Mesenchymal Stem Cells and Cancer: Their Interplay

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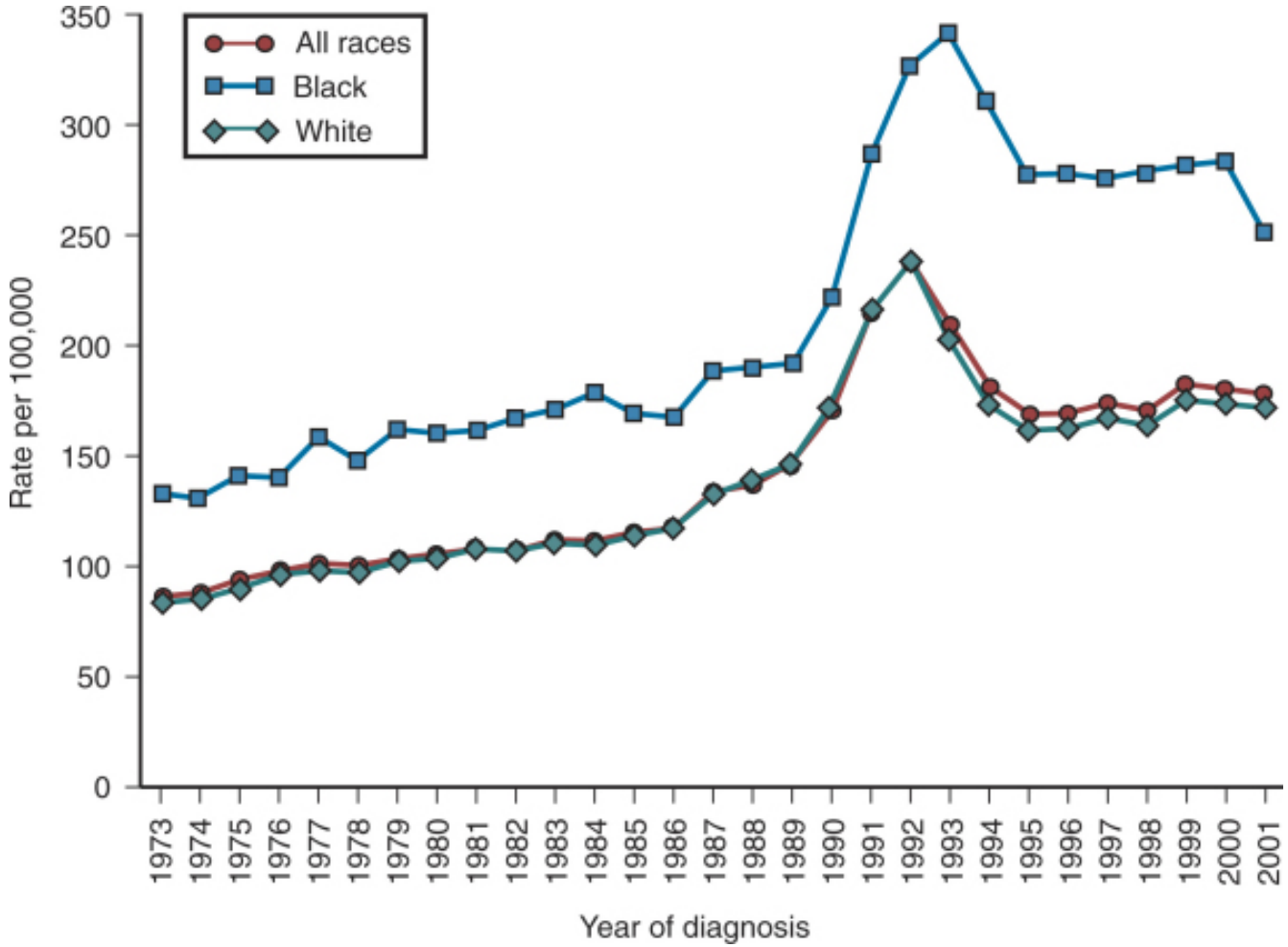
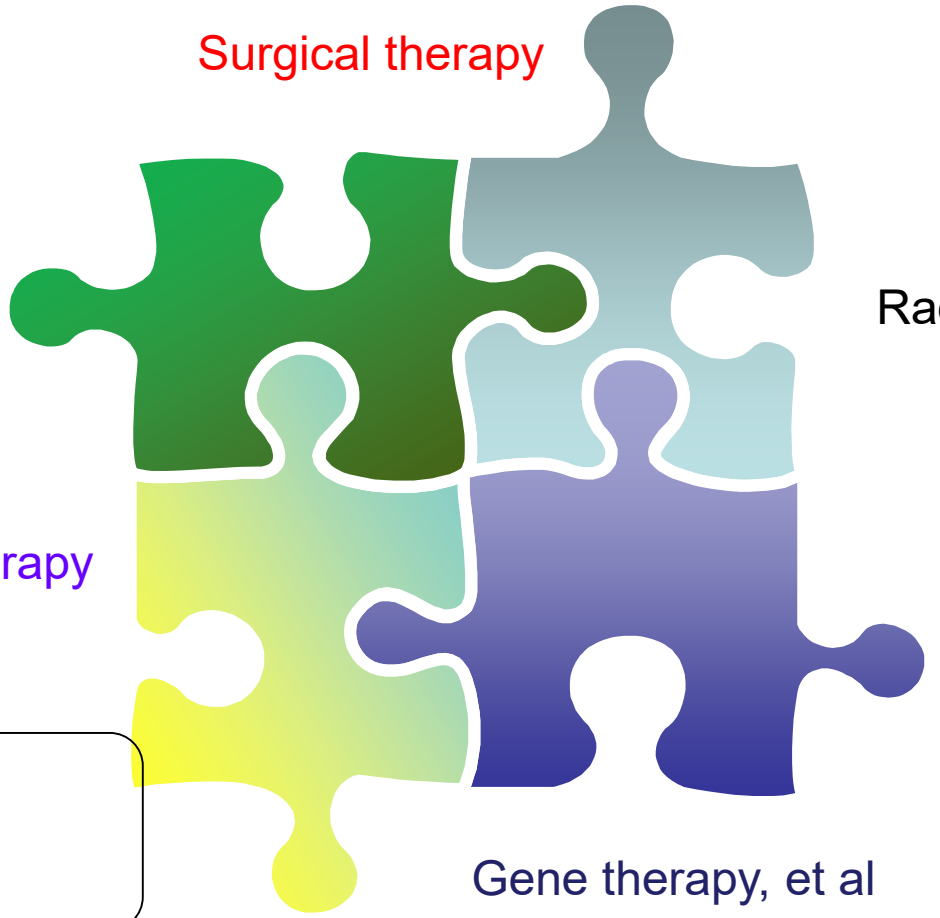
Cancers Are Major Healthcare Challenges



(Campbell-Walsh Urology, 9th ed, 2007, Saunder)

The disadvantages of traditional therapies



Problems of
specific targeting ?

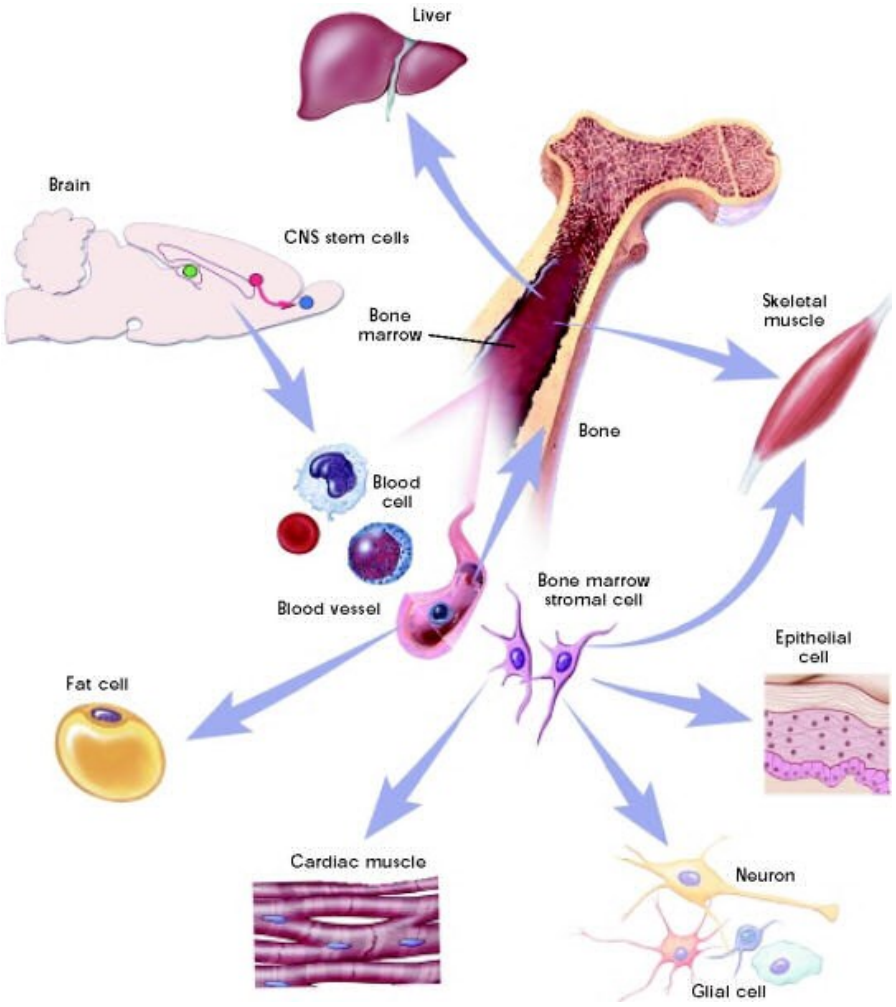
Gene therapy methods are well established

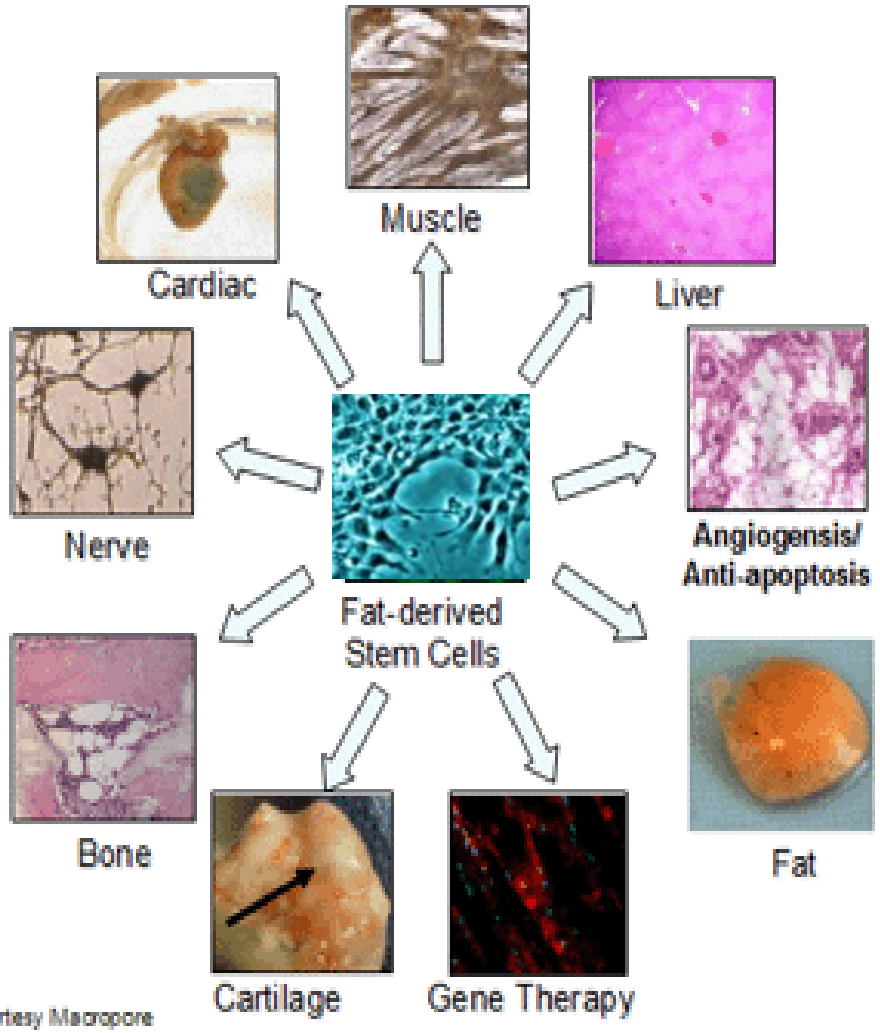
- **As of January 2007, there were a total of 1,260 gene therapy clinical trials approved worldwide, with approximately 90 new trials submitted for approval each year, most of which in the USA and Europe.**

- **Nearly half of all trials use one of two viral-based gene transfer vectors, adenovirus and retrovirus.**

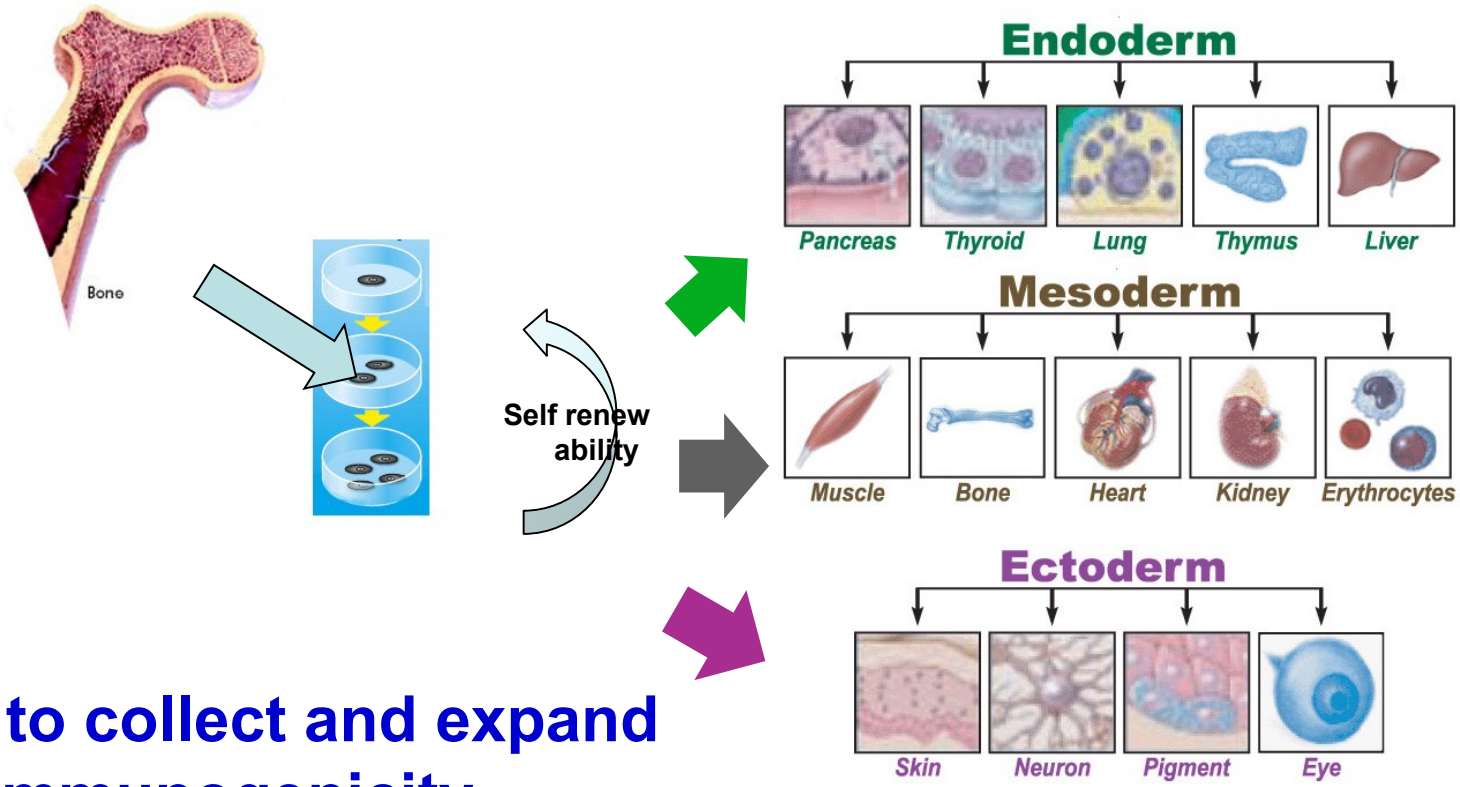
- **Specific homing/targeting is still the challenge.**

Mesenchymal Stem Cells



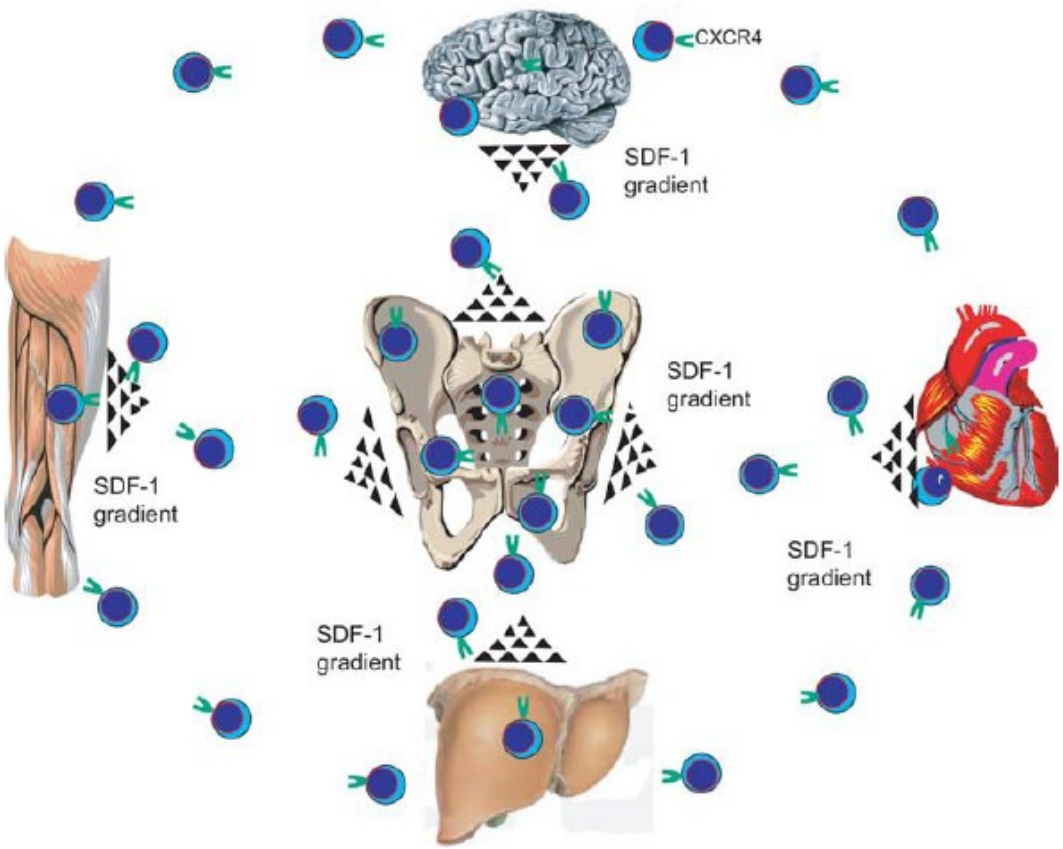


Bone Marrow Mesenchymal Stem Cells



- Easy to collect and expand
- Low immunogenicity
- Low tumorigenic/transformation rate
- Can be gene modified without affecting their phenotype
- Home to injury tissues and tumours/cancer

MSCs Can Home to Injury Tissues



MSCs home to a variety of tissues, particularly after tissue injury and ischemia.

