Bone marrow mesenchymal stem cells increase survival after ionizing irradiation combined with wound trauma

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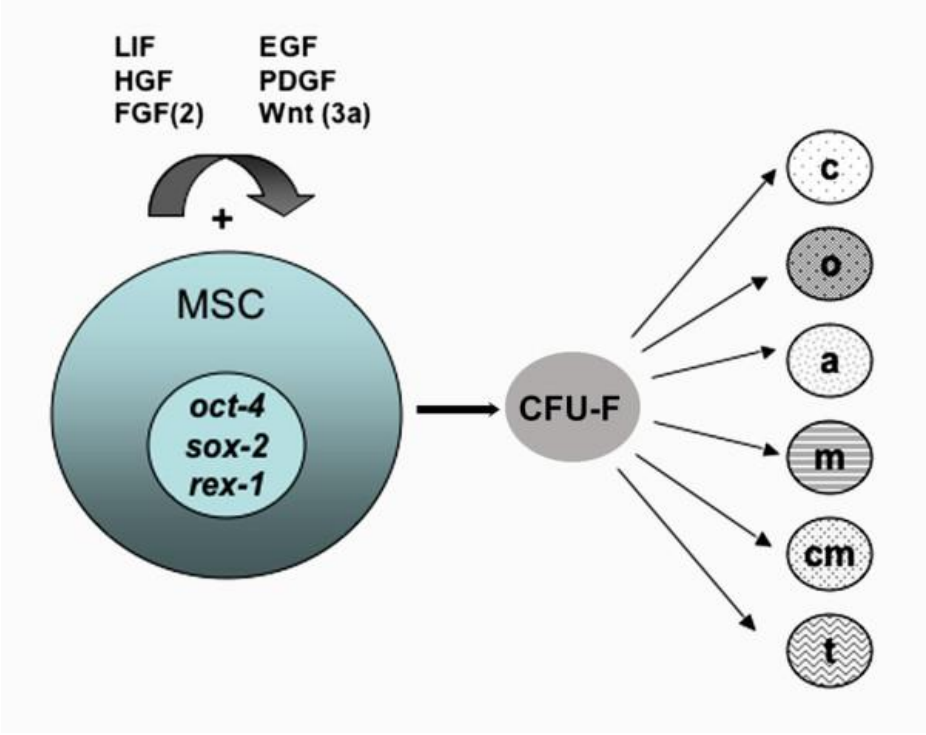
Outline

- Background
- Rationale
- Hypothesis
- MSC isolation and identification
- Animal model of radiation followed by wound trauma
- Survival, body weight, wound healing, and H_2O intake
- MSC challenged by bacteria

Mesenchymal Stem Cell Self-Renewal and Differentiation



CFU-F
colony forming unit-fibroblast

- **chondroblast**
- **osteoblast**
- **adipoblast**
- **myoblast**
- **cardio-myoblast**
- **tenoblast**

BMSC Markers: positive staining for STRO-1, SCA-1, CD44, and CD105 negative staining for CD34 and CD117

Extracellular signaling factors, including growth factors and cytokines, promote and/or maintain mesenchymal stem cell self-renewal. LIF, leukemia inhibitory factor; EGF, epidermal growth factor; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor. Gene markers characteristic of MSC self-renewal include *oct-4*, *sox-2*, and *rex-1*.

Adapted from: Kolf CM, Cho E, Tuan RS. Arthritis Res Ther. 2007;9(1):204.

Rationale for testing BMSCs

1. Promoted wound healing in CI-rat (Hao et al. Gene Ther 16: 34-42, 2009)

2. SOD gene-transfected BMSCs improved RI-mouse survival (Aly et al. Blood 113: 1201-1203, 2009)

3. Improved RI- mouse survival (Hu et al. Br J Radiobiol 83: 52-58, 2010)

4. Attenuated sepsis-induced mortality in mice by increasing IL-10 production from macrophage (Nemeth et al. Nature Medicine 15: 42-49, 2009)

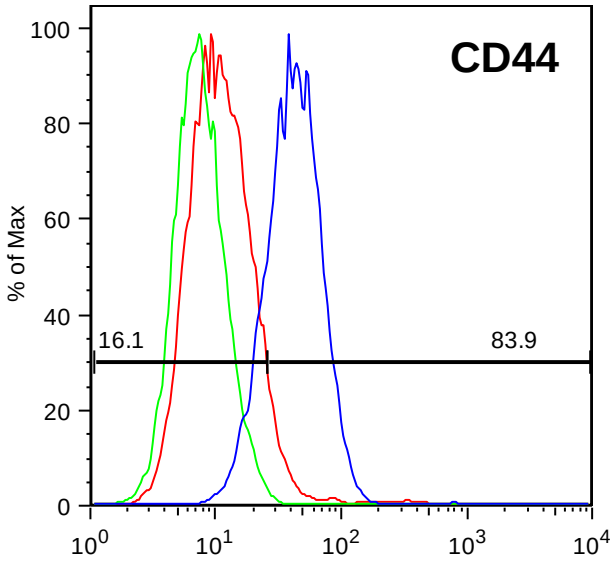
5. Responded to ionizing radiation by activating iNOS pathway (Gorbunov et al. Radiate Res 154: 73-86, 2000)

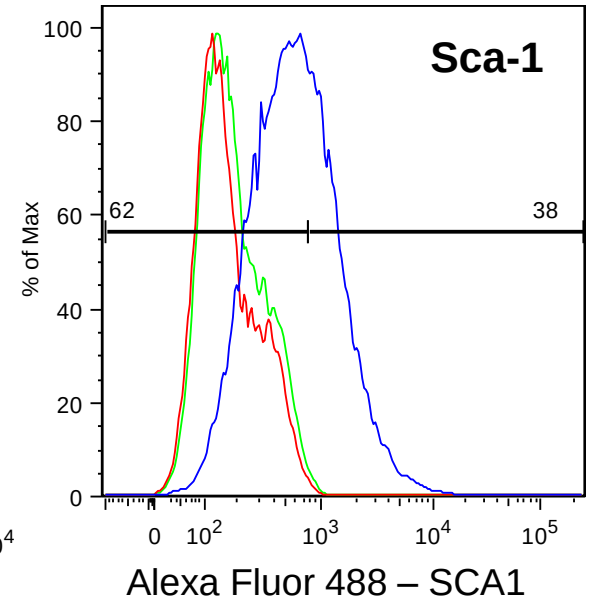
Hypothesis

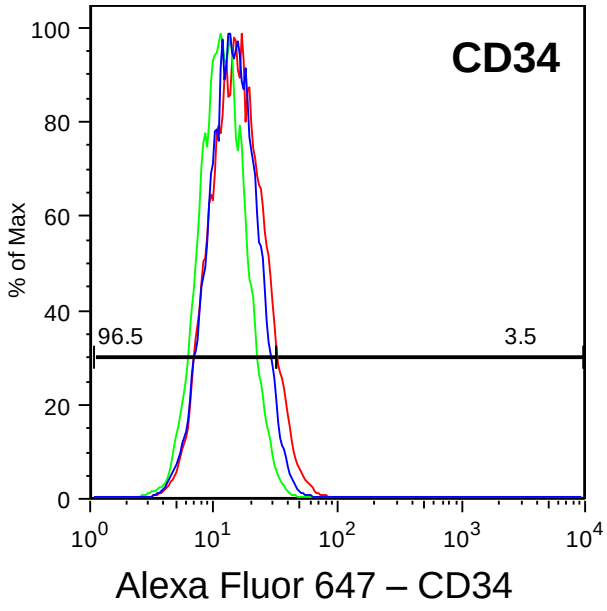
BMSC administration will improve survival after irradiation combined with wound trauma.

