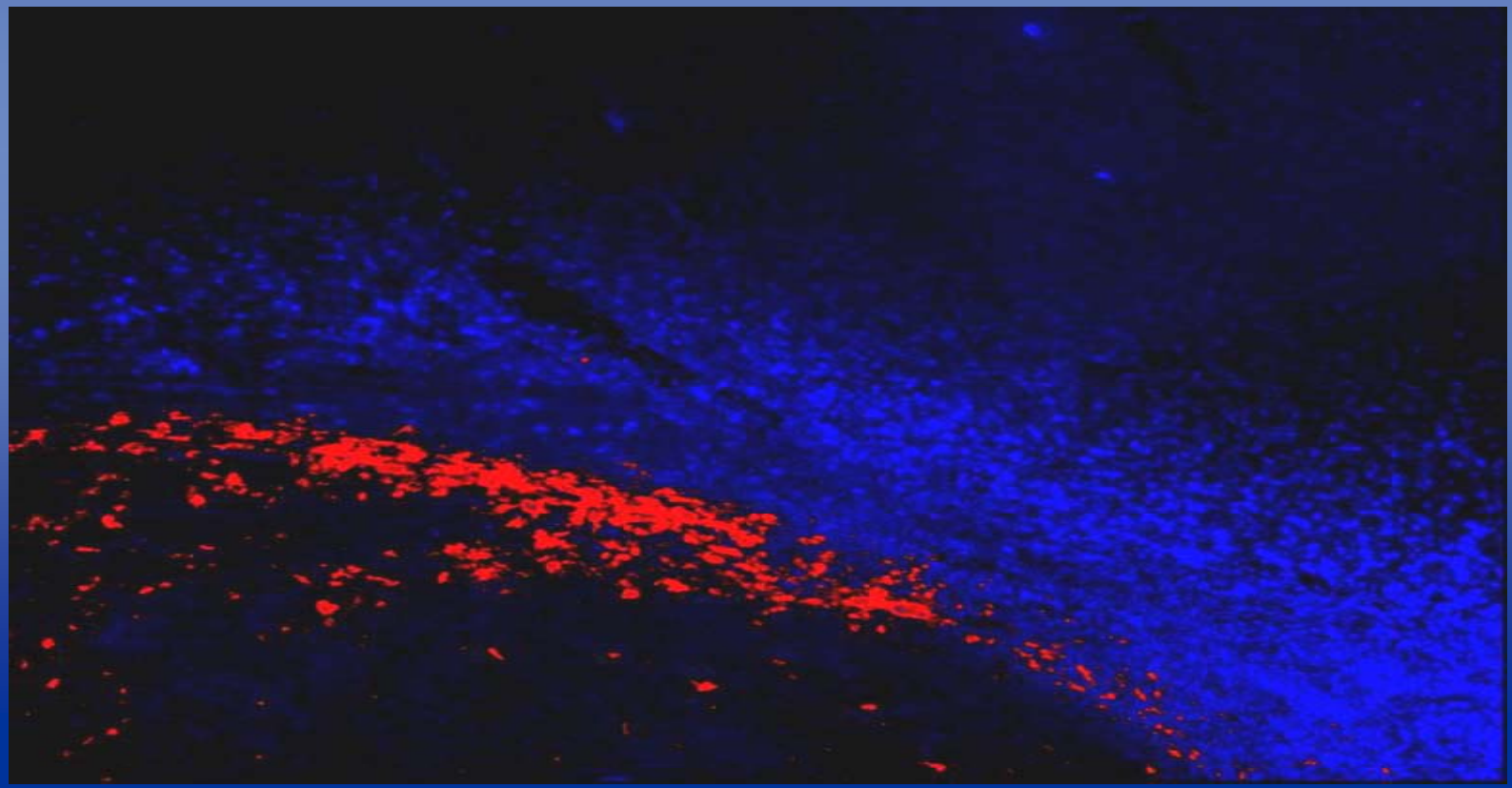
Rotated Image of U87 Tumor in Nude Rat Brain Using Laser Confocal Microscopy 5 Days Following Contralateral Implantation of MSCs