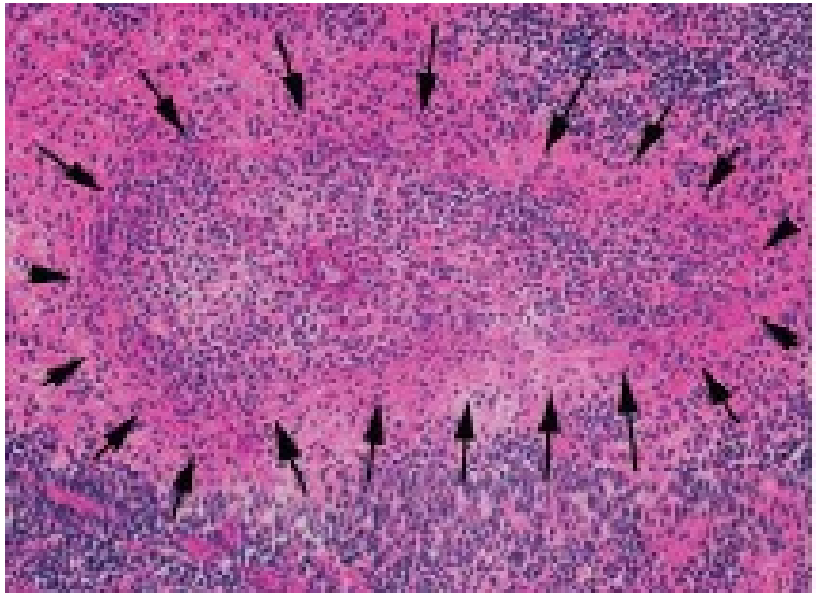
Miyahara Y, Nagaya N, Kataoka M,et al . Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med. 2006 Apr;12(4):459-65.

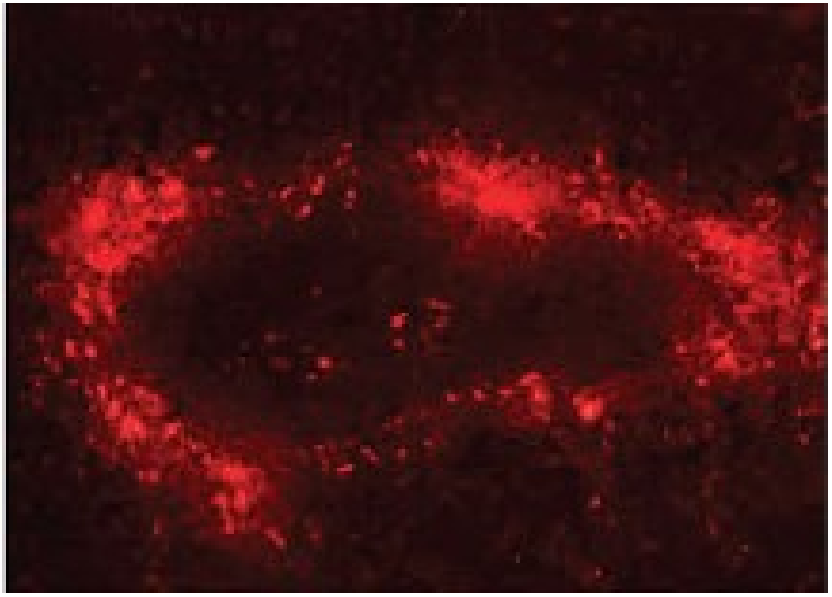
Carvalho KA, Guarita-Souza LC, Hansen P,et al. Cell Transplantation After The Coculture of Skeletal Myoblasts and Mesenchymal Stem Cells in the Regeneration of the Myocardium Scar: An Experimental Study in Rats. Transplant Proc. 2006 Jun;38(5):1596-1602.

Gnecchi M, He H, Noiseux N,et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J. 2006 Apr;20(6):661-9.

Kraitchman DL, Tatsumi M, Gilson WD, et al. Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction. Circulation. 2005 Sep 6;112(10):1451-61.

Systemically Administrated MSCs Specially Home to the Tumours





Journal of the National Cancer Institute, Vol. 97, No. 7, 2005

Similarities Between Tissue Repair and Tumour Development

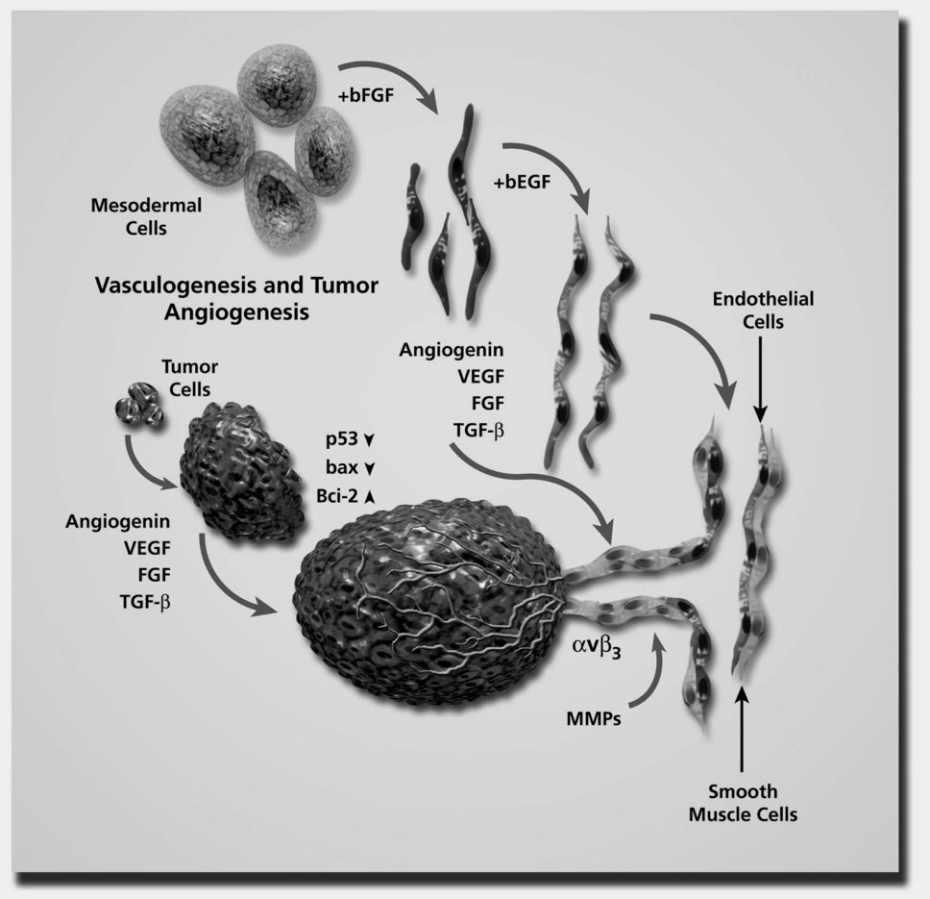
Tumour development shares many common characteristics of wound healing; *Tumours are regarded as wounds that never heal.*

- **Rapid cell proliferation and differentiation**
- **Angiogenesis**
- **Immuno-suppresive nature**

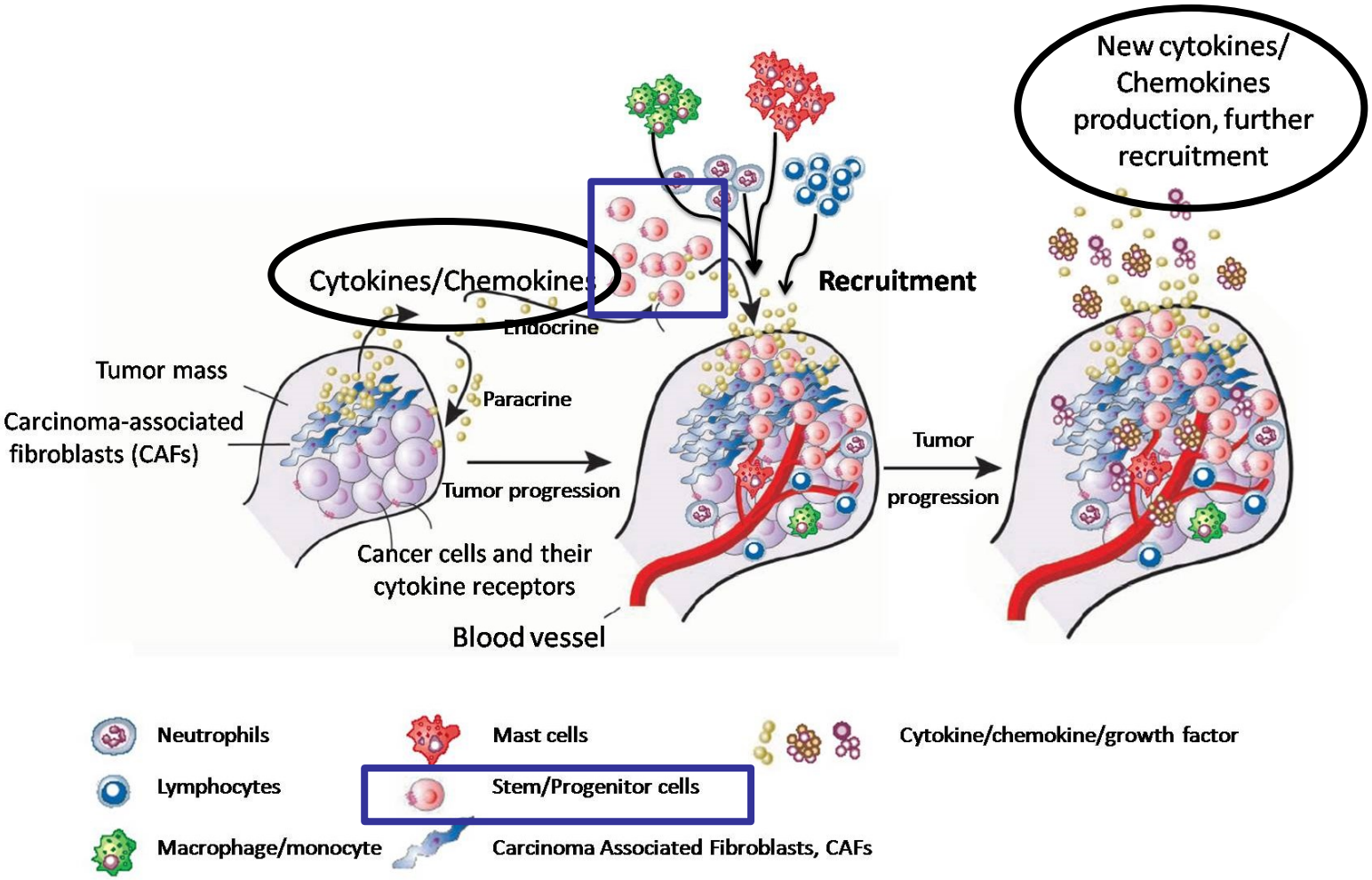
Main Difference: ***Tissue Remodeling: Apoptosis***

MSCs and Tumour Stroma

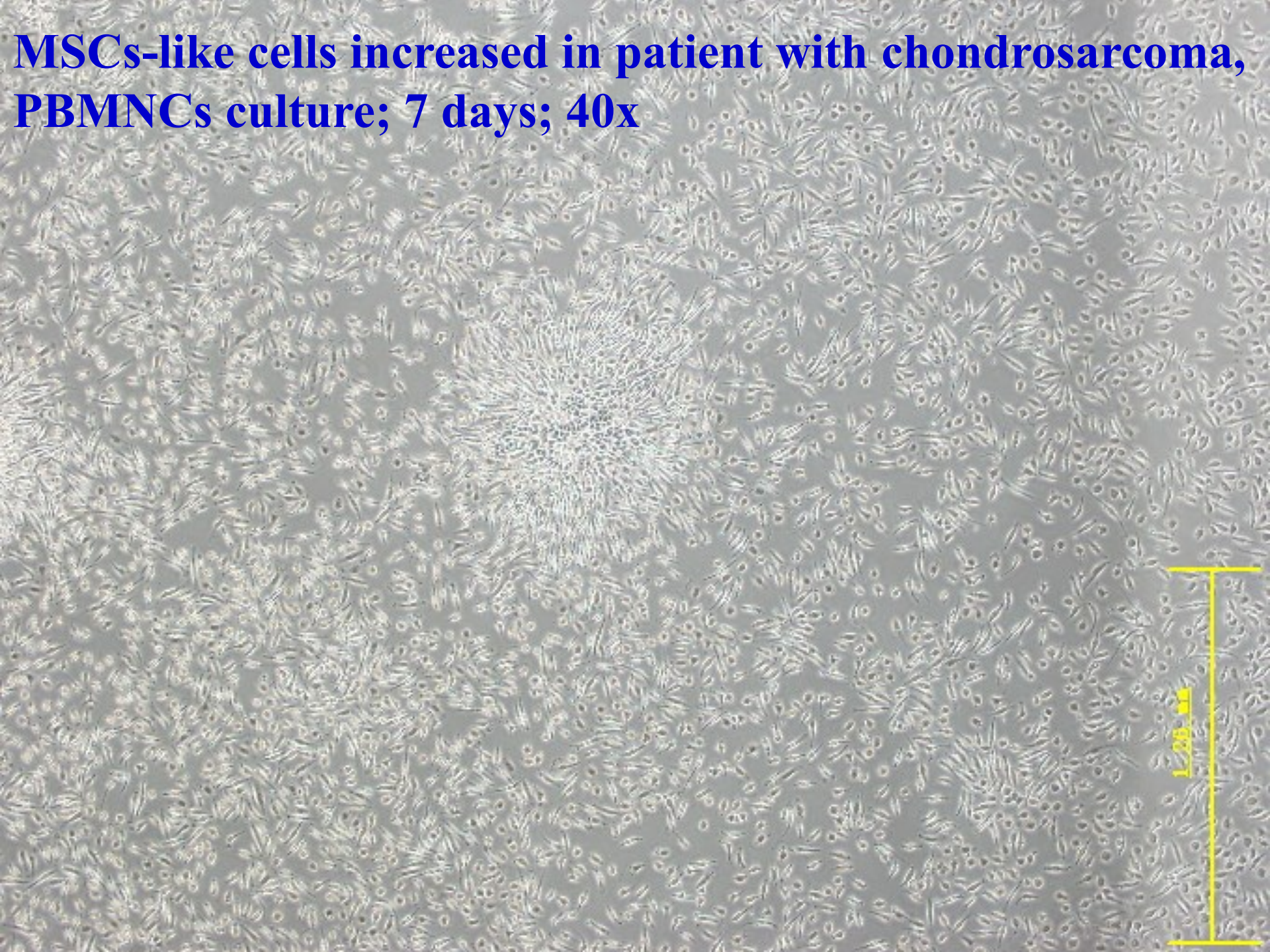
- **Where do the tumour stroma orginiate ?**
  - **Local**
  - **Circulating cells**
- **MSCs form tumour stroma.**
- **Tumor mcroenvironment select MSC engraftment.**



The tumour environment recruits MSCs

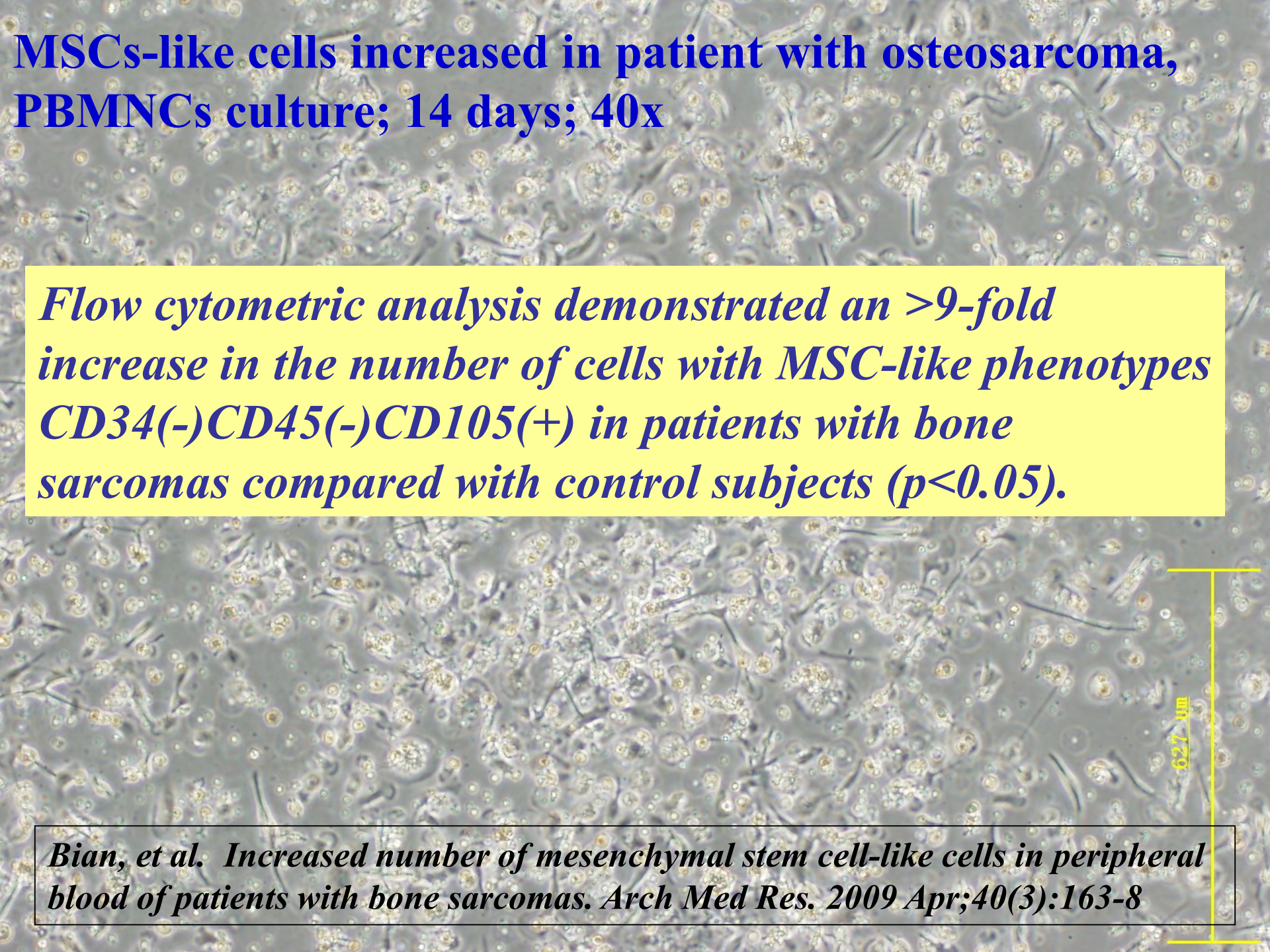


MSCs-like cells increased in patient with chondrosarcoma, PBMNCs culture; 7 days; 40x