Validation of mBMSCs (1)

Flow Cytometry Phenotype Analysis of mouse BMSCs
CD44+, Sca-1+, CD34-





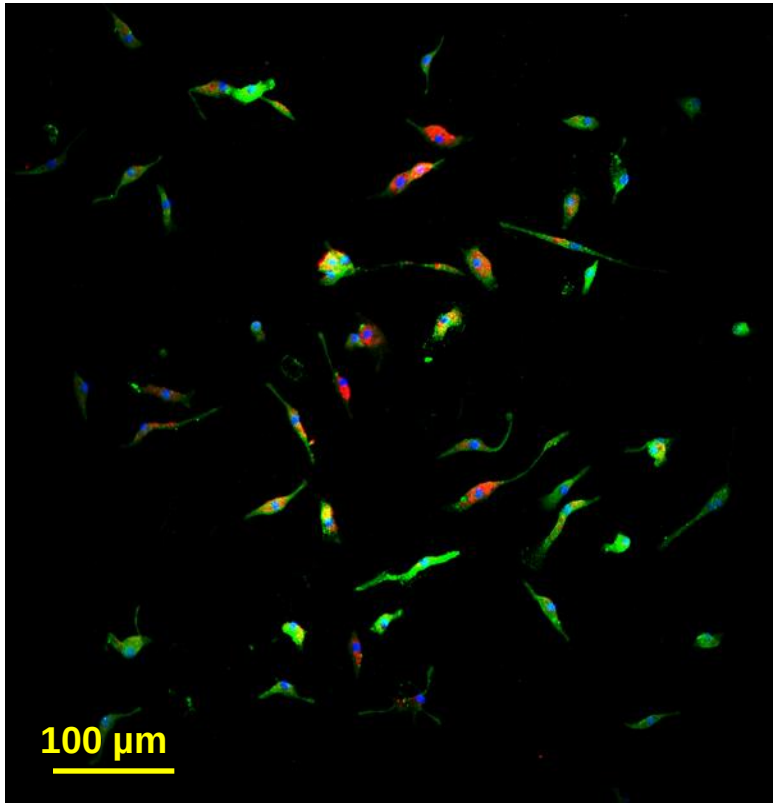


- Phenotype antigen
- Unstained cells
- Isotype Control

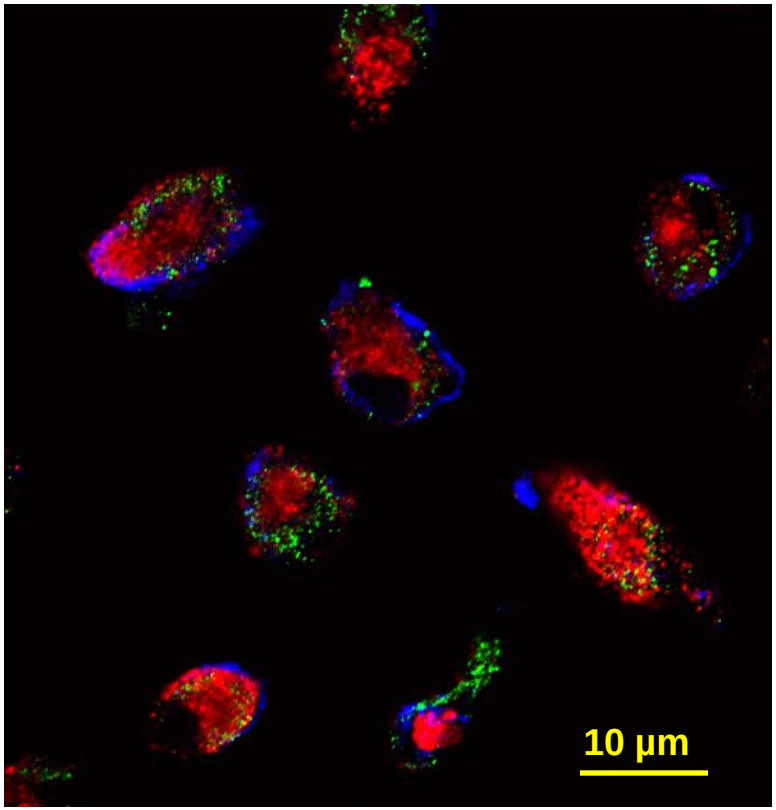
Mouse BMSCs were obtained from bone marrow of femurs, cultivated for 45 days, and subjected to 4 passages.

Validation of mBMSCs (2)

Mouse BMSCs - Cell Phenotype Analysis
Sca-1+, STRO-1+, and CD44+



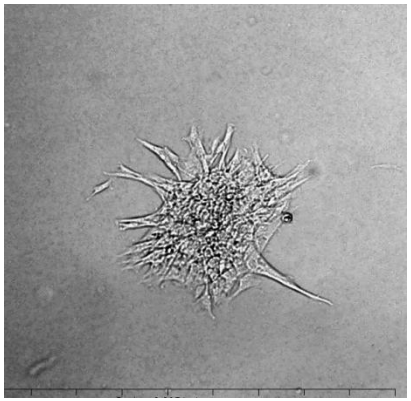
Sca-1 (red) and STRO-1 (green)
Nuclei Counterstaining (blue)

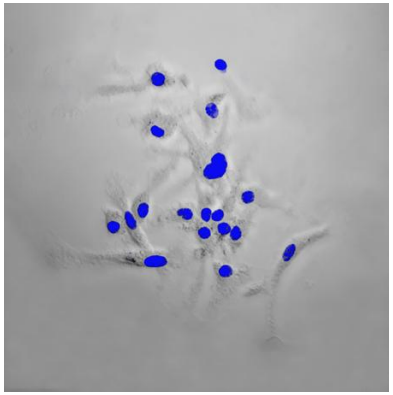


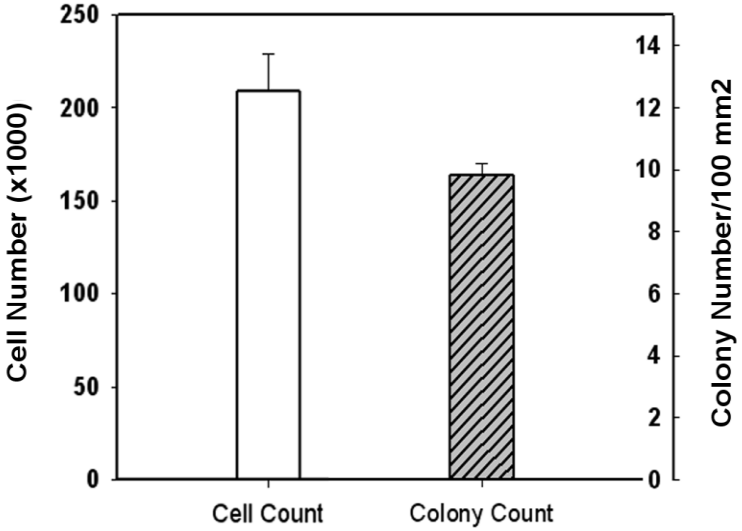
CD44 (blue) and STRO-1 (green)
Glycerol-3-Phosphate Dehydrogenase (GPDH) (red)