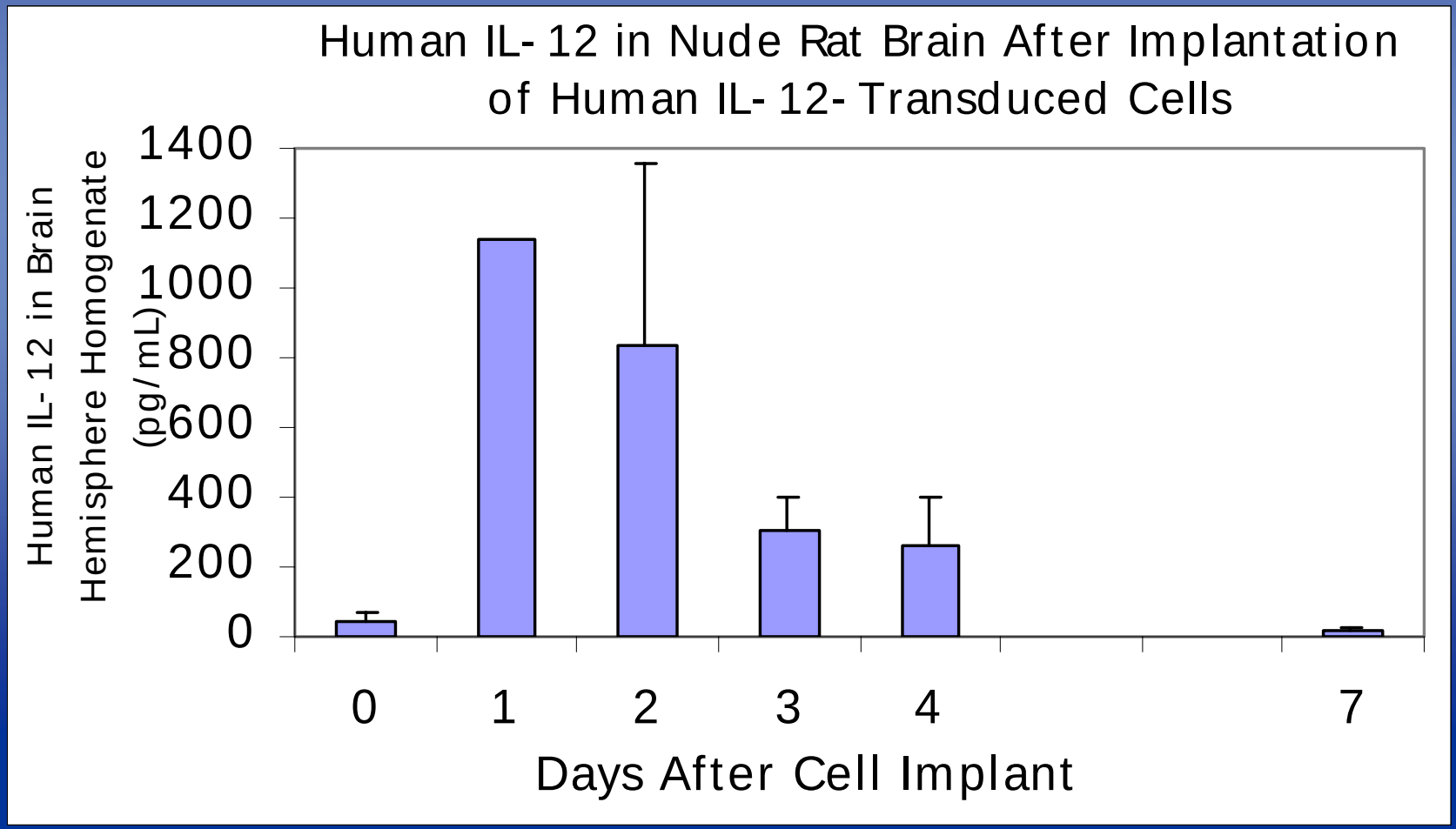
Rotated Image of U87 Tumor in Rat Brain Using Laser Confocal Microscopy 5 Days Following Contralateral Implantation of MSCs