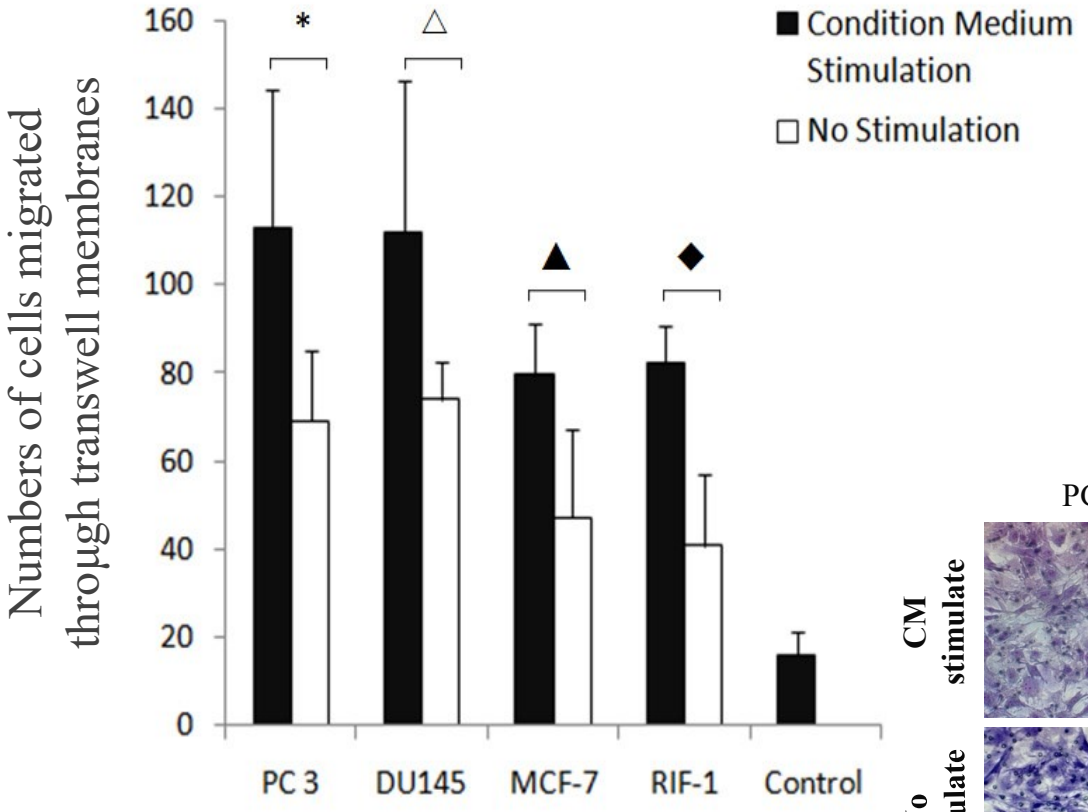
MSCs-like cells increased in patient with osteosarcoma, PBMNCs culture; 14 days; 40x

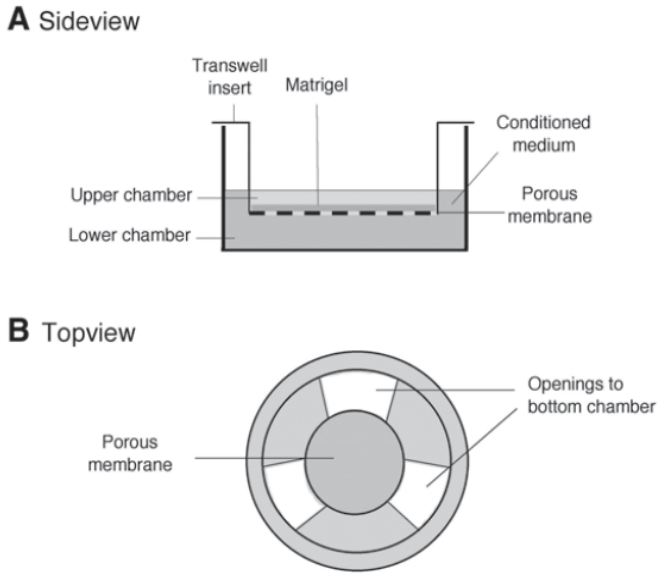
Flow cytometric analysis demonstrated an >9-fold increase in the number of cells with MSC-like phenotypes CD34(-)CD45(-)CD105(+) in patients with bone sarcomas compared with control subjects ($p<0.05$).

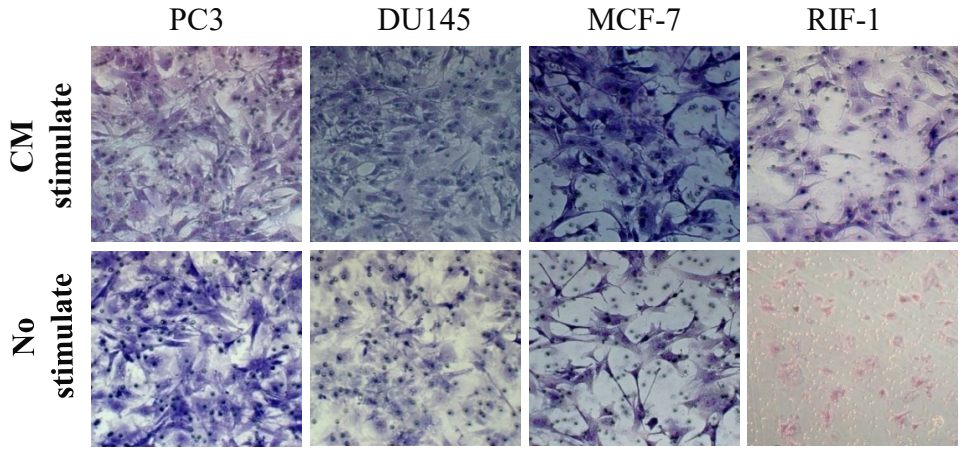


Bian, et al. Increased number of mesenchymal stem cell-like cells in peripheral blood of patients with bone sarcomas. Arch Med Res. 2009 Apr;40(3):163-8

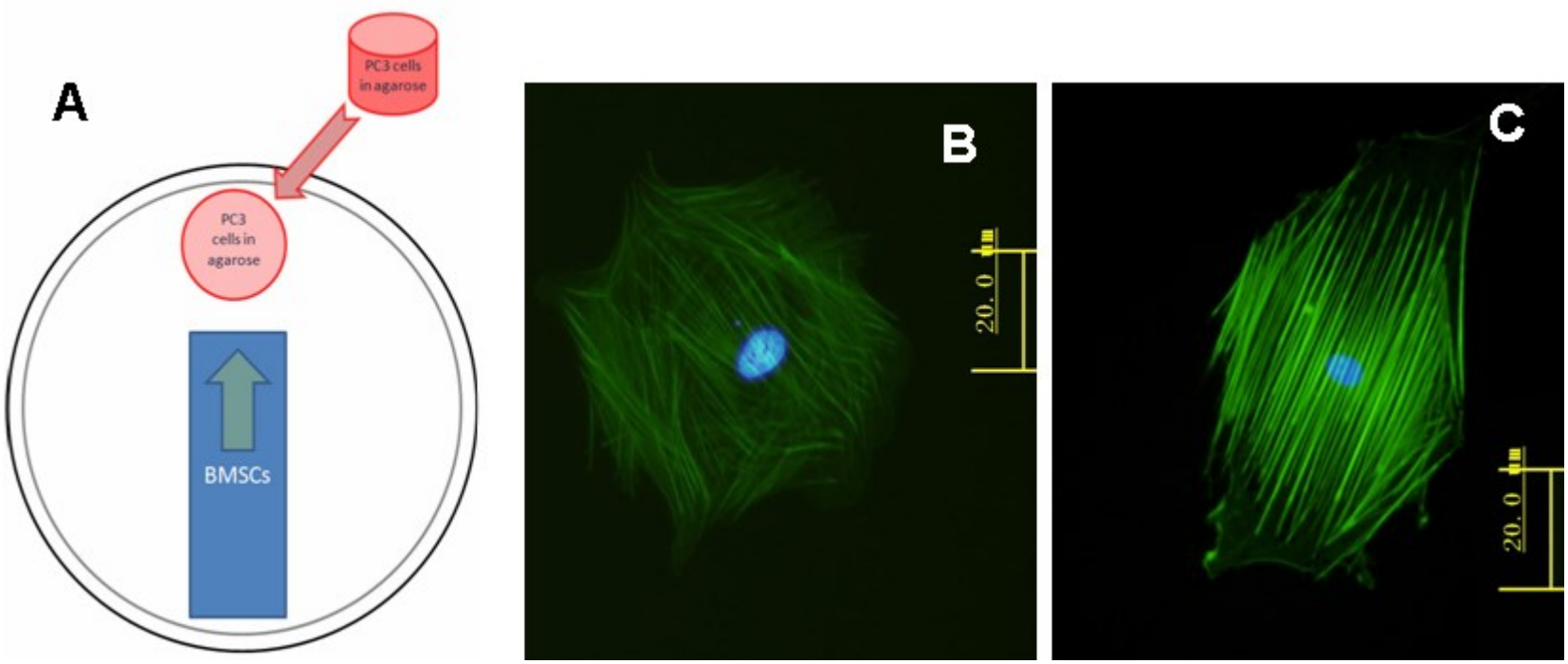
BMSCs migration toward tumor cell lines before and after tumor condition medium stimulation