mBMSCs form colonies and

Naïve mouse BMSCs proliferation



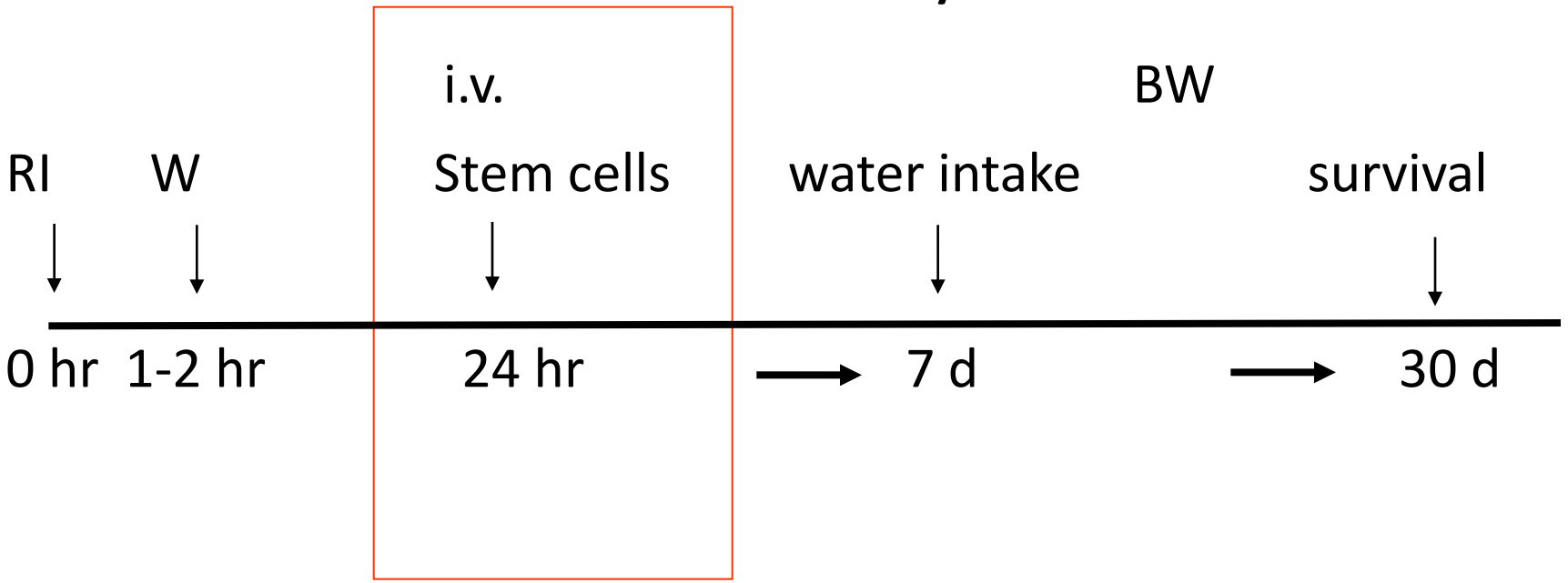




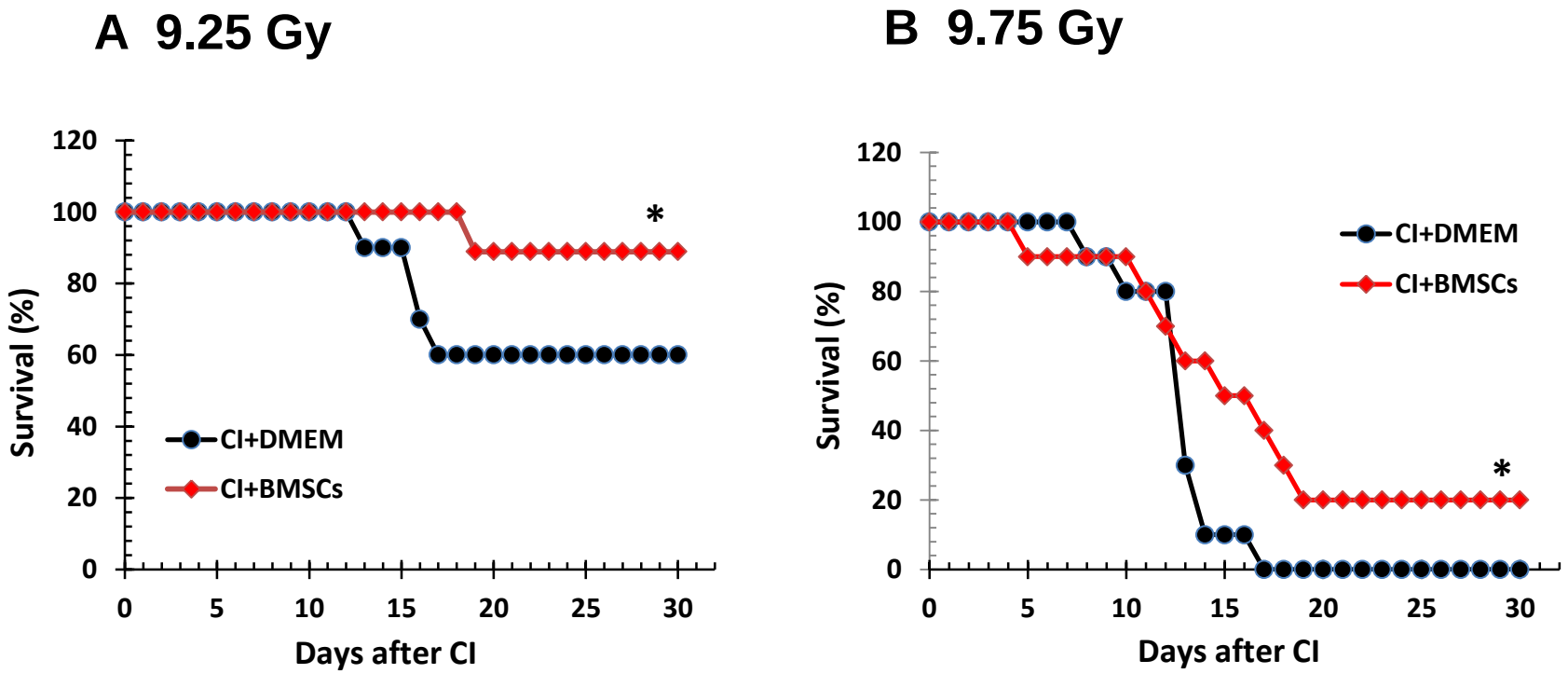
$100x10^3$ cells were plated in 60 mm Petri dishes and
Cultivated for 10 days. DAPI stains nucleus and shows in blue color