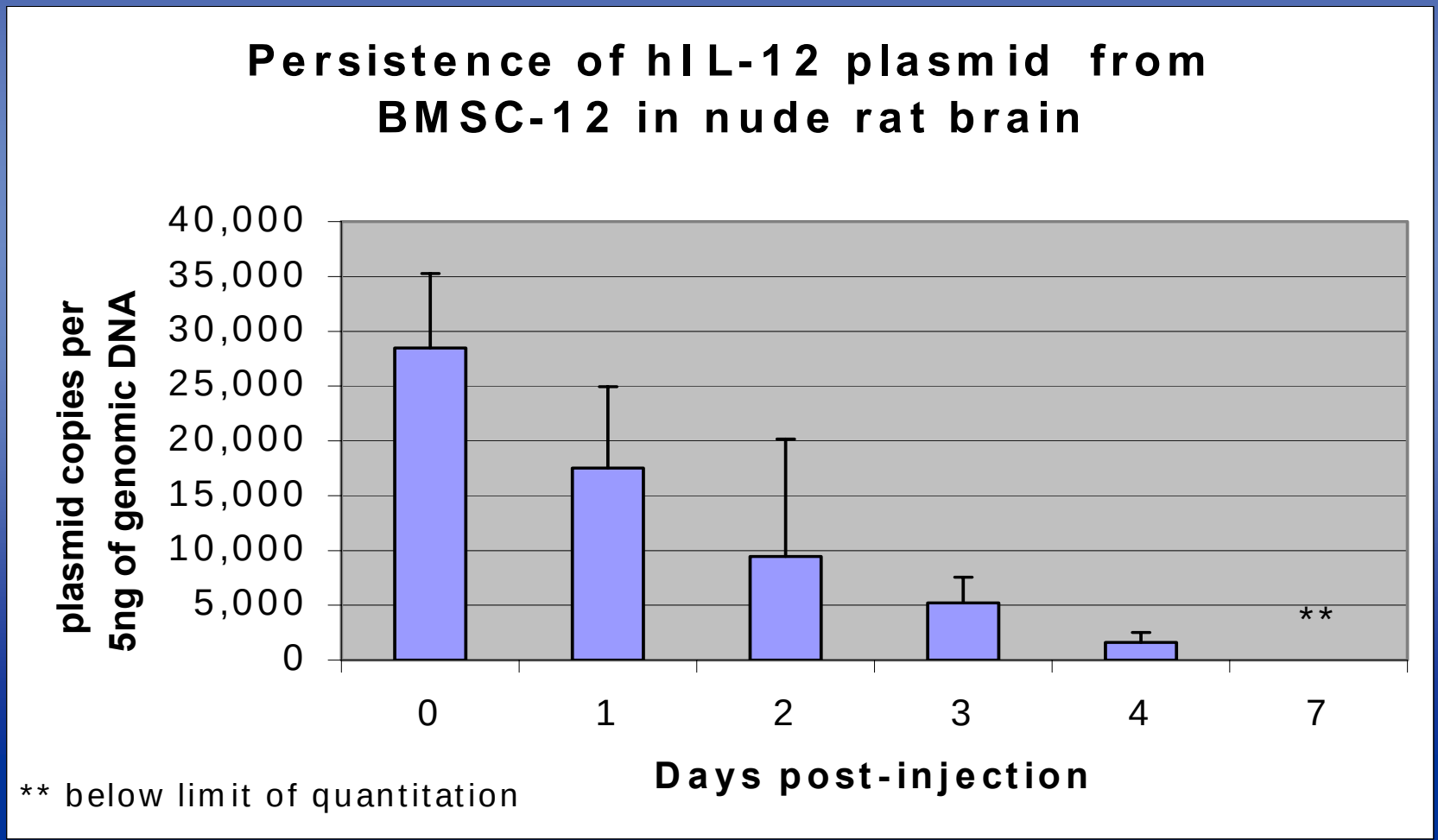
Homing of BMSCs to Contralateral Rat Tumor 5 Days Following Implantation