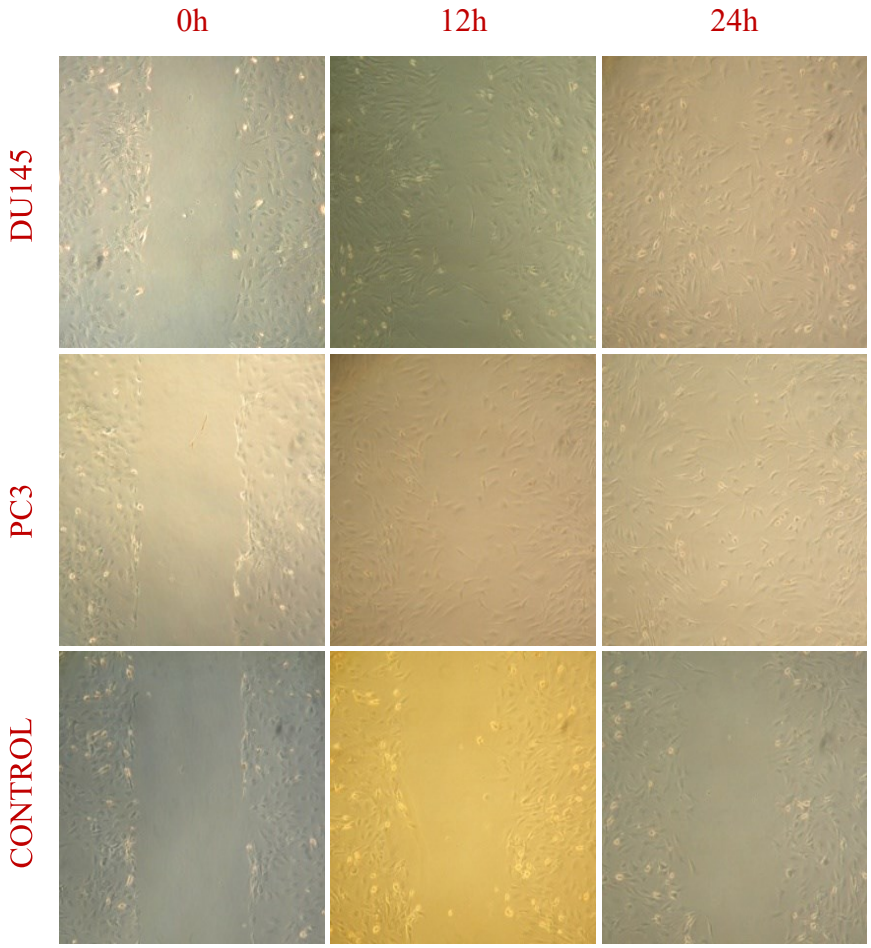


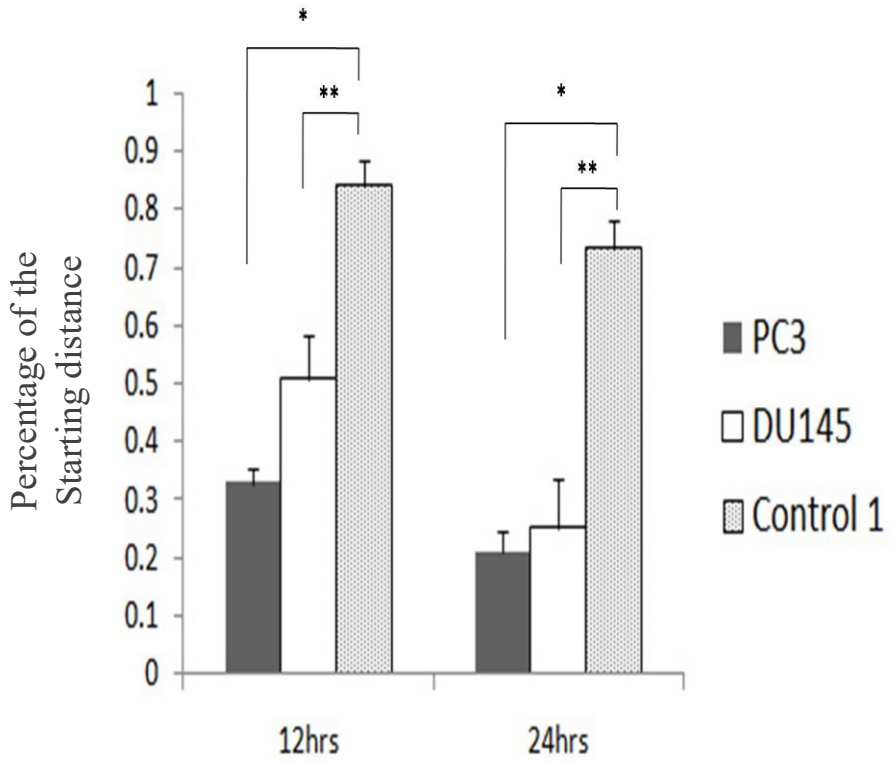


BMSCs response to tumor cells in close contact

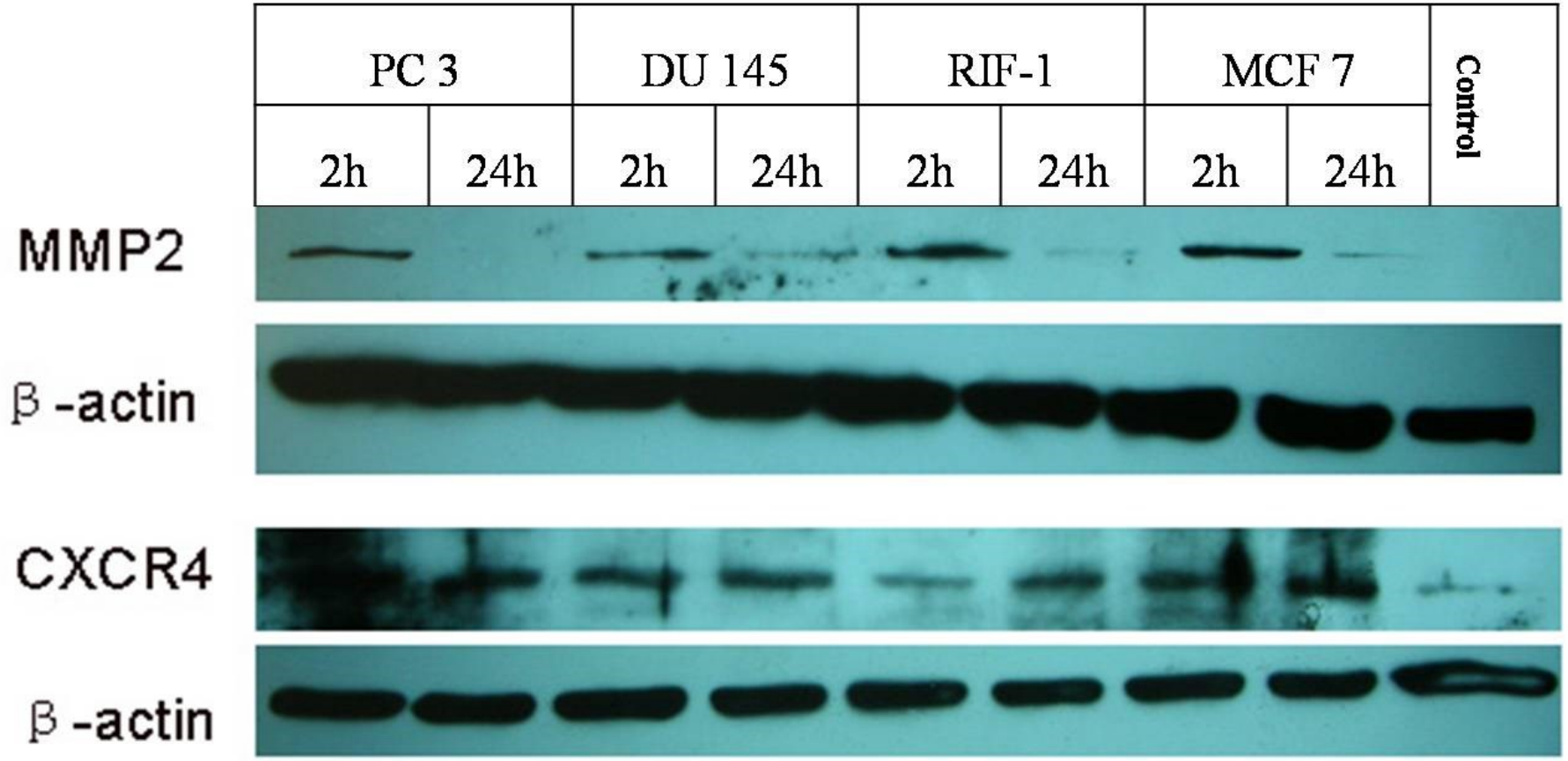


Wound healing assay of BMSCs in tumor cells condition media

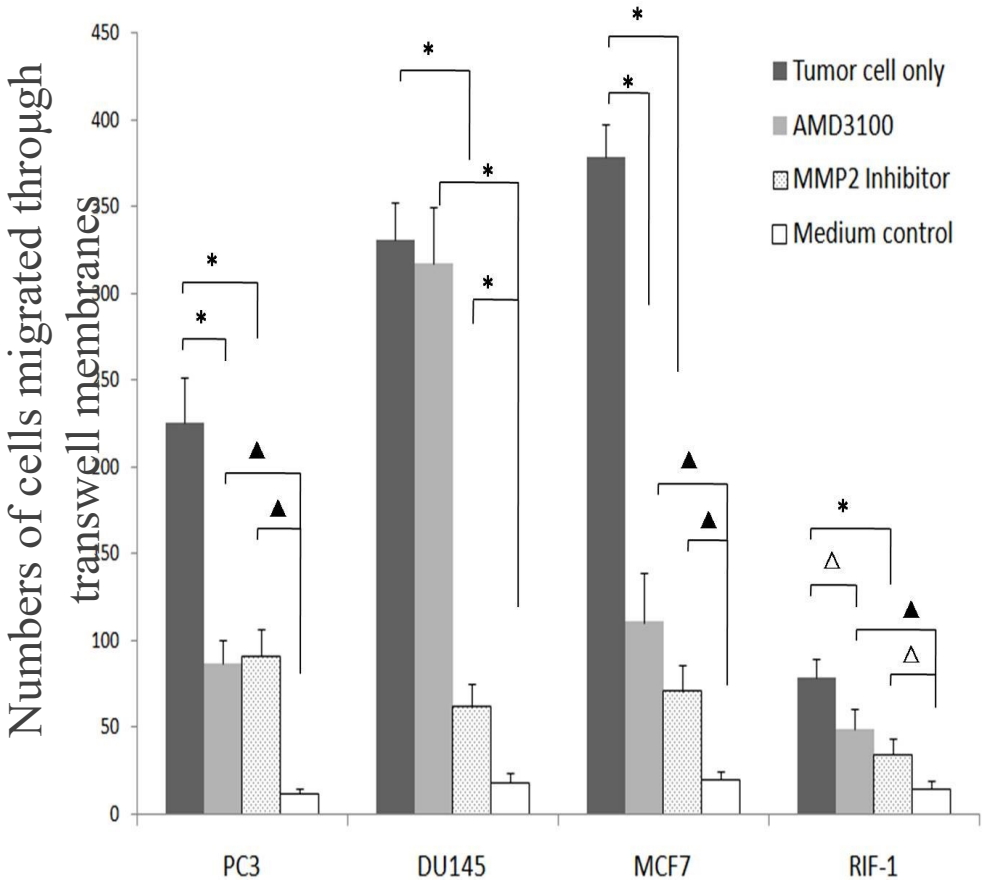


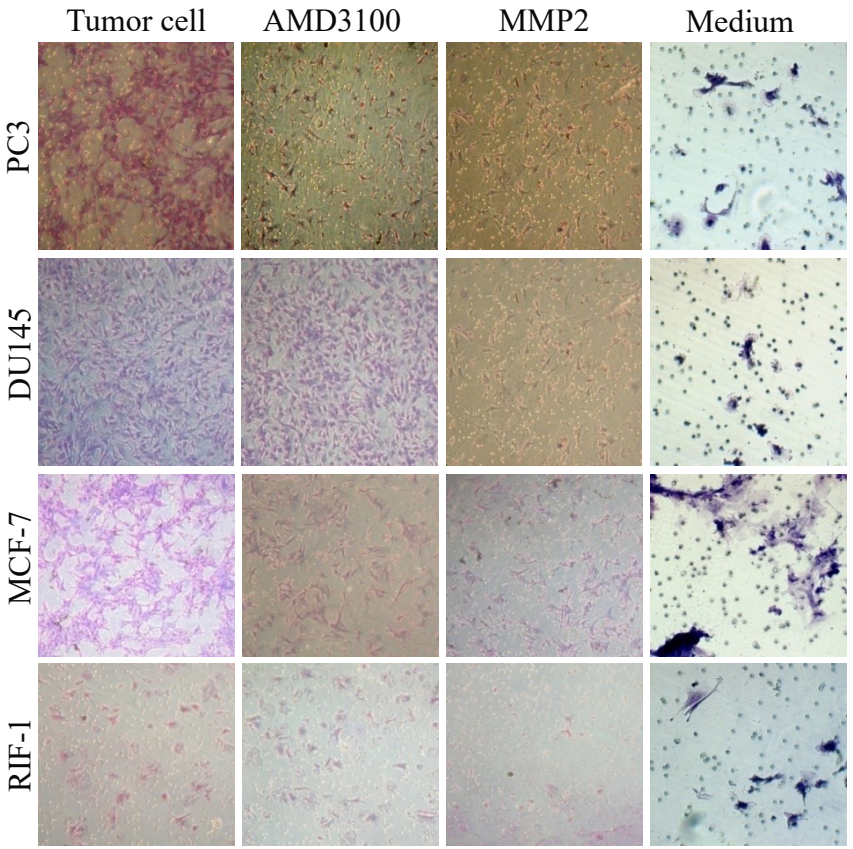


The changes of CXCR4 and MMP-2 expression were confirmed by Western blot

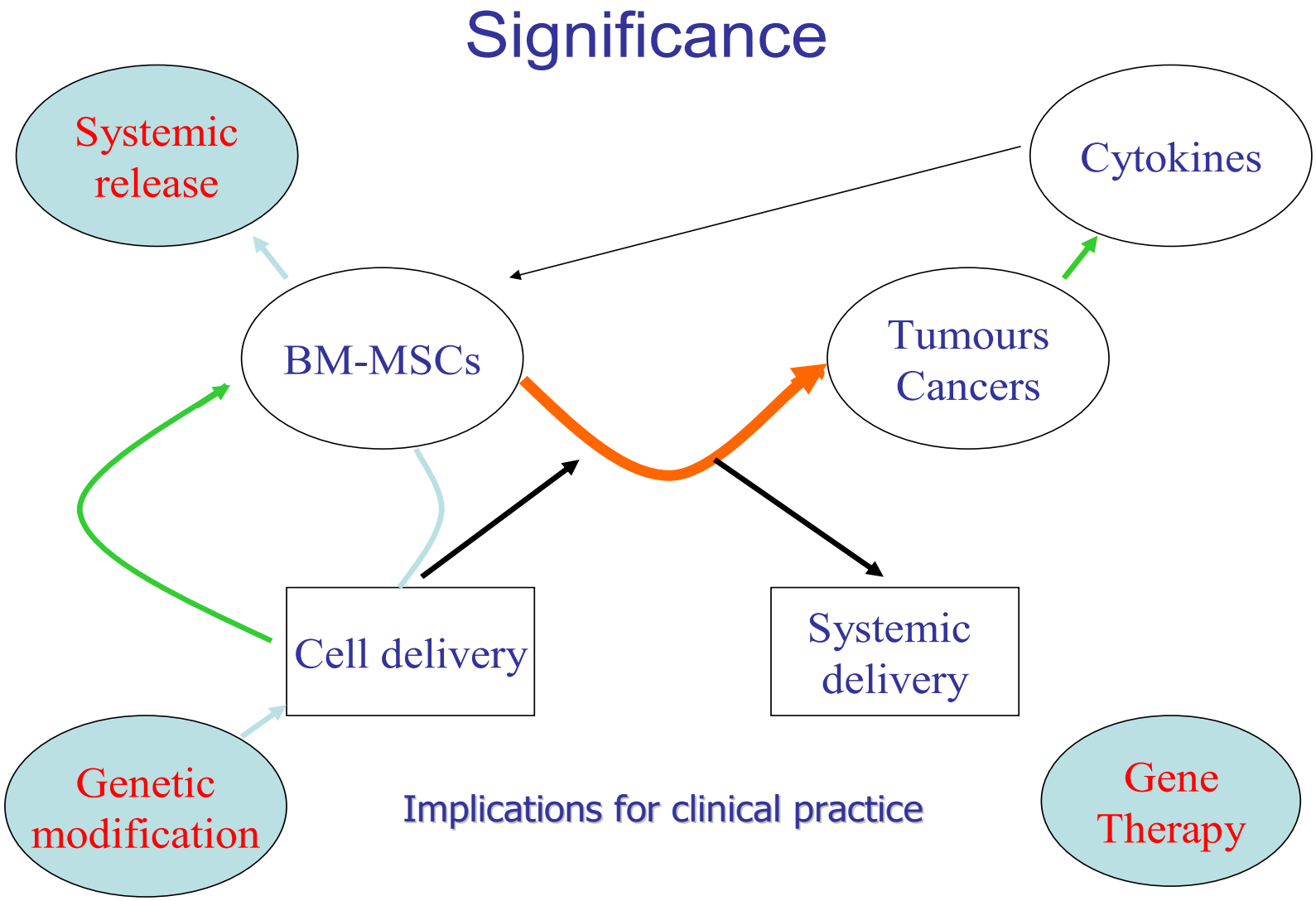


BMSCs migration towards to tumor cells before and after treating with CXCR4 and MMP2 inhibitor



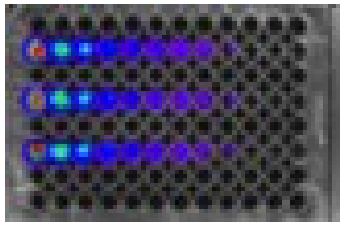


MSCs as carrier for anti-tumour/cancer therapy



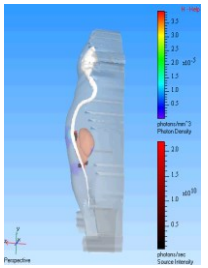
IVIS ® 200 Imaging System: Tracing Stem Cells in vivo