Procedure

Wound size: 15% of total body surface area



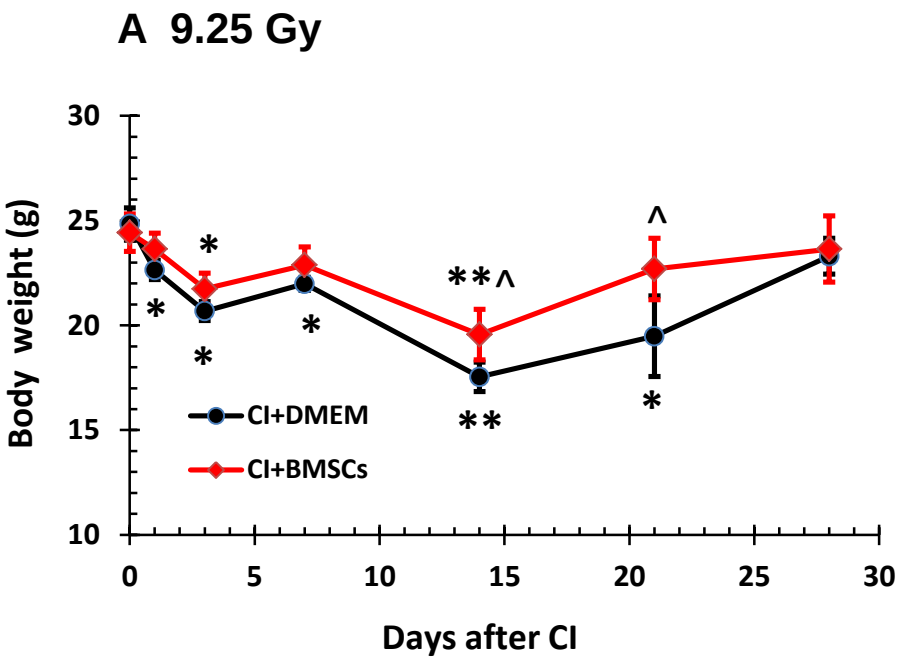
mBMSCs improve survival after CI

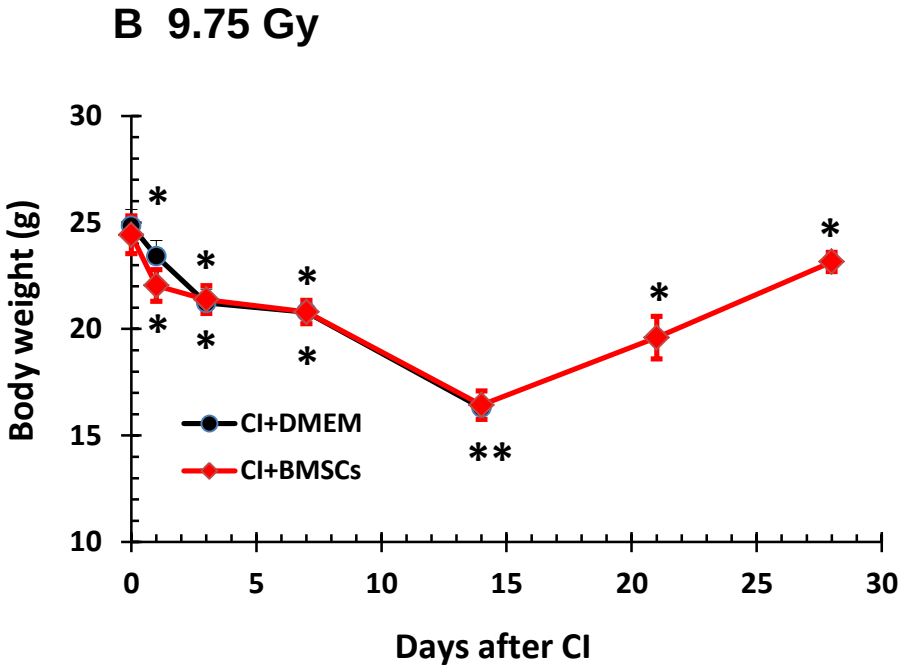


A. B6D2F1/J mice; 9.25 Gy; BMSCs $3x10^6$ / 0.4 ml DMEM; i.v. +24 hr
30% survival improvement

B. B6D2F1/J mice; 9.75 Gy; BMSCs $2x10^6$ / 0.4 ml DMEM; i.v. +24 hr
20% survival improvement

mBMSCs improve Body weight after CI

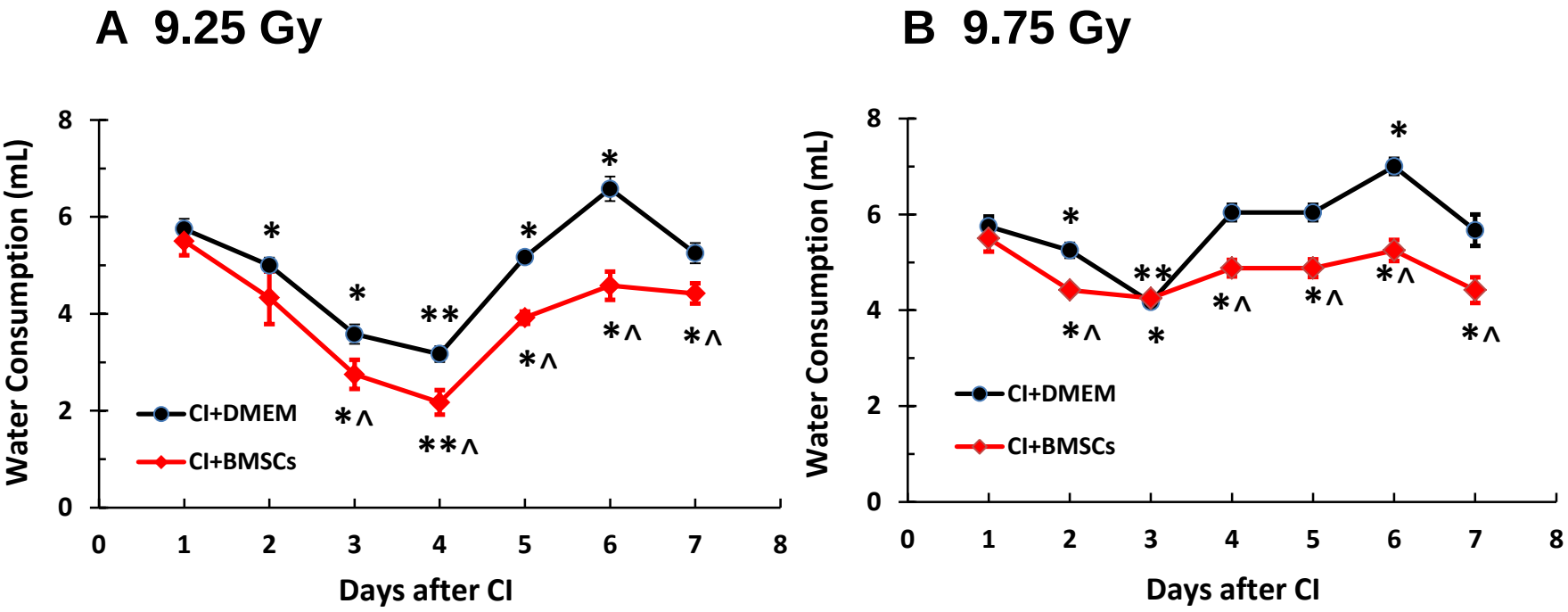




A. B6D2F1/J mice; 9.25 Gy; BMSCs $3x10^6$ / 0.4 ml DMEM; +24 hr;
30% survival improvement

B. B6D2F1/J mice; 9.75 Gy; BMSCs $2x10^6$ / 0.4 ml DMEM; +24 hr;
20% survival improvement

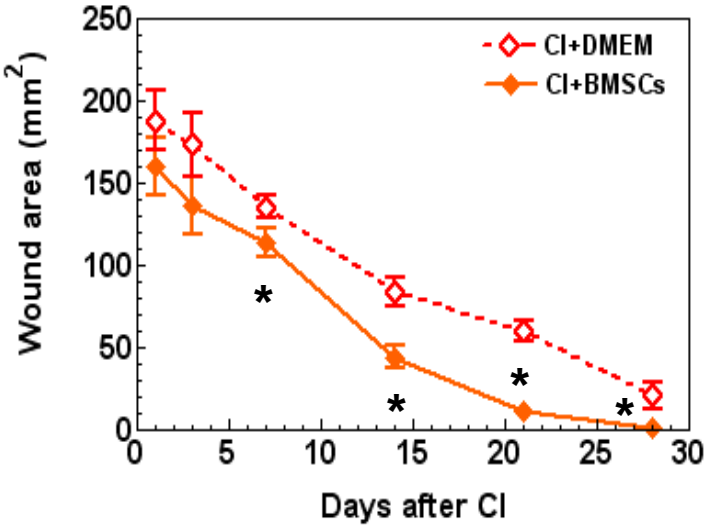
mBMSCs reduce water intake after CI



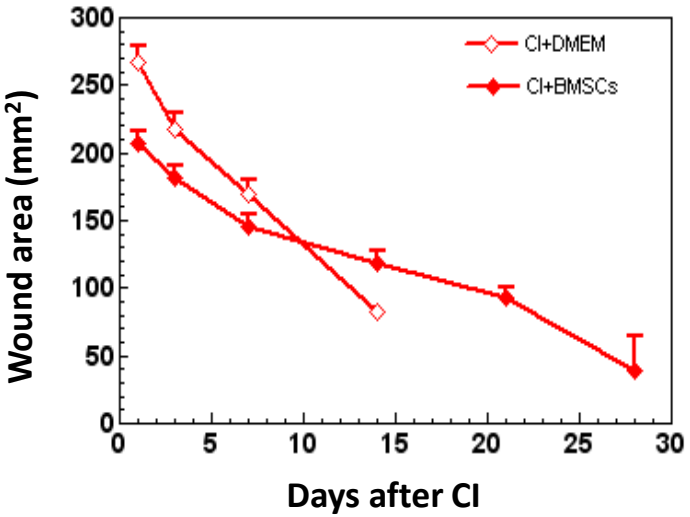
A. B6D2F1/J mice; 9.75 Gy; BMSCs 2x10E6 / 0.4 ml DMEM; +24 hr; 20% survival improvement
B. B6D2F1/J mice; 9.25 Gy; BMSCs 3x10E6 / 0.4 ml DMEM; +24 hr; 30% survival improvement