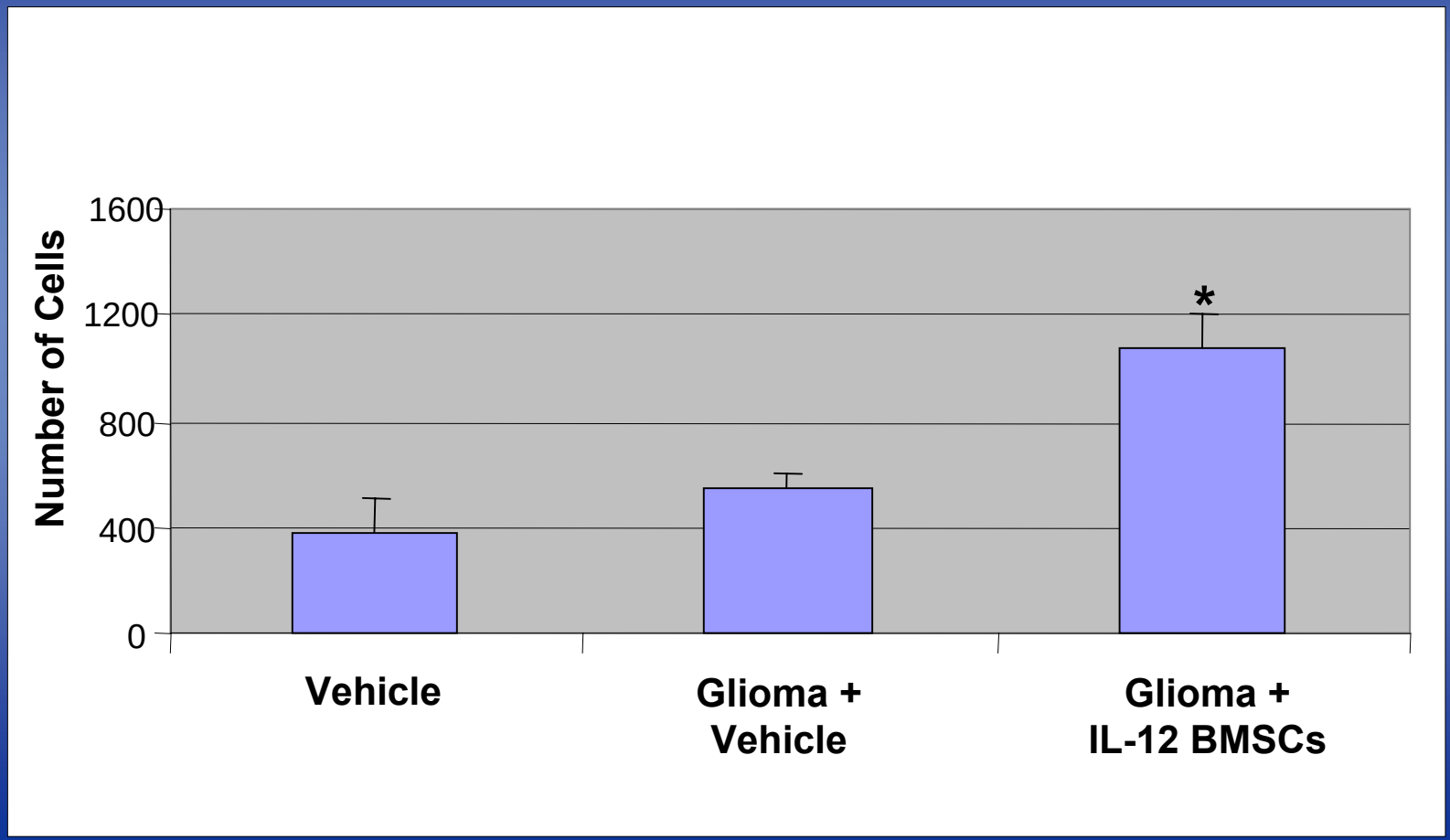
IL-12 Expression Following Implantation of BMSC12 in the Nude Rat



The Rapid Decrease in IL-12 Expression Results From the Transient Nature of the Transfection