In vitro

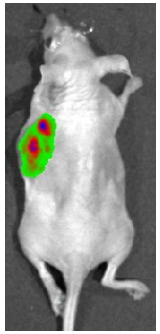


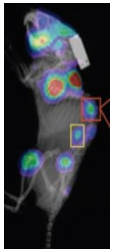
GFP Rat
Luc-CMV Mouse
Luc-BMP-4 Mouse

3D reconstruction



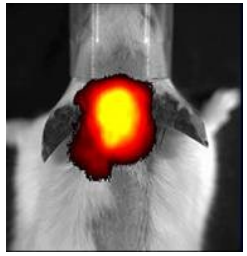
In vivo bioluminescence

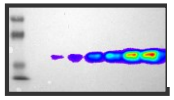


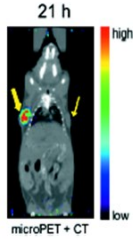


Co-registration with X-Ray

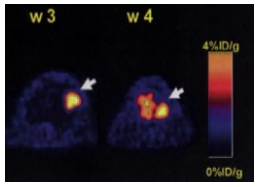
In vivo fluorescence

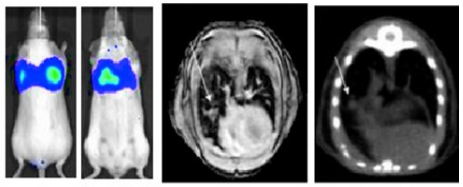






with PET & SPECT

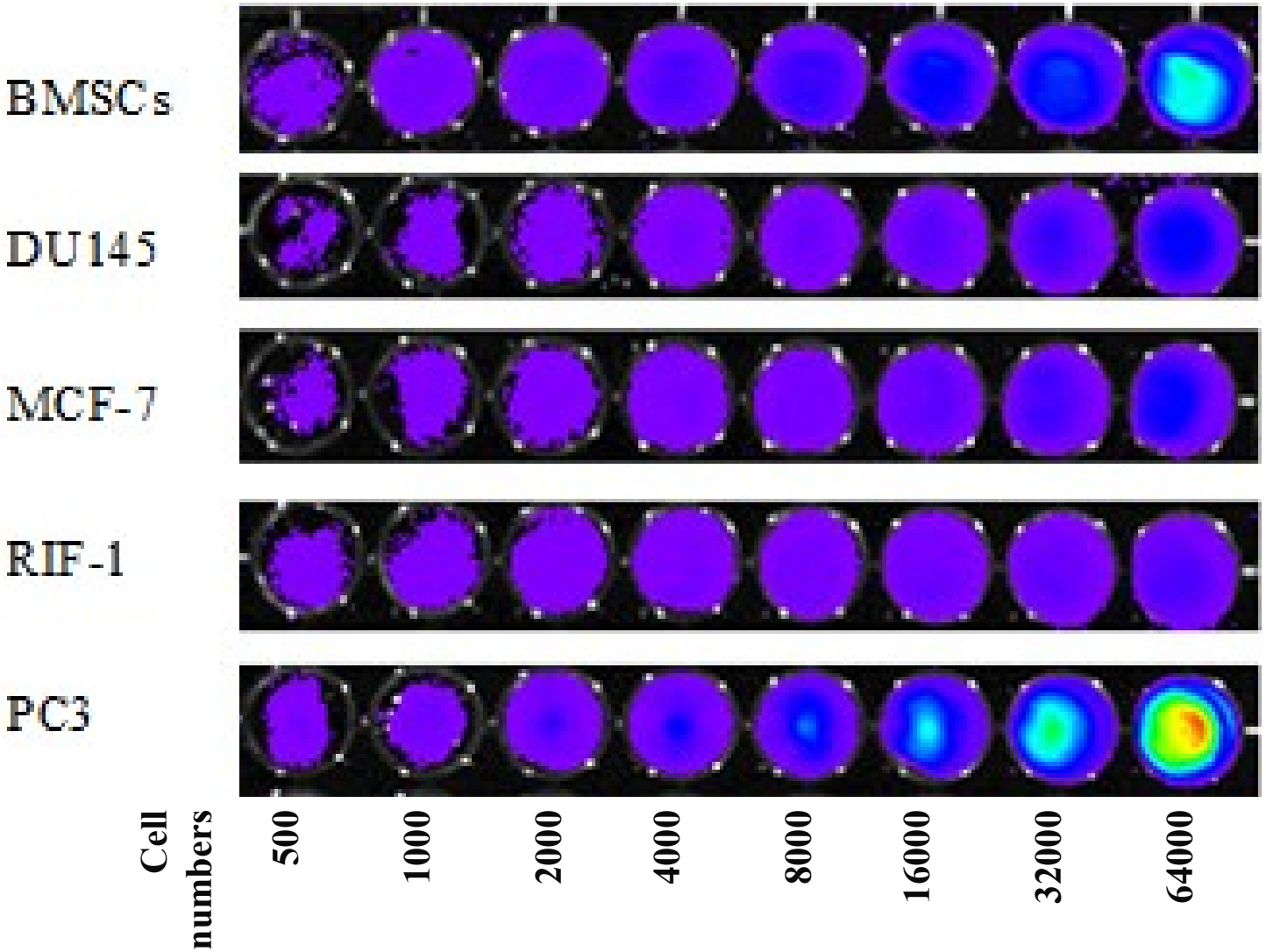




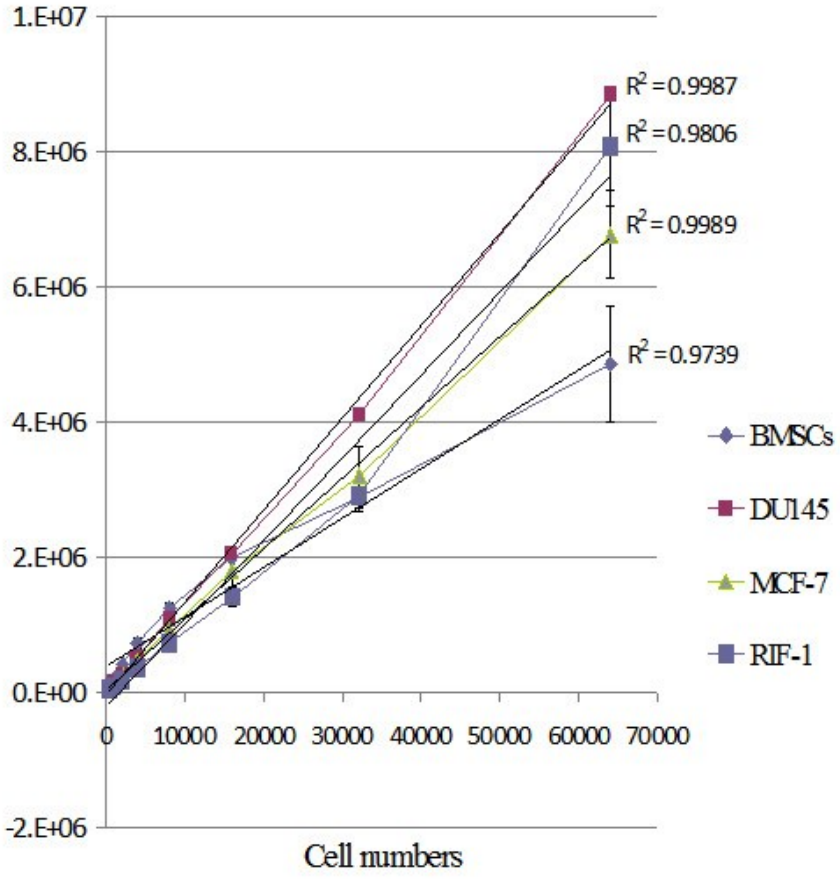
with MRI & CT

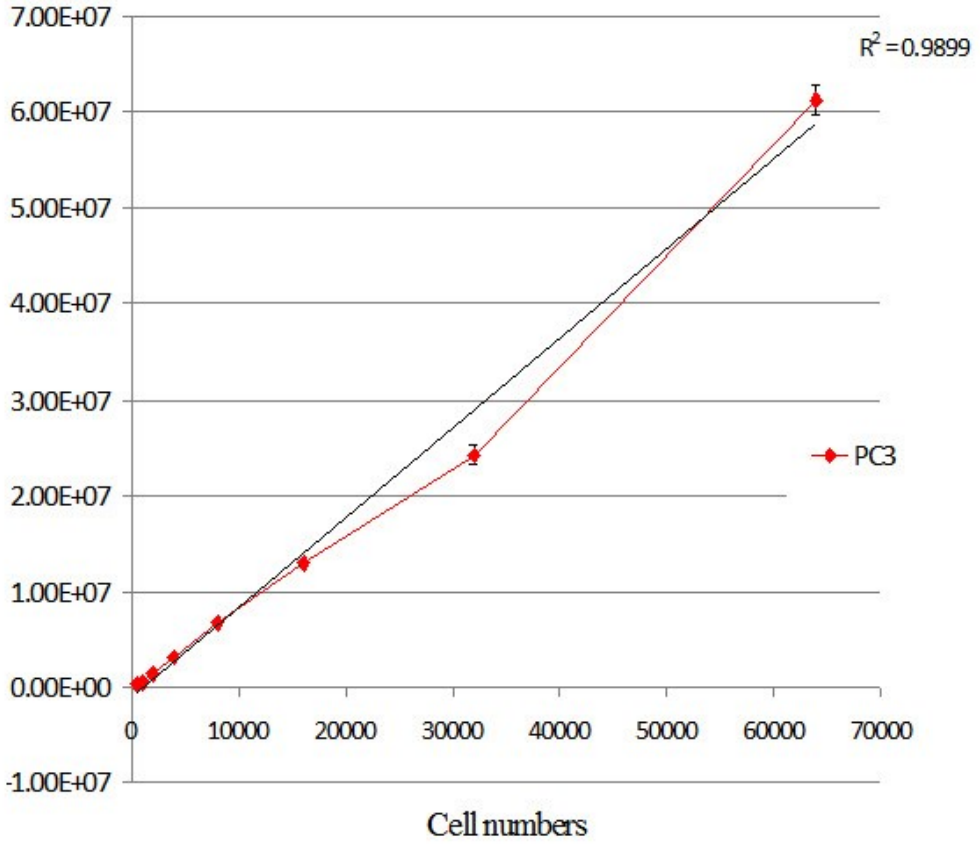
Chemiluminescence

Luciferase gene was stably transduced into BMSCs and tumour cell lines



Good correlations were found between cell numbers and bioluminescence in all cells

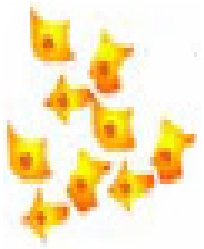




Study MSCs Homing to Tumours

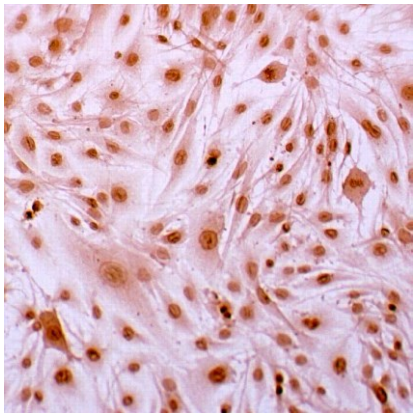
TUMOUR CELLS SUBCUTANEOUS IMPLANTATION

Luciferase labelled MSCs

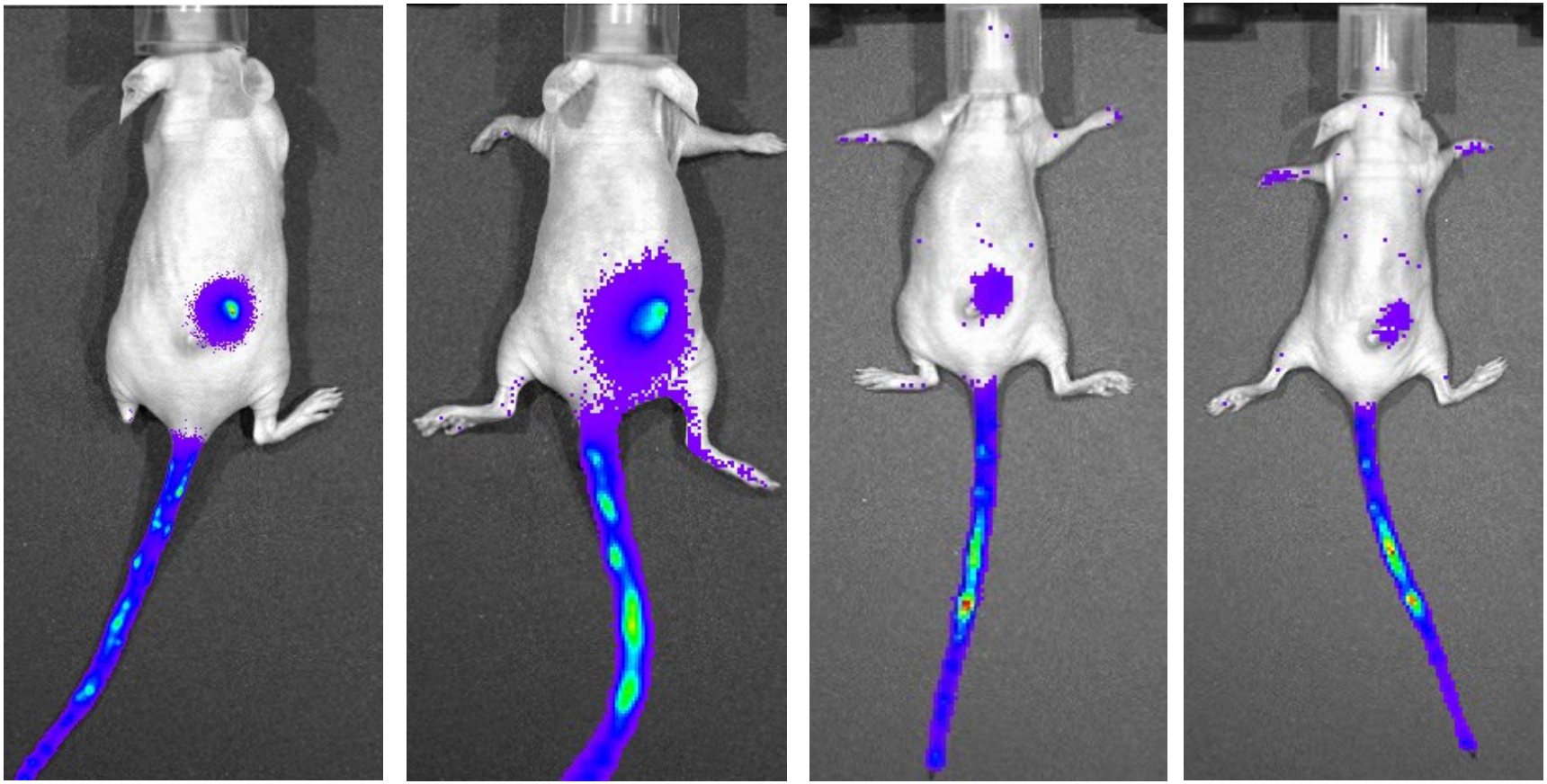


Tumour xenograft model

Luciferin injection
In-vivo imaging



MSCs distribution in PC3 tumor bearing mice

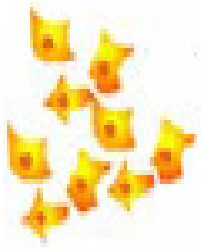


Day 3 Day 6 Day 9 Day 12

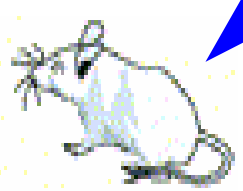
Study MSCs Homing to Tumours

TUMOUR CELLS SYSTEMIC INJECTION

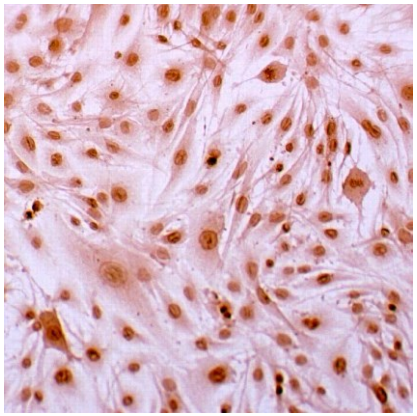
Luciferase labelled tumour cells

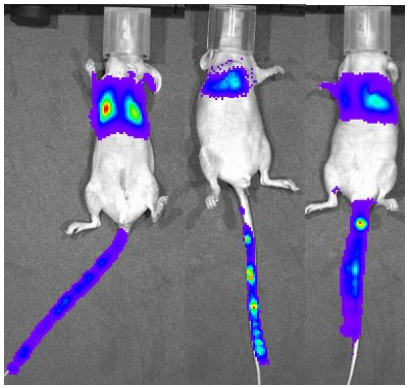


Tumour metastasis model

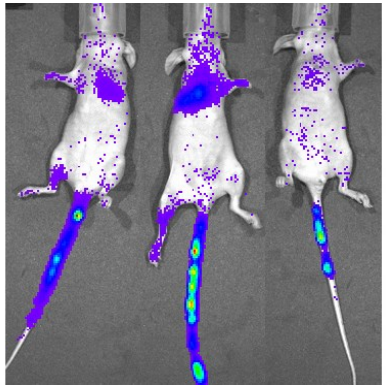


Luciferin injection
In-vivo imaging

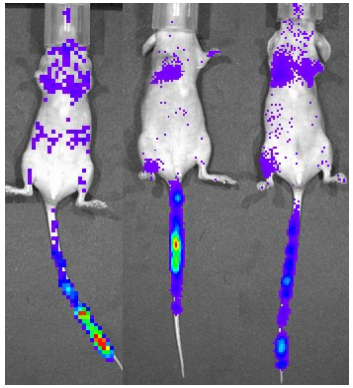




Day 1



Day 3



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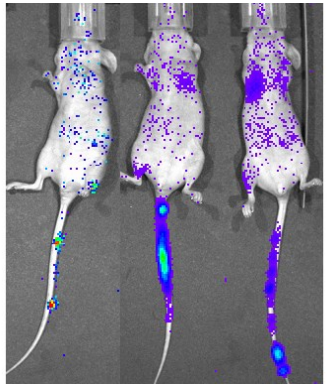
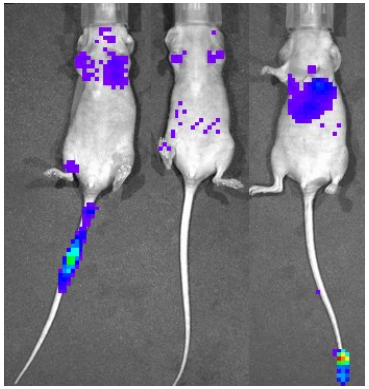
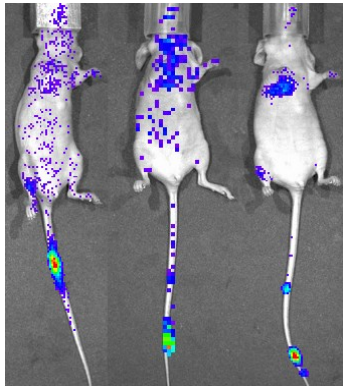


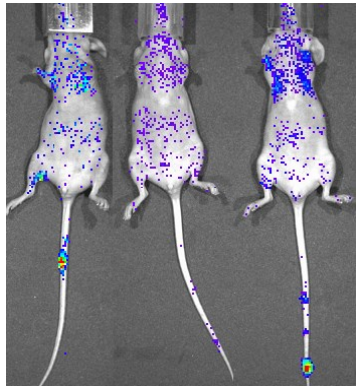
Day 7



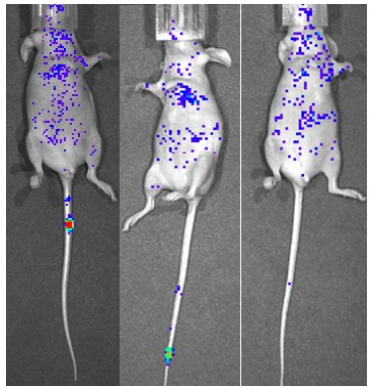
Day 9



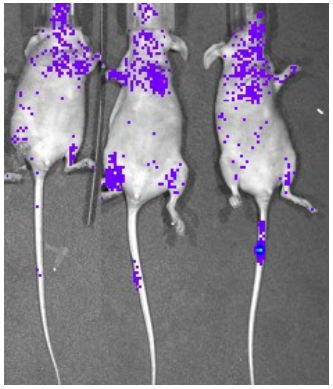
Day 12



Day 14

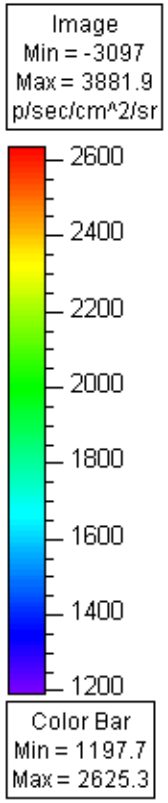


Day 20



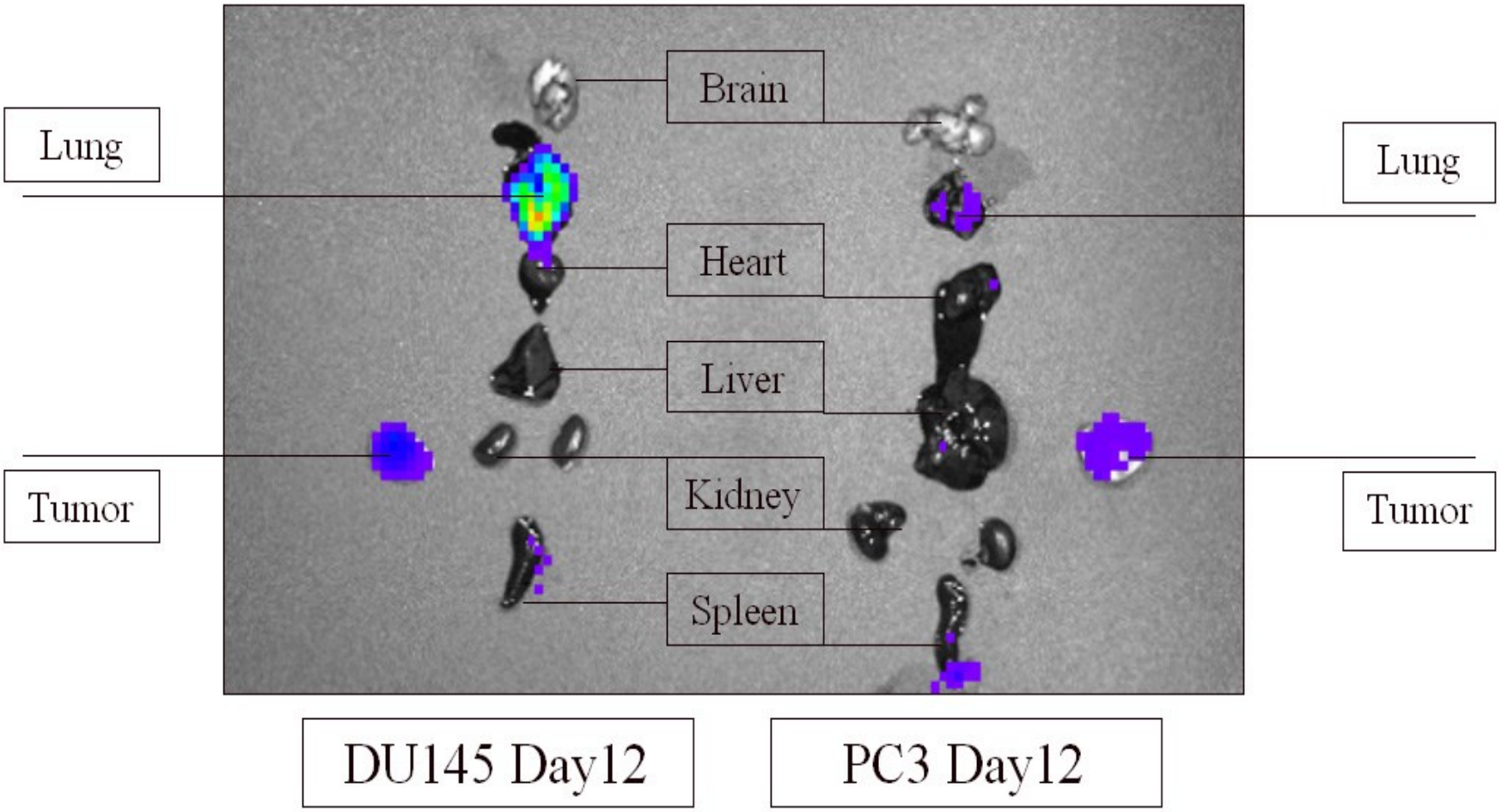
Day 30

MSCs distribution in PC3 lung tumor model from day 1 to day30. The cells mainly stay in lung where the tumor located. Proved by histology.