mBMSCs accelerate wound healing after CI

A 9.25 Gy



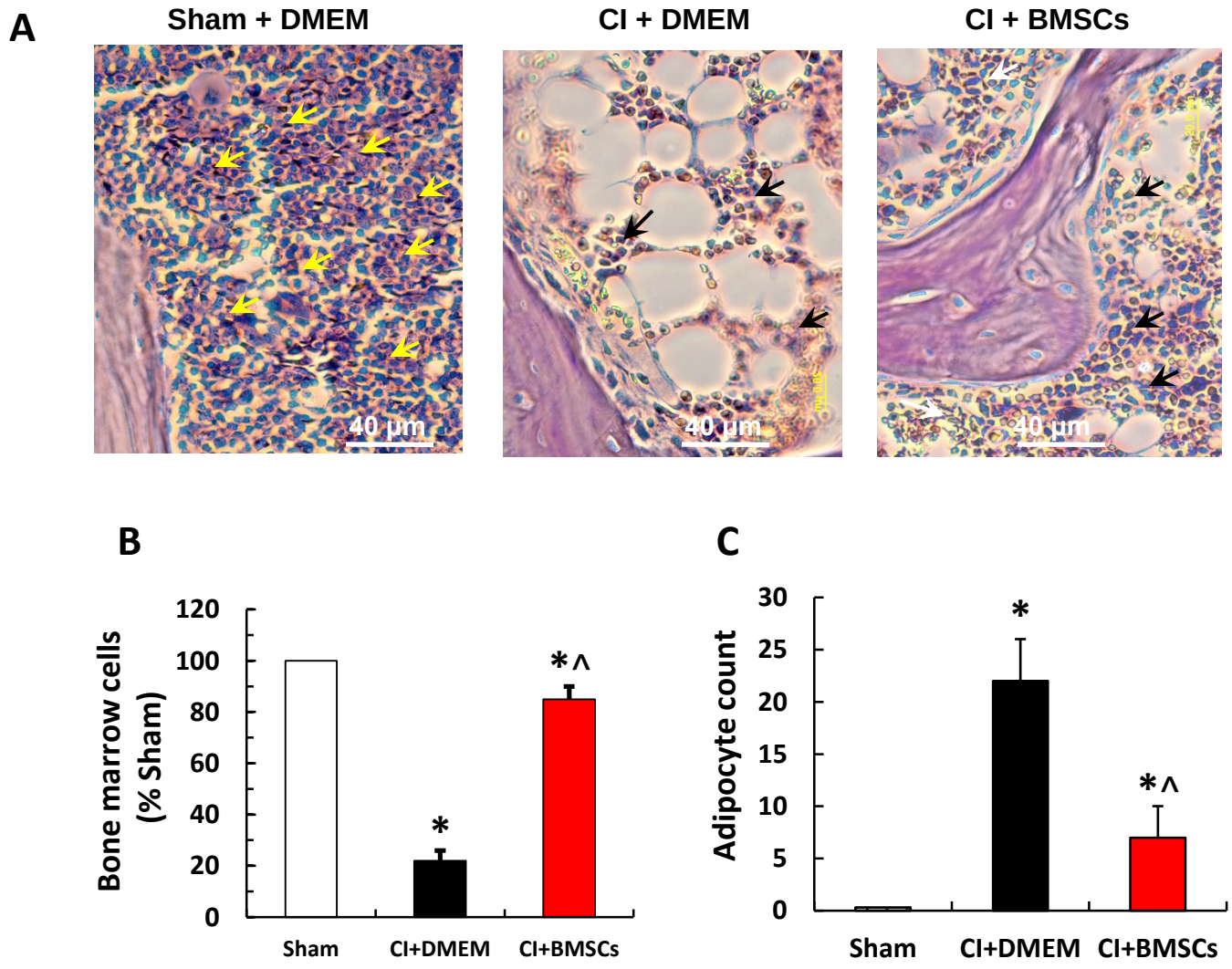
B 9.75 Gy



C 21 days after CI

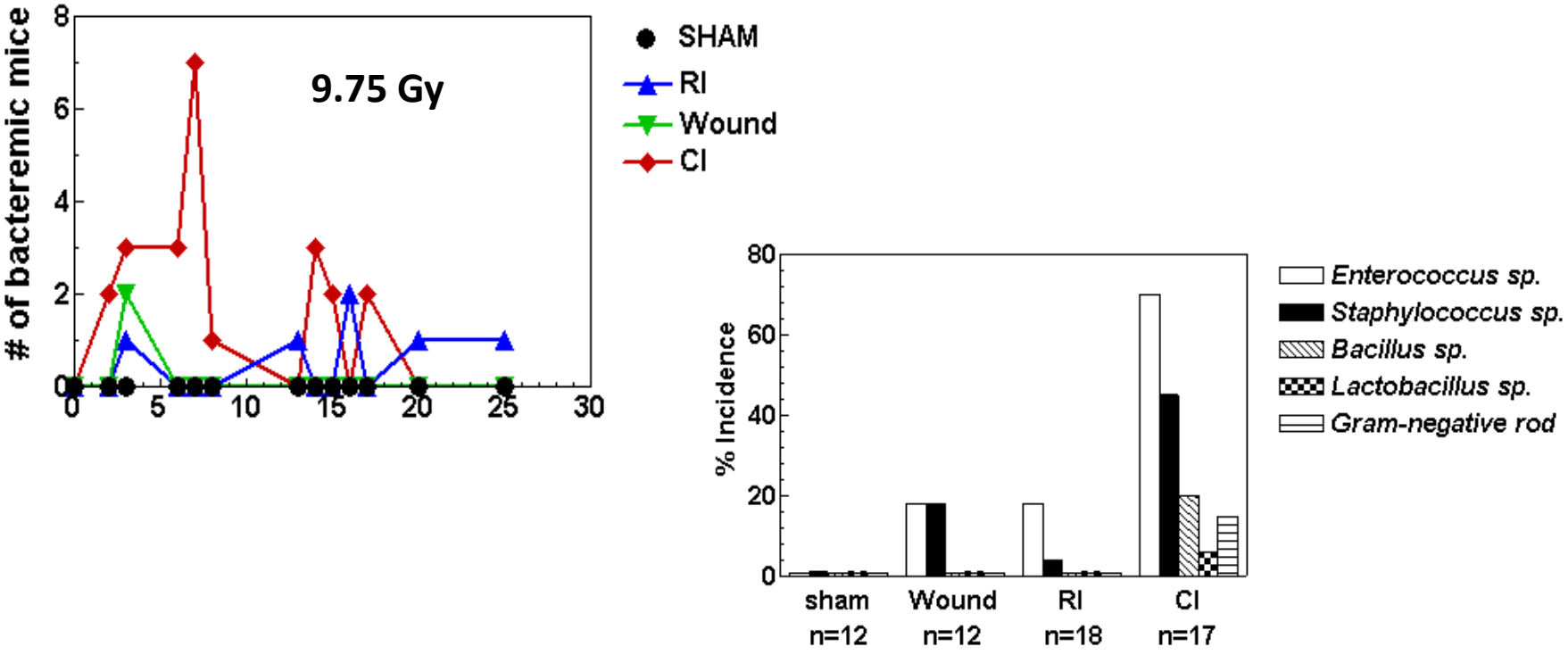
CI + DMEM **CI + BMSCs**

mBMSCs regenerate bone marrow after CI



Wounding

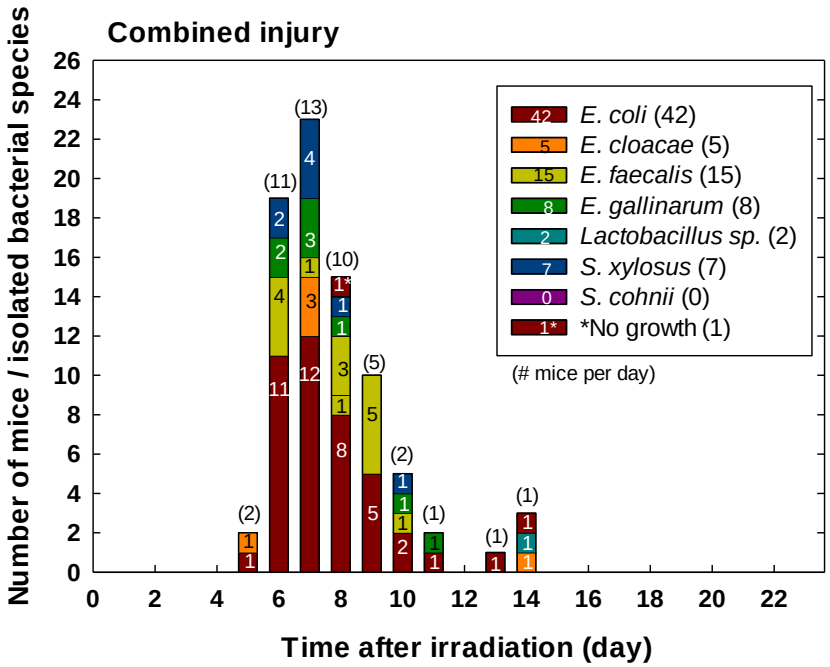
- induces early onset of RI-induced bacterial infection; and