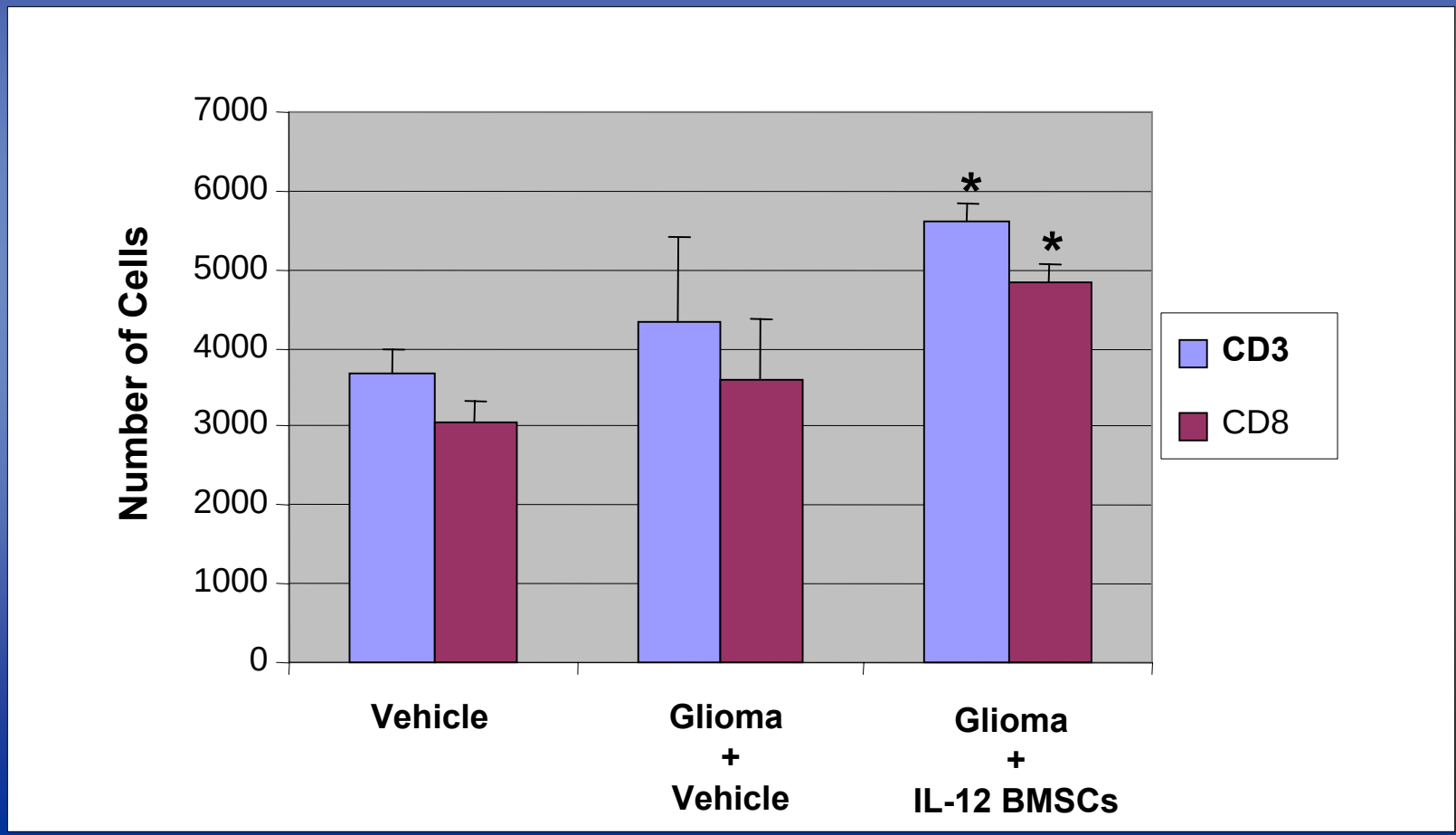


Macrophage Recruitment by IL-12 BMSCs



* Significantly different from Vehicle and Glioma + Vehicle controls (p<.005)

T Cell Recruitment by IL-12 BMSCs



* Significantly different from Vehicle control ($p<.05$)

Recruitment of NK Cells by rIL-12 or BMSC-12

Treatment Tumor + IL-12 or BMSC	Duration of Treatment	No. of Animals In Group	No. of Animals With Tumor	No. Animals Stained for NK
200 ng, IL-12,	0	2	2	0
200 ng IL-12,	1 day	2	2	1
200 ng IL-12,	3 days	2	0	*
Vehicle	4 days	2	0	N/A
5 ng Cont. infusion	4 days	4	4	4
200 ng Cont. infusion	4 days	2	2	2
Control BMSC, 50K	8 days	3	3	0
Control BMSC, 500K	8 days	3	0	N/A
BMSC-12, 50K	8 days	3	1	1
BMSC-12, 500K	8 days	3	3	3