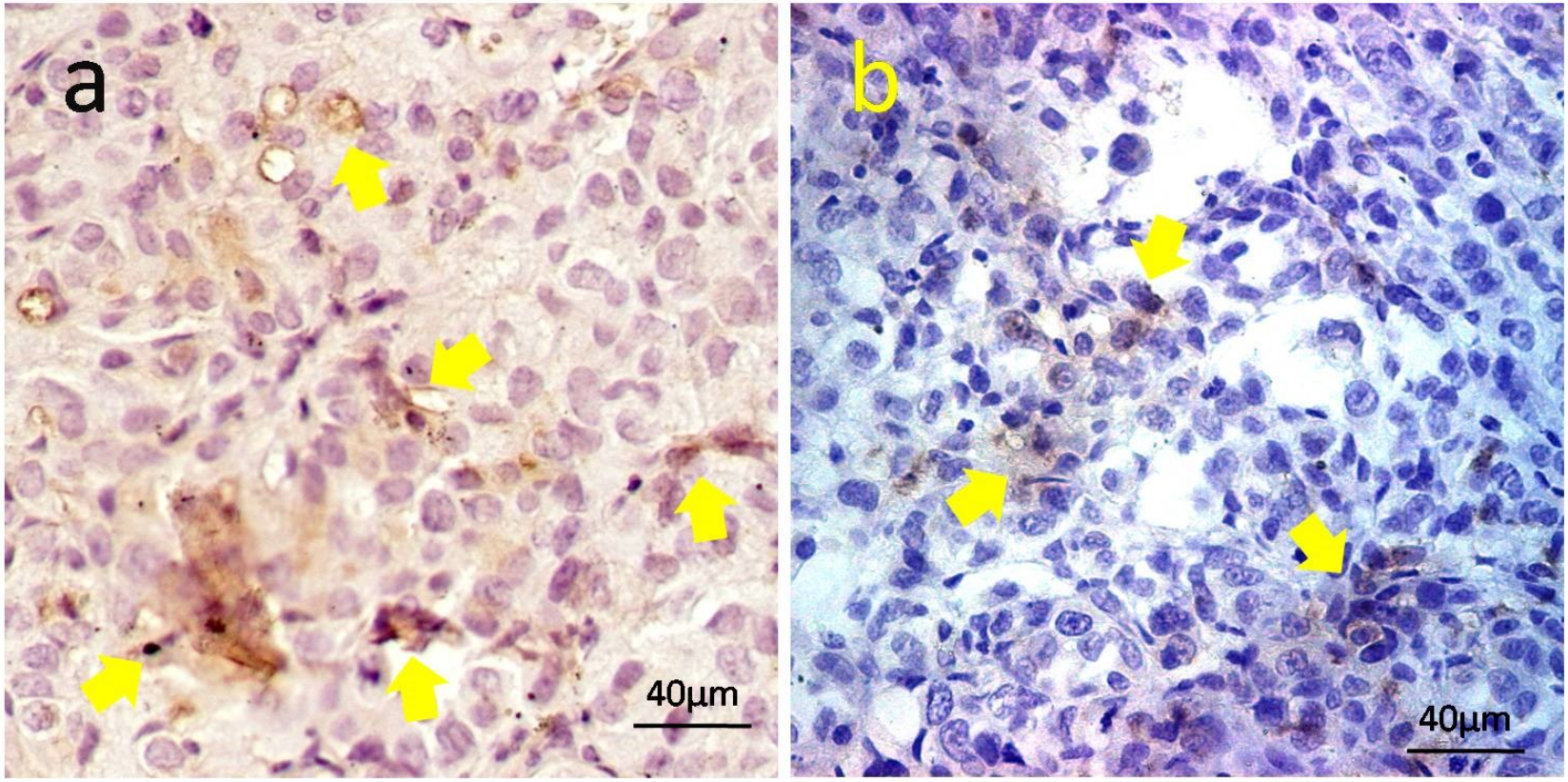


bkg sub
flat-fielded
cosmic

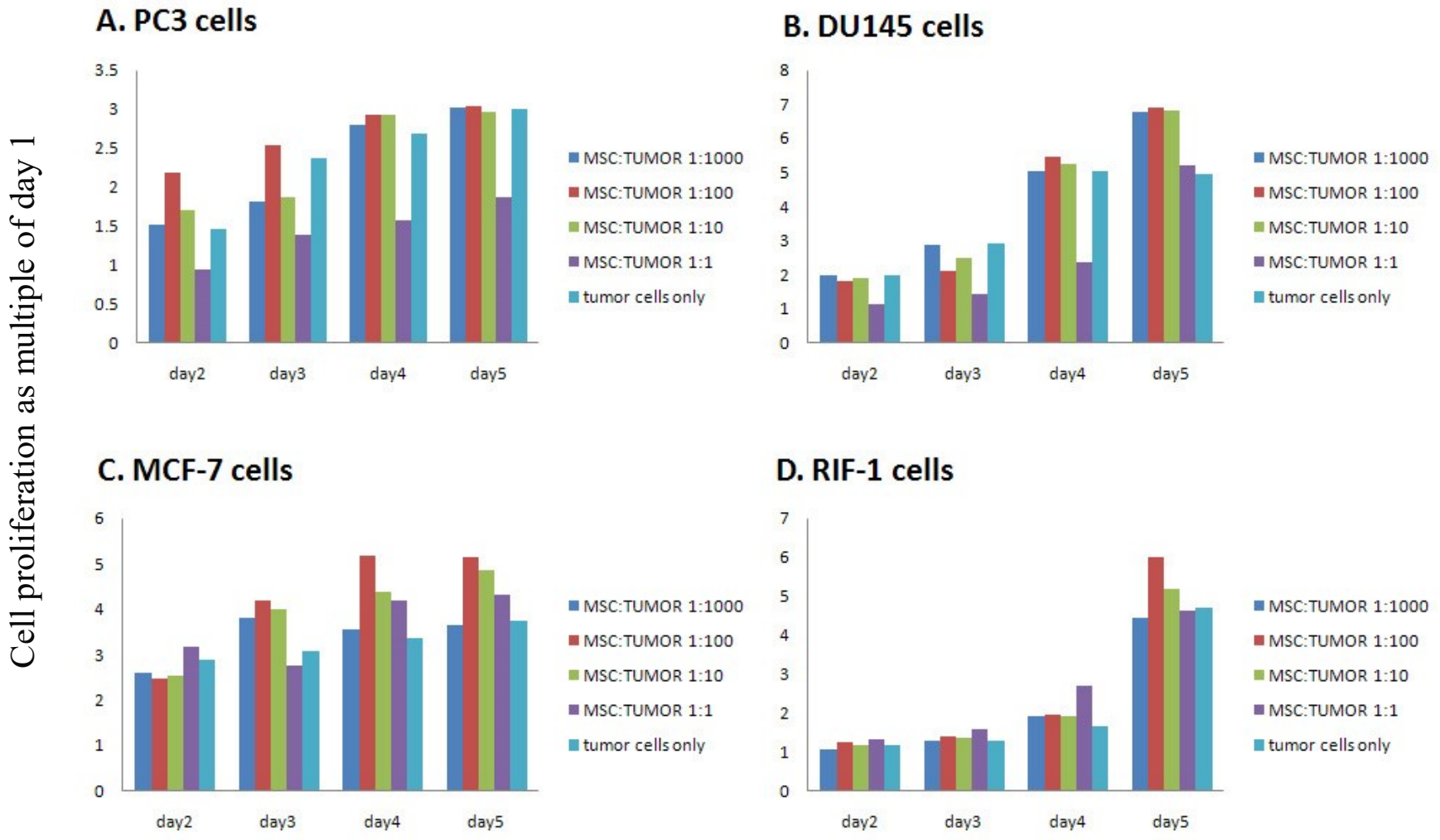
MSCs mainly home to tumours following systemic administration



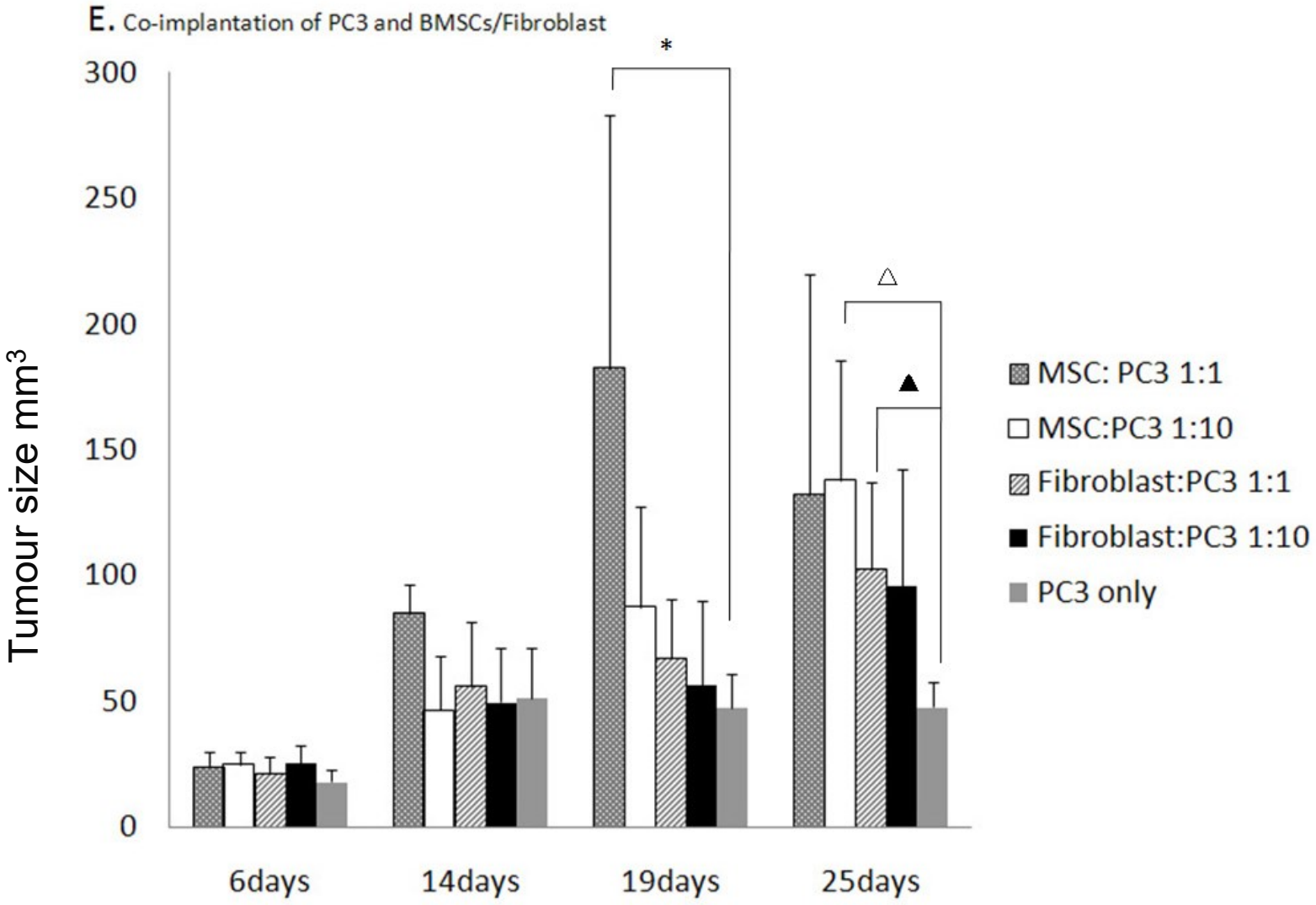
The engraftment of BMSCs in the tumour parenchyma was confirmed by immunostaining of GFP positive cells



BMSCs did not favor tumour growth in short term co-culture



BMSCs favour PC3 tumour growth in vivo

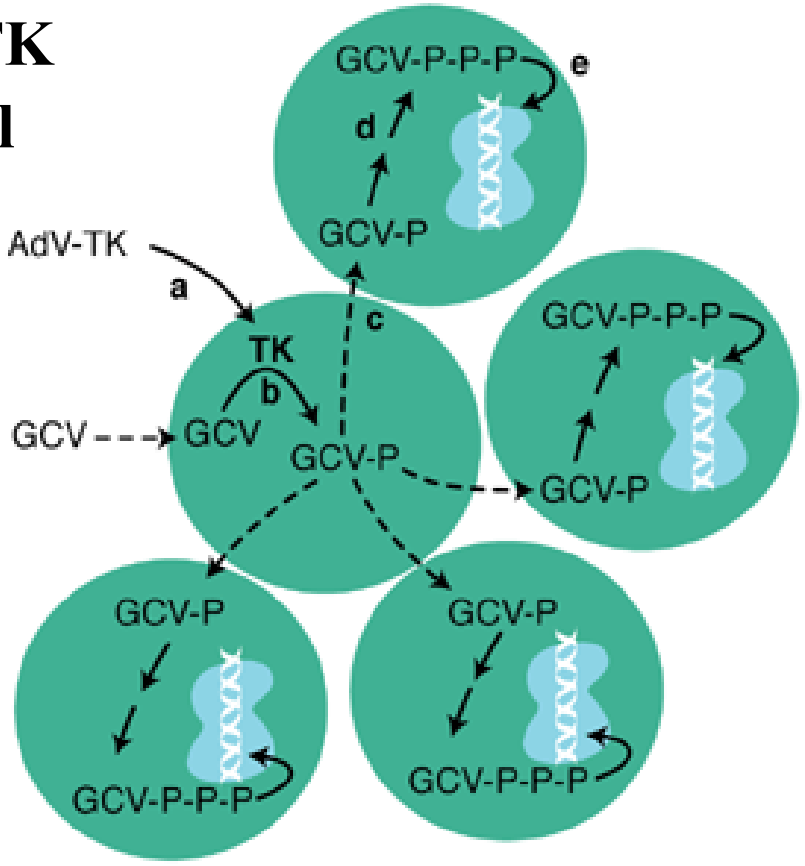


TK (Thymidine Kinase)/GCV (Ganciclovir) System

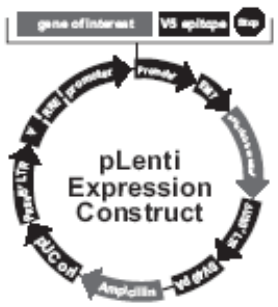
Herpes simplex virus 1 (HSV-1) TK enzyme is not expressed in normal cells.

HSV-TK converts GCV to triphosphate GCV (GCV-TP), which is cytotoxic.

GCV-TP causes cell death by apoptosis

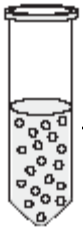


Suicide gene therapy

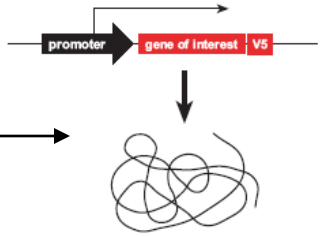


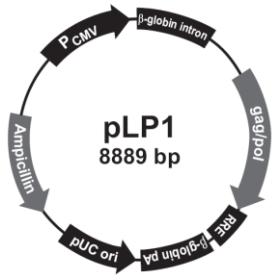
293FT OR 293T

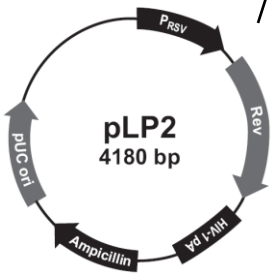
producer cell line

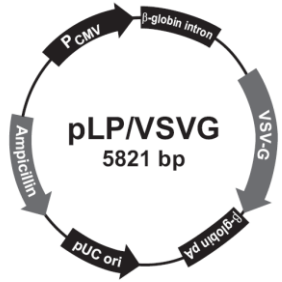


Your Mammalian Cell Line of Interest



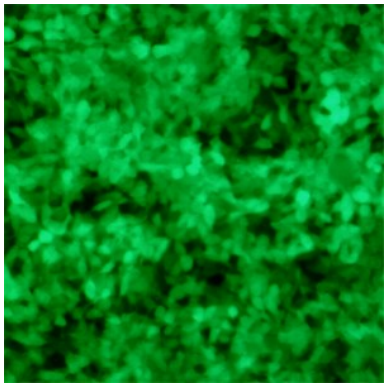


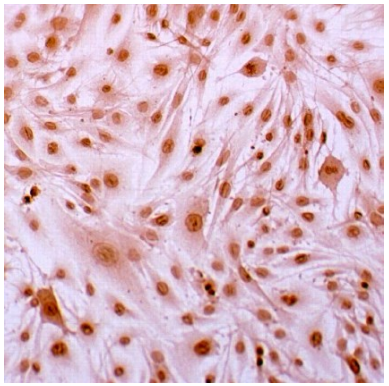




Stem cells for gene therapy

- **Stem cells as gene delivery vehicles**
- **TK gene was tranfected into MSCs using Lentiviral system**

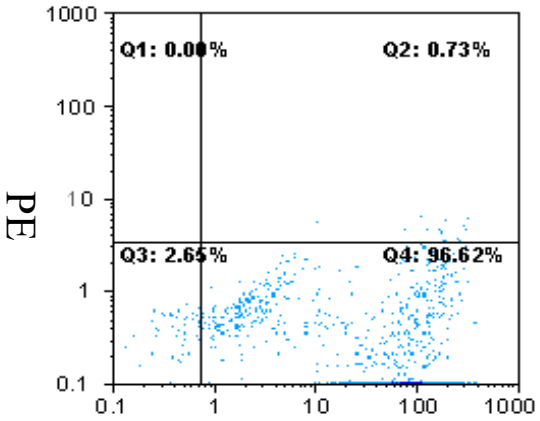




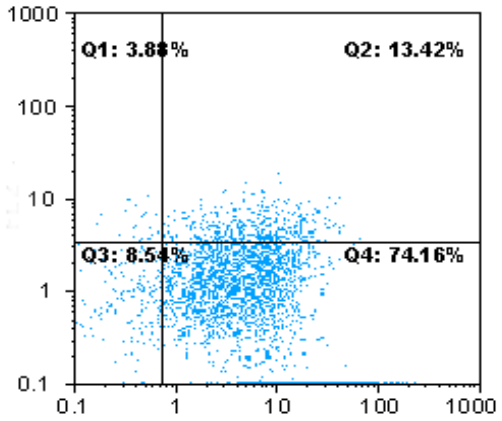
48h after C3H10T1/2 cells transfected with Lenti-CMV-luciferase-GFP virus.