- causes more bacterial infection.

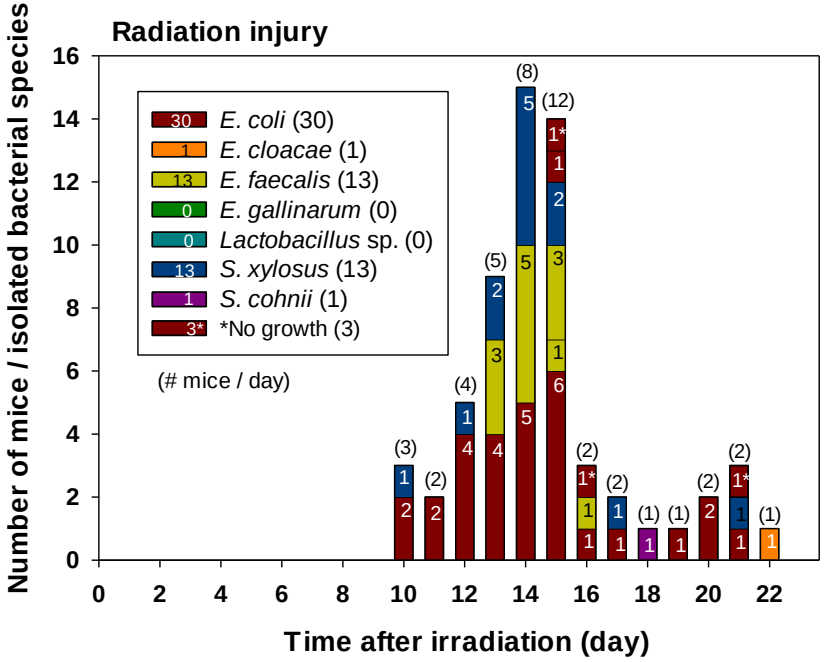


Kiang *et al*., Radiat Res 173: 319, 2010

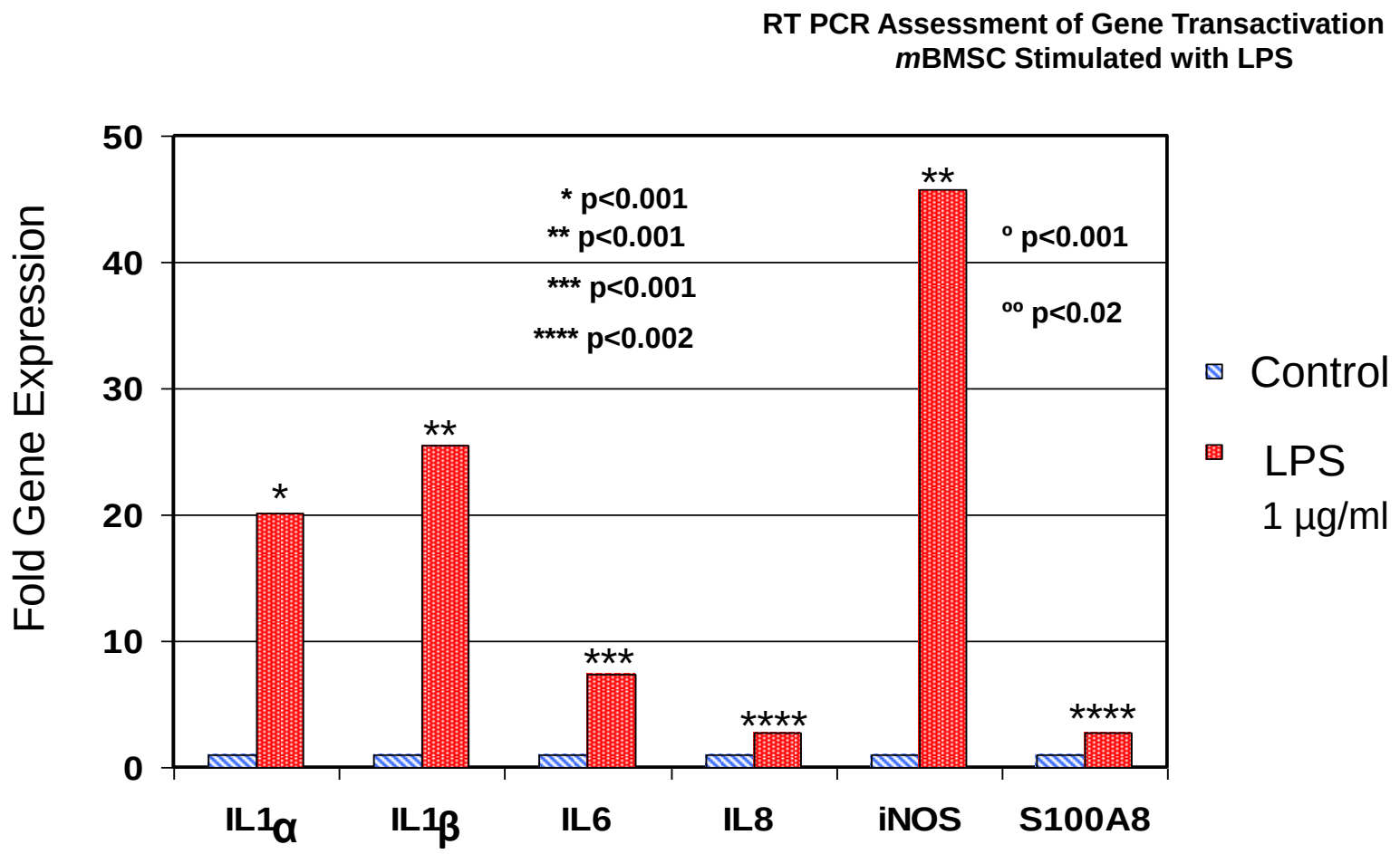
Incidence and Frequency of Polymicrobial Infection after Lethal Irradiation (9.75 Gy ^{60}Co γ photons) in B6D2F$_1$/J Female Mice