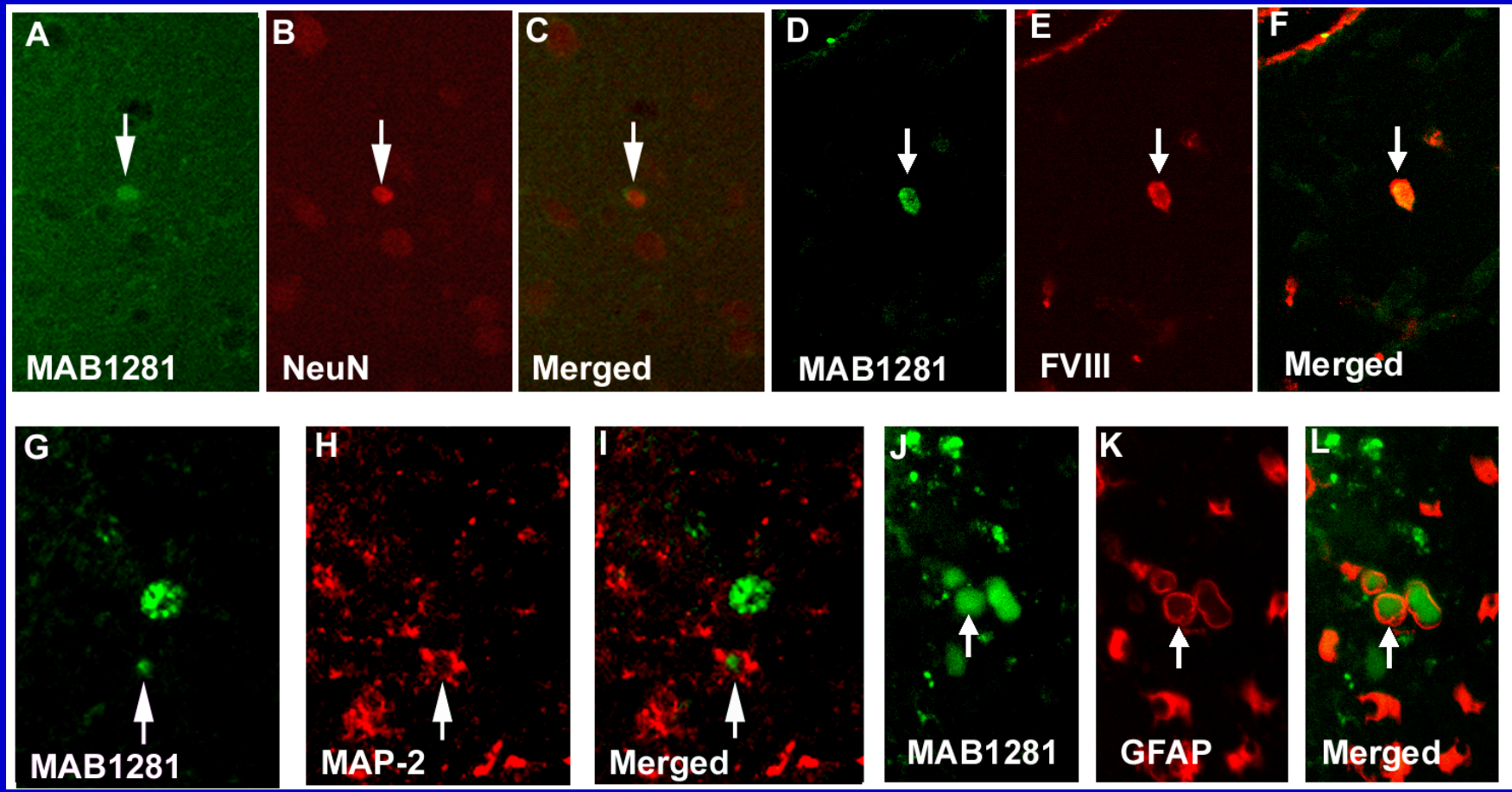
*no tumor visualized 7-2, but localized stained cells present

What Would a Survival Study Mean and What Dosage Regime Would Appropriately Mimic the Proposed Treatment

- F98 tumor is quantitatively lethal in untreated rats in 60 days
- Monthly treatment regime in rats is feasible but irrelevant
- Weekly treatment is feasible but it is unclear how precisely it relates immunologically to the proposed human therapy
- Based on this uncertainty, it is unclear how one would interpret a survival study using weekly or twice weekly treatment