The phenotype of Lenti-Luciferase-TK transduced BMSCs did not change

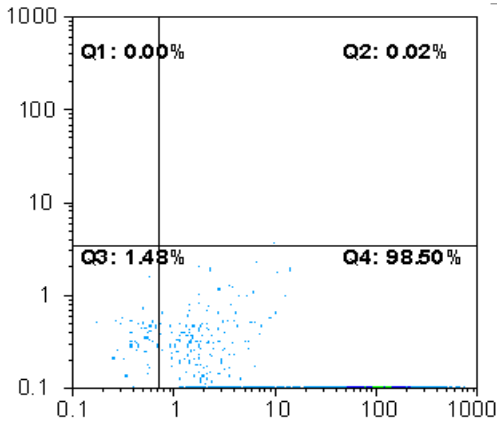
Negative Control



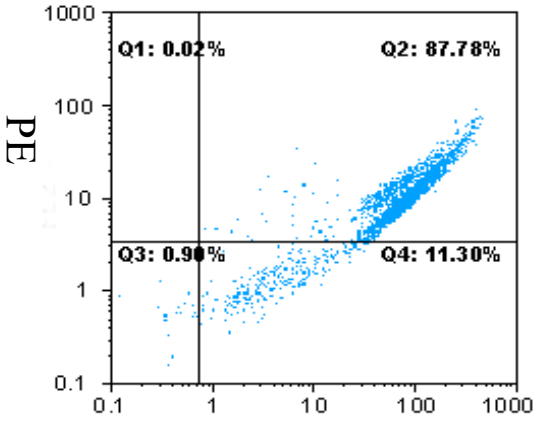
CD45



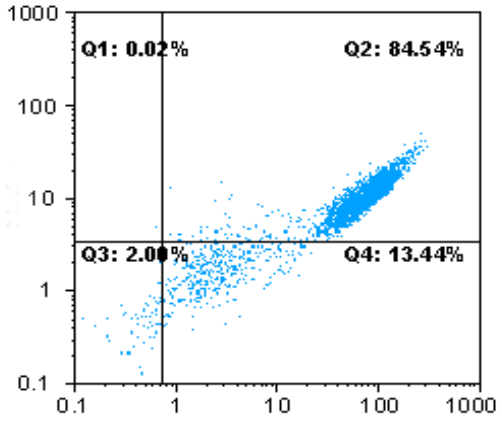
CD 31



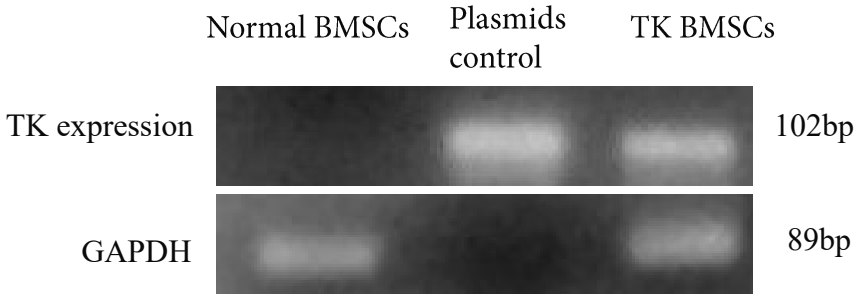
CD90

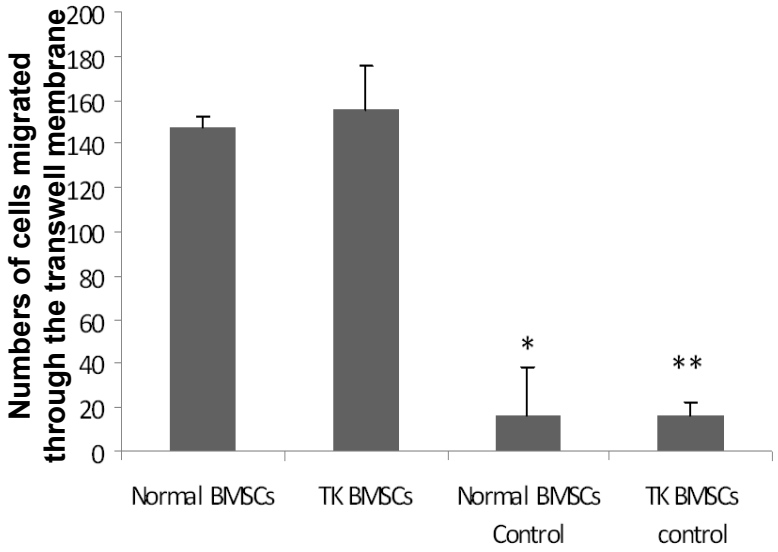


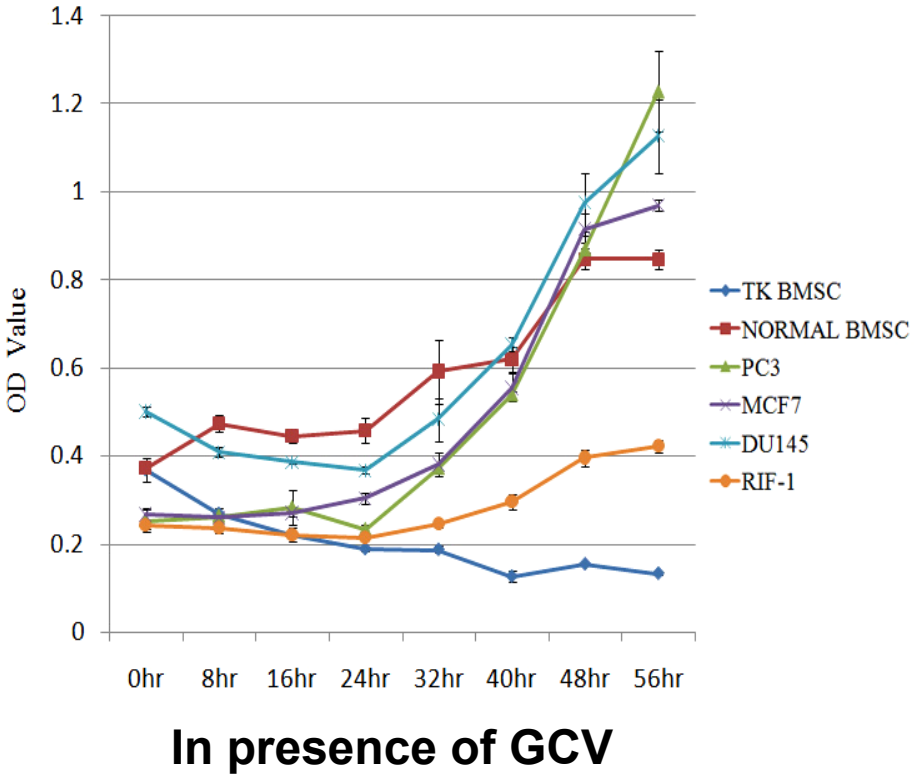
CD44



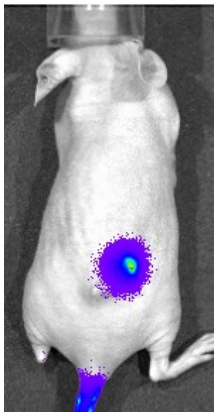
TK transduced BMSCs respond to GCV in 24 h

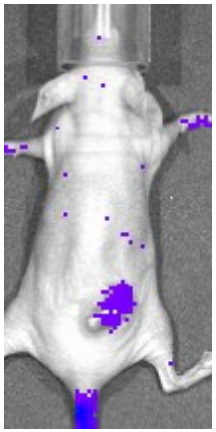


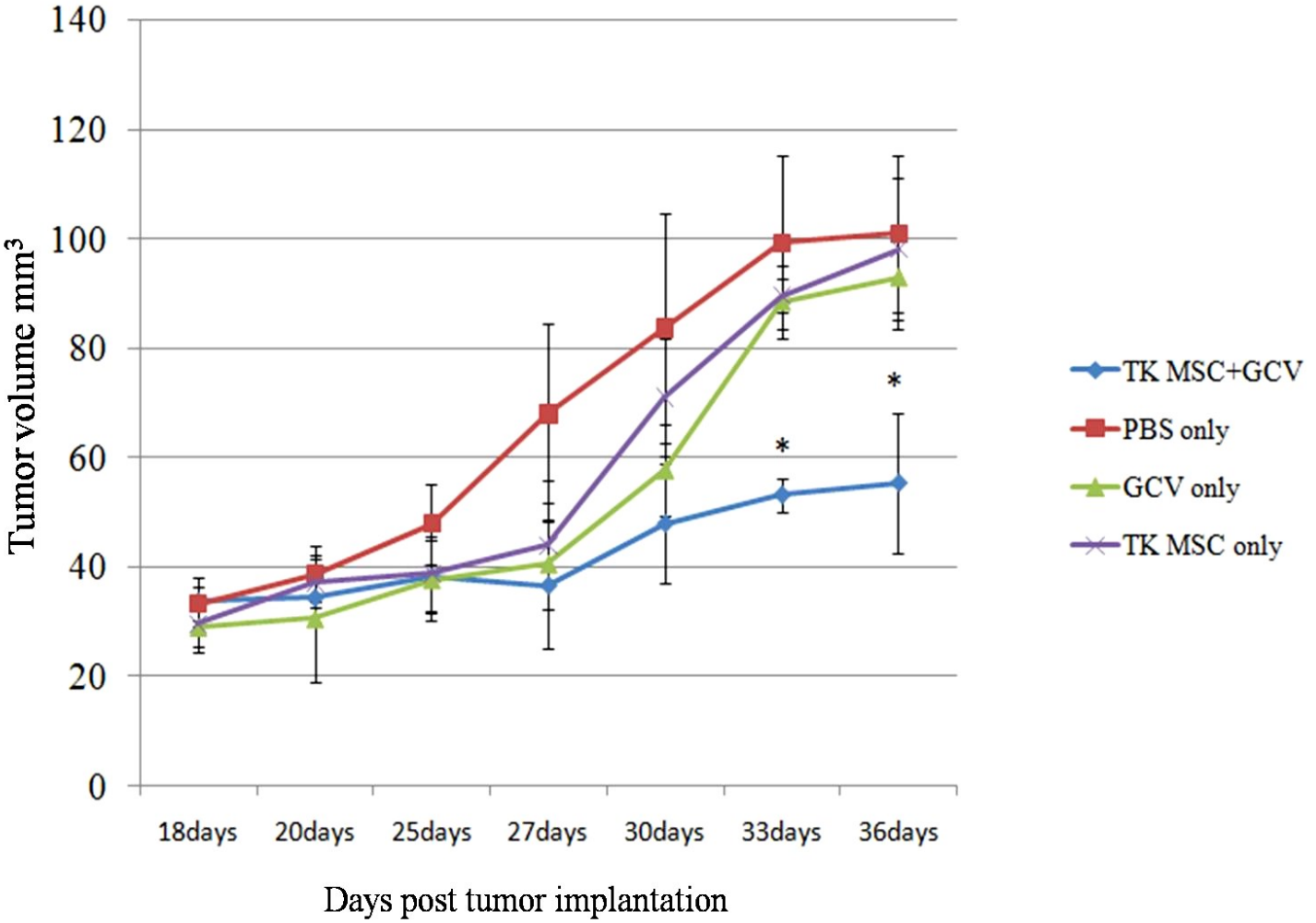


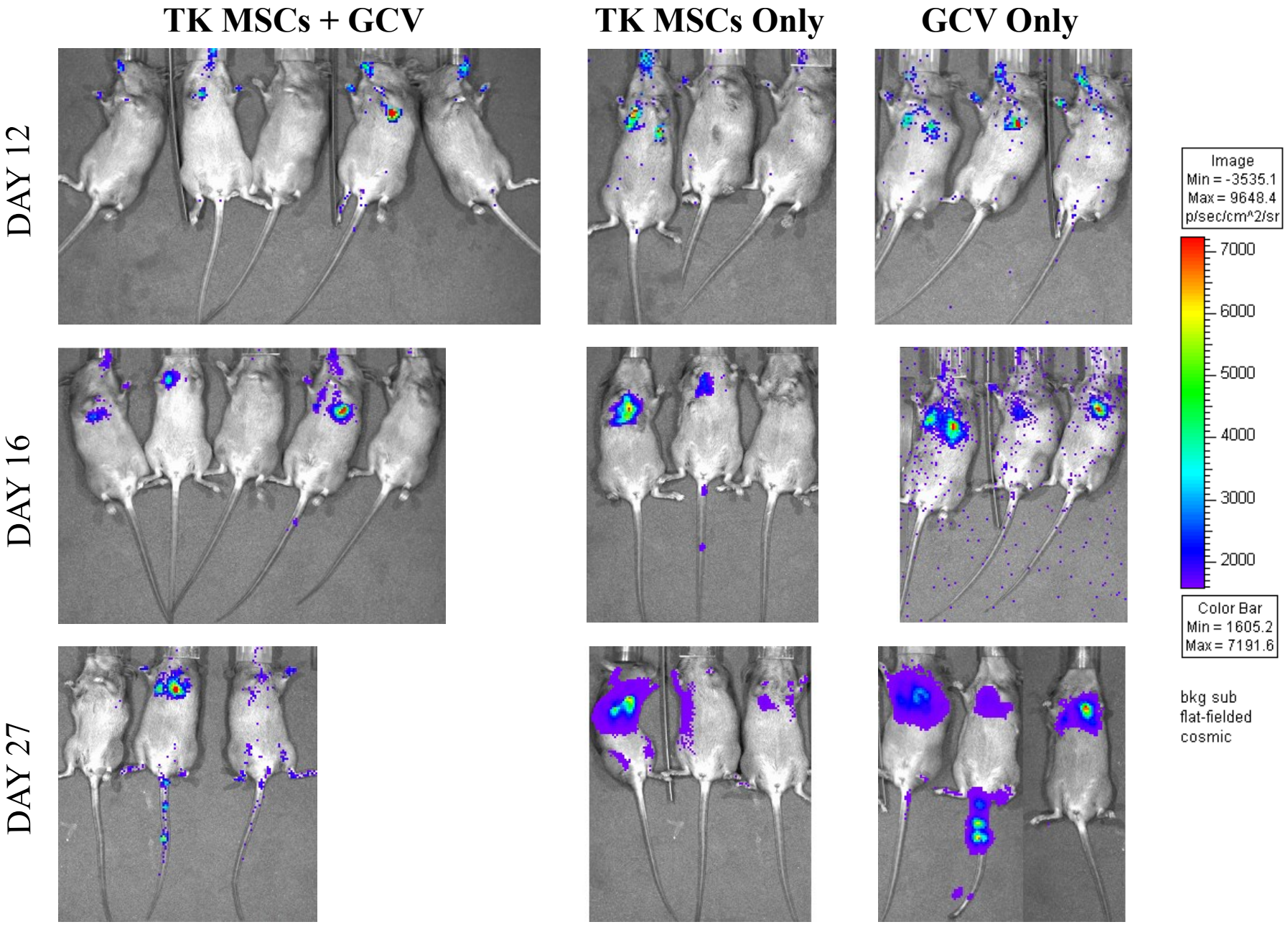


Anti-tumour effect of systemically administered TK-MSCs in the presence of GCV in tumour bearing mice.