- Collected aseptically ventricular heart blood, liver, and spleen from moribund mice.
- Homogenized tissue.
- Isolated and identified bacteria.





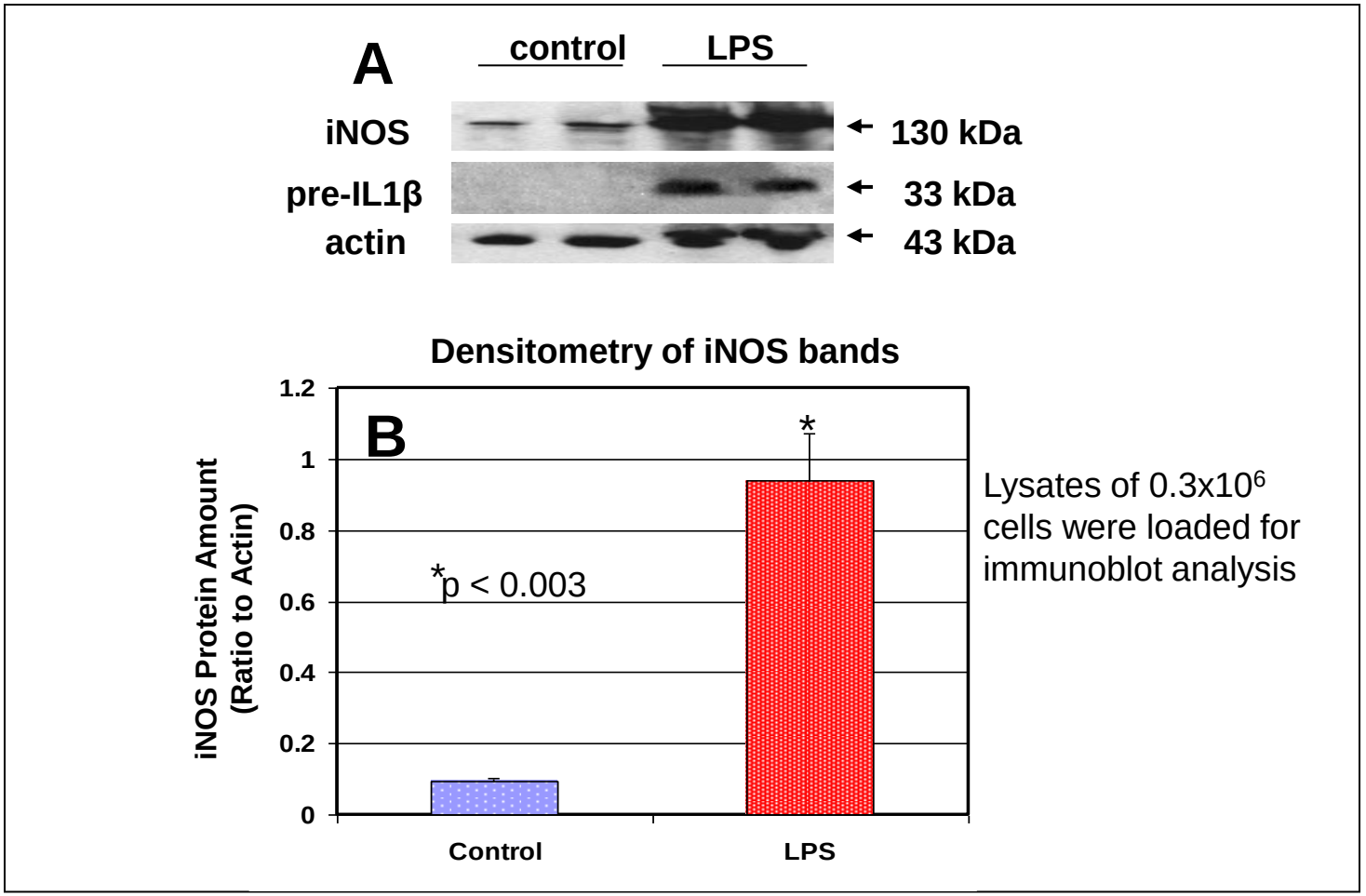
LPS induces increases in gene expression of cytokines and iNOS in mBMSCs



Conditions: mBMSC were incubated with 1 µg/ml LPS for 3h. The cells were harvested at 24 h after incubation and subjected to RT PCR analysis.

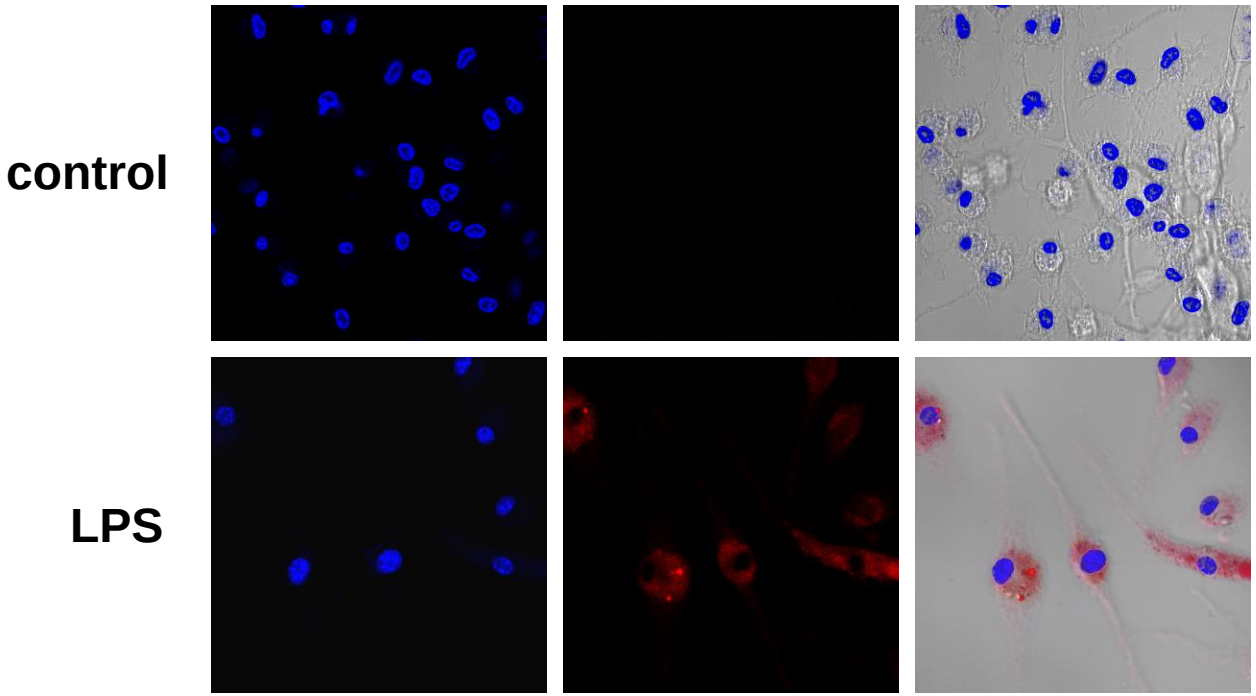
In a preliminary experiment we have found that response of *m*BMSC occurred in a dose-dependent manner in the LPS concentration range of 0.05 µg/ml – 2.5 µg/ml.