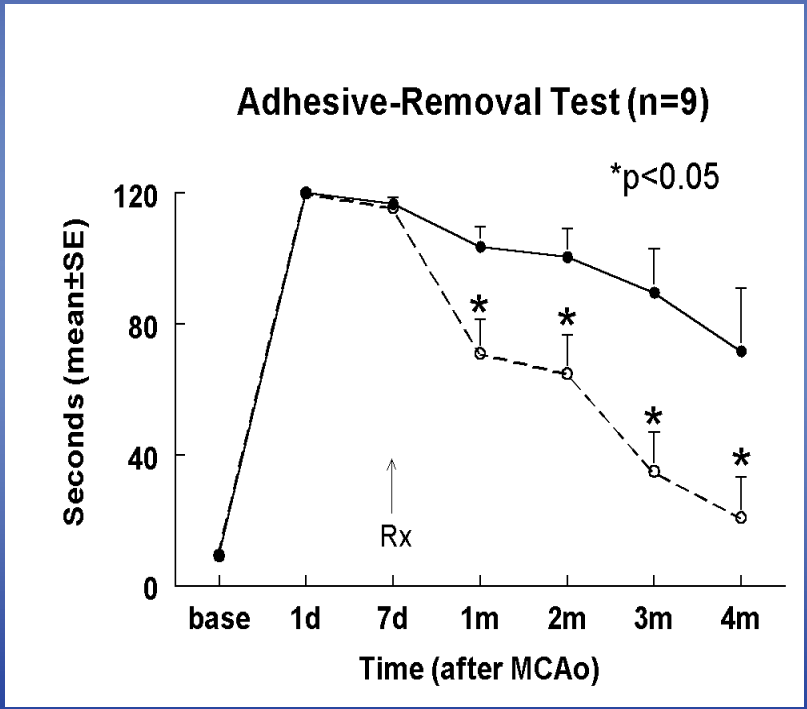
Additional Reviewer's Comments

Human BMSCs + Neural Markers in MCAO Rat Model System with IV Delivery



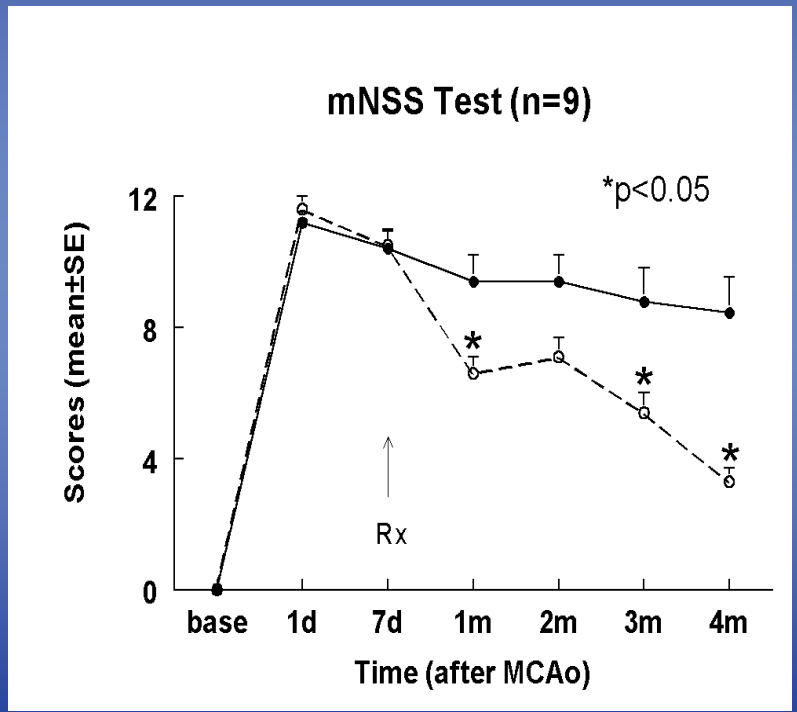
Direct injection of BMSCs into the brain yielded similar results.

Treatment of stroke with hBMSCs in rats



Motor skills

Time required to remove adhesive paper from paws



Modified Neurological Severity Scores

Assesses motor, sensory, balance and reflexes

Ad Hoc Reviewer Comments

Comment	Response
1.Engraftment and differentiation of BMSCs into neural elements may be problematic	Unlikely due to low long-term survival and low propensity to differentiate into neural cells.
2. Cytotoxic pro-drug approach is better than cytokine approach	We disagree. Cytokine approach is safer (no bystander killing) and doesn't require homing to every metastatic site by gene modified cells. Activated immune cells will target metastatic sites.

Ad Hoc Reviewer Comments (Continued)

Comment	Response
3. Allogeneic approach may be superior to autologous approach	Autologous approach -- safer for Phase I study -- large inflammatory response may be detrimental to the brain. Induction of an allo-immune response to the cells may potentially complicate interpretation of study and may prevent multiple dose administration
4. NSCs are more suitable for clinical application than BMSCs	Suitability of BMSCs for the clinic has already been demonstrated-- cells have been used previously in human subjects (other indications). NSCs are not ready for the clinic due to issues of heterogeneity and ability to scale-up production without immortalization of cells to achieve clinical dose, etc.