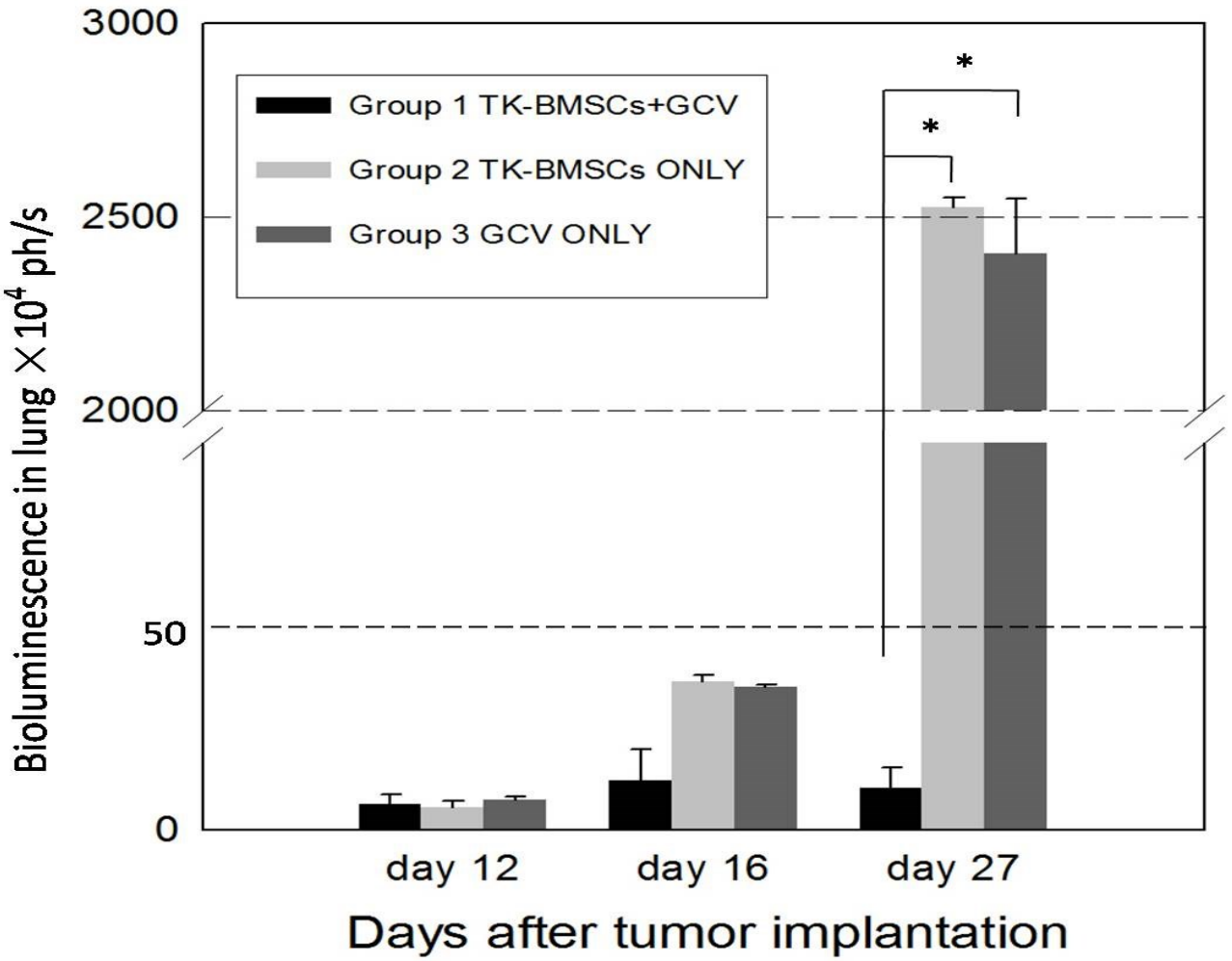




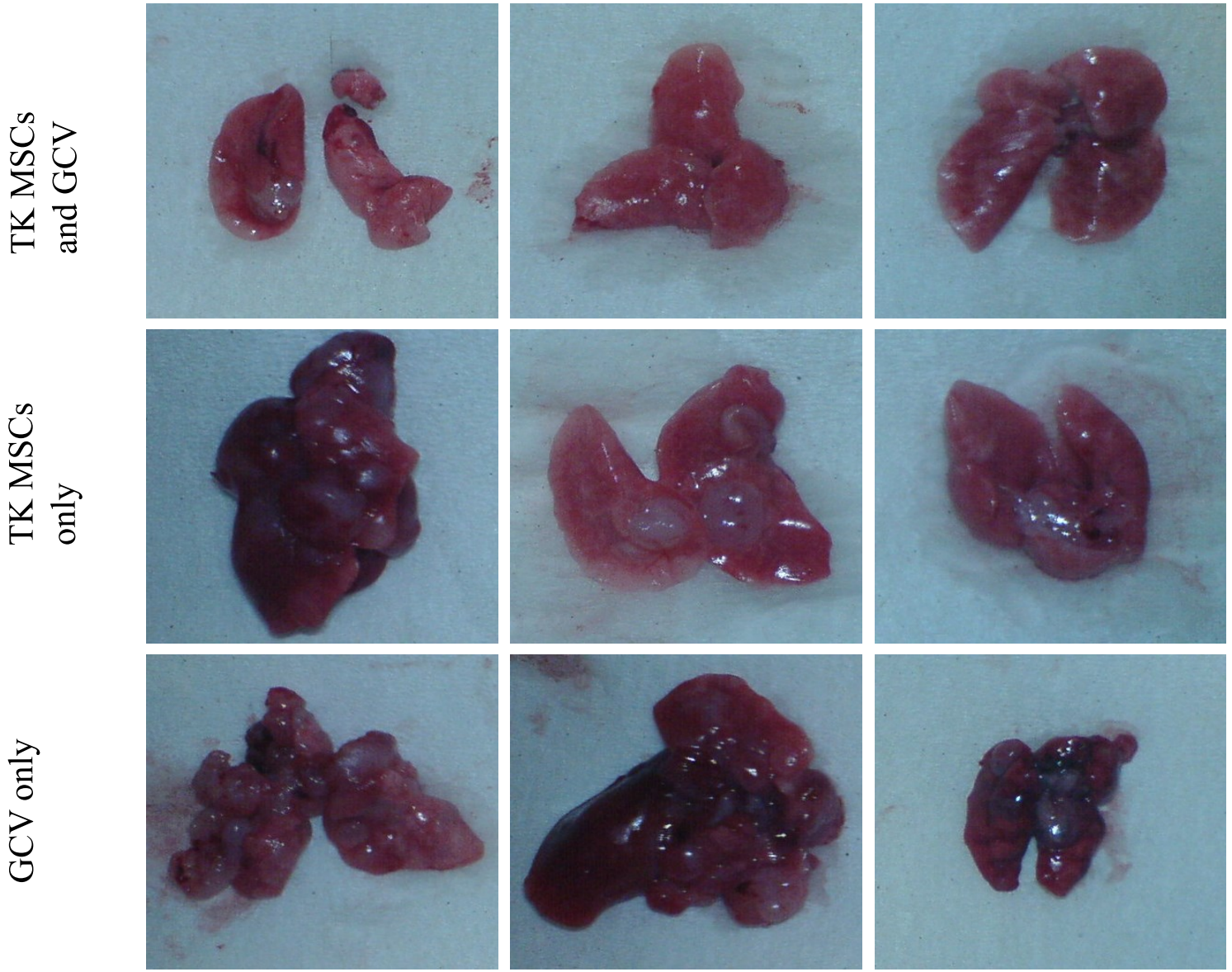



TK MSCs + GCV
TK MSCs Only
GCV Only
DAY 12
DAY 16
DAY 27
Image
Min = -3535.1
Max = 9648.4
p/sec/cm^2/sr
7000
6000
5000
4000
3000
2000
Color Bar
Min = 1605.2
Max = 7191.6
bkg sub
flat-fielded
cosmic

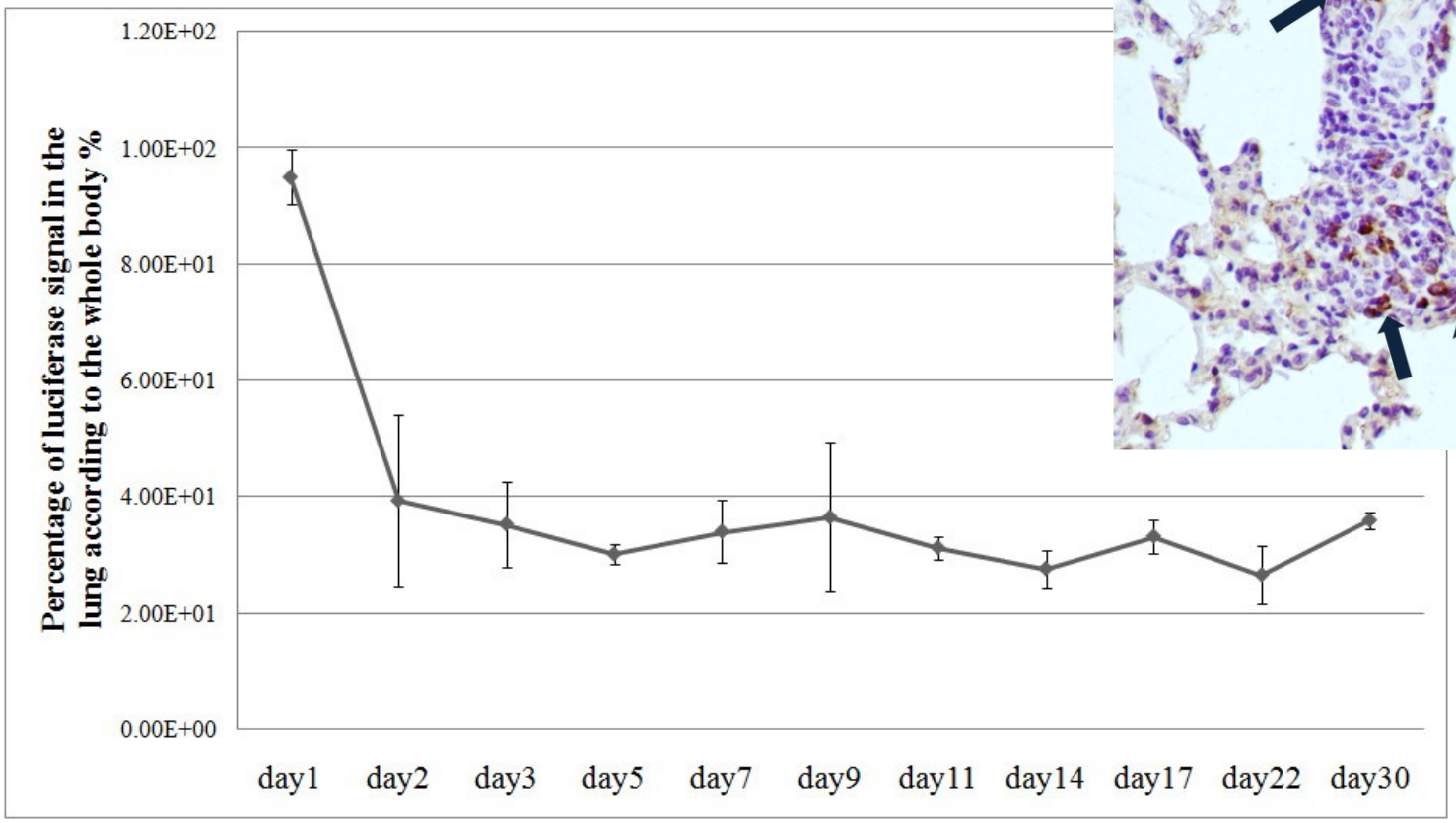
Systemic administration of TK-MSCs and GCV significantly inhibited metastatic tumour growth

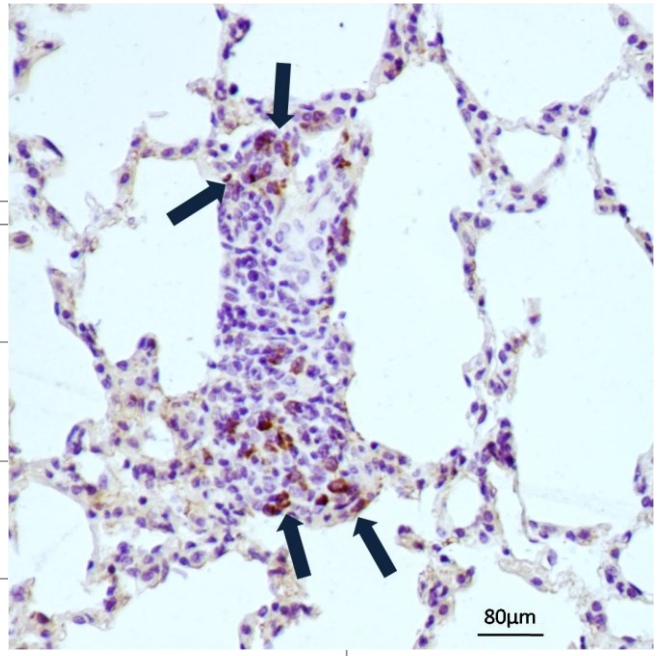


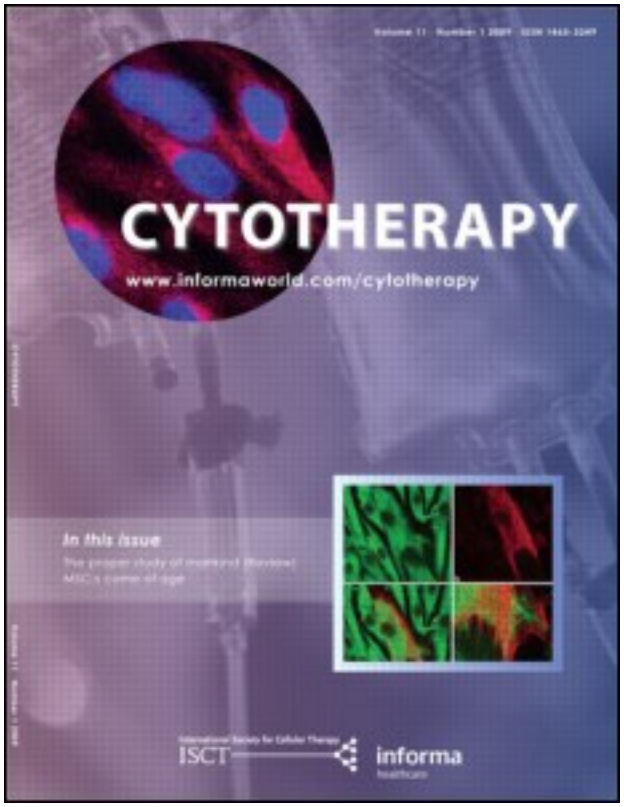
Gross Sample of RIF-1 tumor in lung day 27



Percentage of BMSCs in the lung compared to total BMSCs in the body







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Mesenchymal stem cells as a gene therapy carrier for treatment of fibrosarcoma

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Background aims

Cell-based gene therapy is an alternative to viral and non-viral gene therapy. Emerging evidence suggests that mesenchymal stem cells (MSC) are able to migrate to sites of tissue injury and have immunosuppressive properties that may be useful in targeted gene therapy for sustained specific tissue engraftment.

Methods

In this study, we injected intravenously (i.v.) 1×10^6 MSC, isolated from green fluorescent protein (GFP) transgenic rats, into Rif-1 fibrosarcoma-bearing C3H/HeN mice. The MSC had been infected using a lentiviral vector to express stably the luciferase reporter gene (MSC-GFP-luci). An in vivo *imaging system (IVIS 200) and Western blotting techniques were used to detect the distribution of MSC-GFP-luci in tumor-bearing animals.*

Results

We observed that xenogenic MSC selectively migrated to the tumor site, proliferated and expressed the exogenous gene in subcutaneous fibrosarcoma transplants. No MSC distribution was detected in other organs, such as the liver, spleen, colon and kidney. We further showed that the FGF2/FGFR pathways may play a role in the directional movement of MSC to the Rif-1 fibrosarcoma. We performed in vitro *co-culture and* in vivo *tumor growth analysis, showing that MSC did not affect the proliferation of Rif-1 cells and fibrosarcoma growth compared with an untreated control group. Finally, we demonstrated that the xenogenic MSC stably expressing inducible nitric oxide synthase (iNOS) protein transferred by a lentivirus-based system had a significant inhibitory effect on the growth of Rif-1 tumors compared with MSC alone and the non-treatment control group.*

Conclusions

iNOS delivered by genetically modified iNOS-MSC showed a significant anti-tumor effect both in vitro *and* in vivo*. MSC may be used as a target gene delivery vehicle for the treatment of fibrosarcoma and other tumors.*

Keywords

Fibrosarcoma, gene therapy, inducible nitric oxide synthase, mesenchymal stem cells.

Introduction

Solid tumors comprise two distinct but interdependent compartments: neoplastic cells and the stroma that the neoplastic cells induce and in which they are dispersed. Stem cells are mainly referred to as tumor-supporting fibroblasts and they may derive from resident fibroblasts in the organ/tissue [1] or circulating mesenchymal progenitor cells [2–5].

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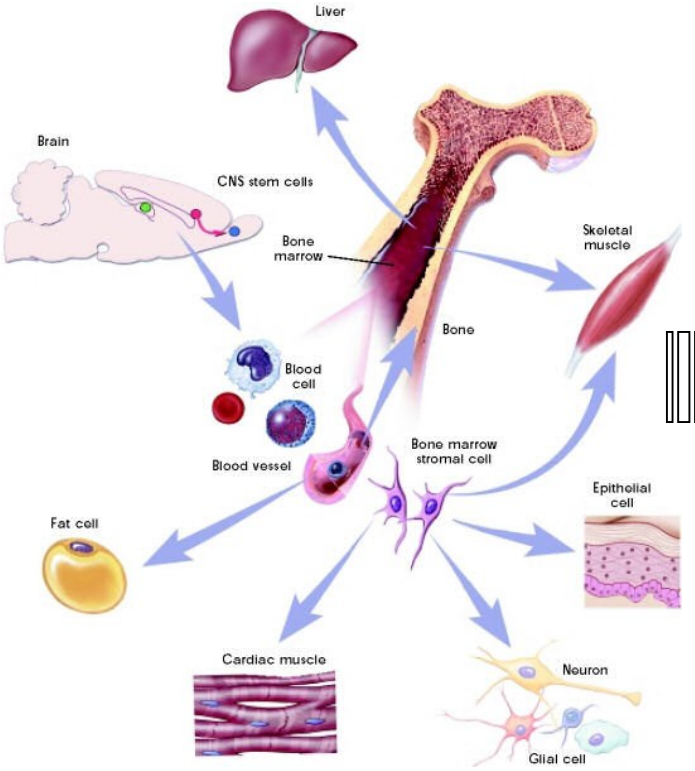
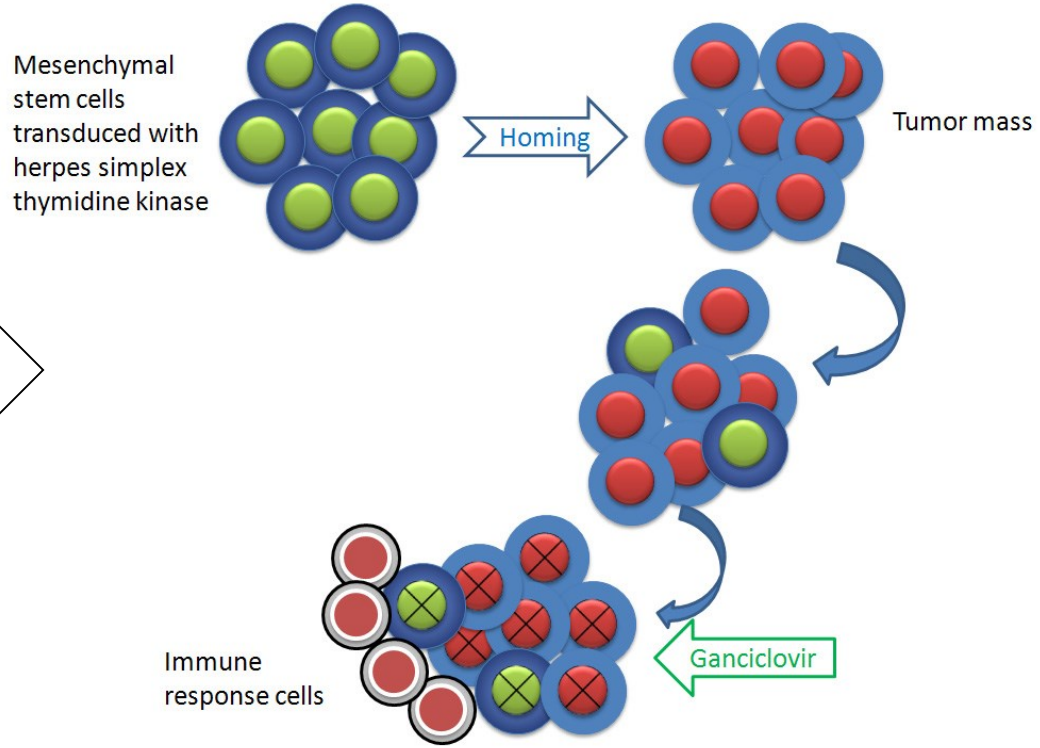
Correspondence to: Professor Gang Li, The Li Ka Shing Institute of Health Sciences, Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China. E-mail: gangli@ort.cuhk.edu.hk

DOI: 10.1080/14653240902960429

Using Mesenchymal Stem Cells as Vehicles for Anti-cancer Therapy





MSCs as anti-tumour gene therapy vehicle

- Transduction of the HSV-TK gene into BMSCs did not change their MSCs phenotype and tumour homing potential.
- Potent cytotoxic effects of TK-BMSCs/GCV was proved on tumour cells in vitro.
- TK-BMSCs together with GCV can greatly inhibit tumour growth in both local and metastasis tumour models in vivo.


Thank you !
谢谢！