LPS increases iNOS and pre-IL1β proteins in mBMSCs



Western Immunoblot Analysis of iNOS and IL-1β Proteins in *m*BMSC Stimulated with LPS

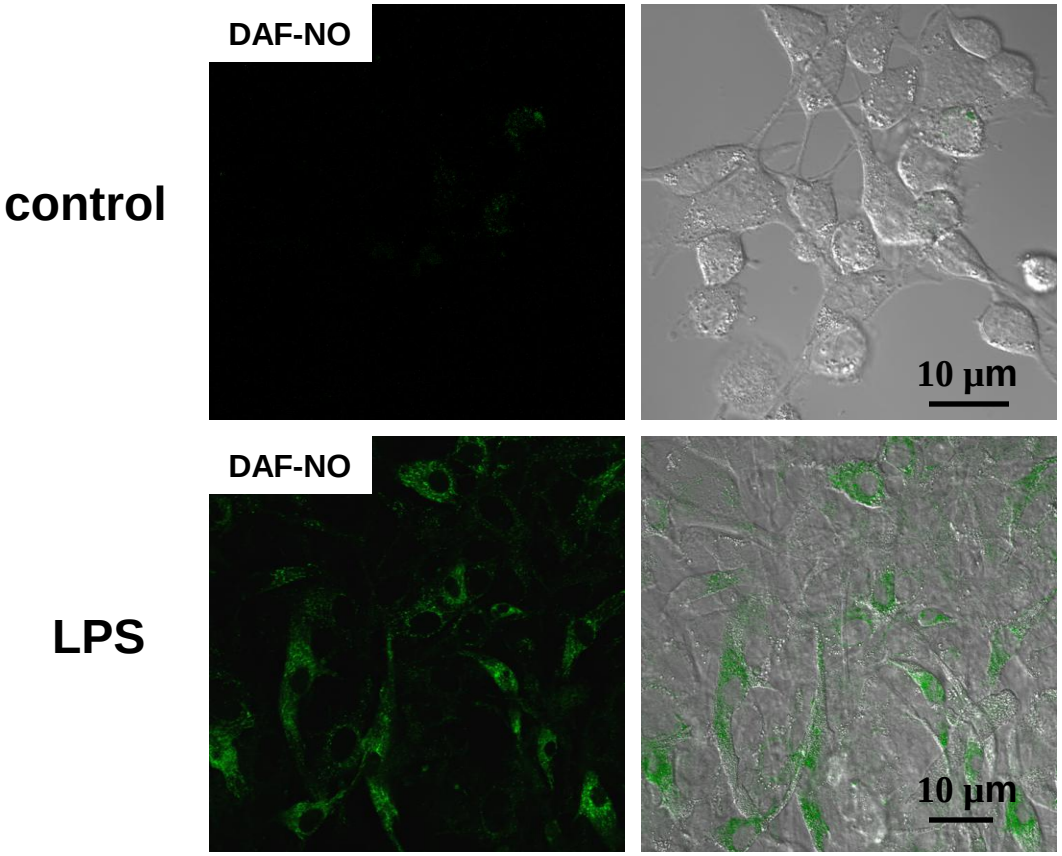
LPS induces iNOS increase that is present in cytosol of mBMSCs



iNOS is presented in red color
Nuclei counterstaining with Hoechst 33342

Immunofluorescence Imaging of iNOS Protein in *m*BMSC Stimulated with LPS

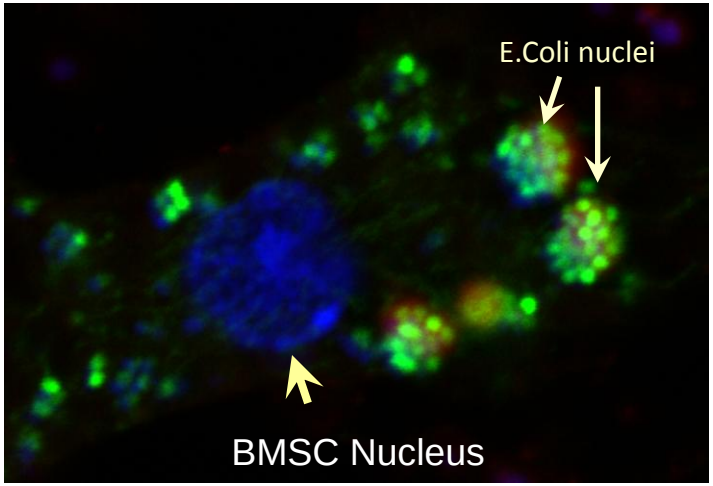
LPS increases NO in mBMSCs



Fluorescence Imaging of the LPS-Induced NO Production in *m*BMSC with DAF2 Fluorescent Probe: The formation of the adduct of DAF2 with NO was detected by appearance of green fluorescence in mBMSC.

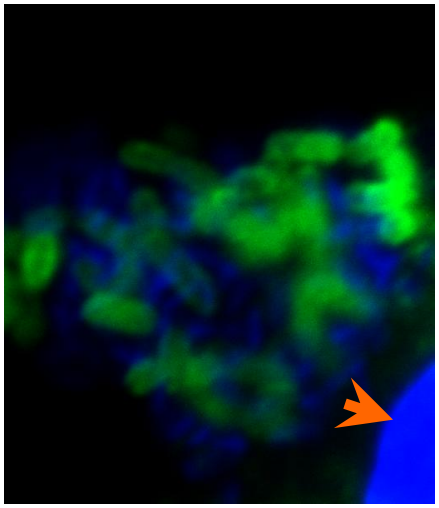
TUNEL-Positive Staining of *E.Coli* Pinocytized by BMSCs

Nucleus DNAs are stained with Hoechst 33342.
TUNEL staining is shown in green.
BMSC nucleus is indicated with arrow head.



E.Coli Pinocytized by BMSCs Are Subjected to Autophagy

Nucleus DNAs are stained with Hoechst 33342.
LC3 immunostaining is shown in green.
A fragment of BMSC nucleus is indicated with arrow head.



Summary: Therapy

mBMSCs

1.Significantly improved survival.
2.Reduced body weight loss.
3.Accelerated wound healing.
4.Were anti-bacterial.

Conclusion

Bone barrow mesencymal stem cells are effective as therapy for radiation injury combined with wound trauma.

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