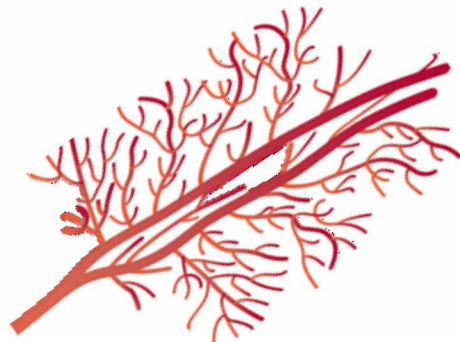


IRSN

INSTITUT
DE RADIOPROTECTION
ET DE SÛRETÉ NUCLÉAIRE

Faire avancer la sûreté nucléaire

Effects of $^{137}\text{Cesium}$ chronic low dose exposure on neovascularization process



Dimassi S, Kereselidze D,
Magneron V, Beugnies L,
Sache A, Ebrahimian T.G

ICRR 2019

25-29 August 2019, Manchester

MEMBRE DE

ETSON

EUROPEAN
TECHNICAL SAFETY
ORGANISATIONS
NETWORK

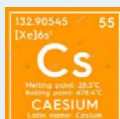
Post-accident situations



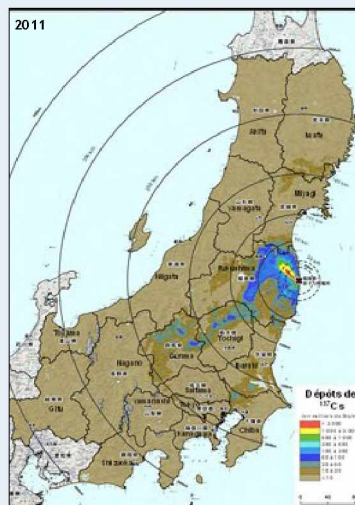
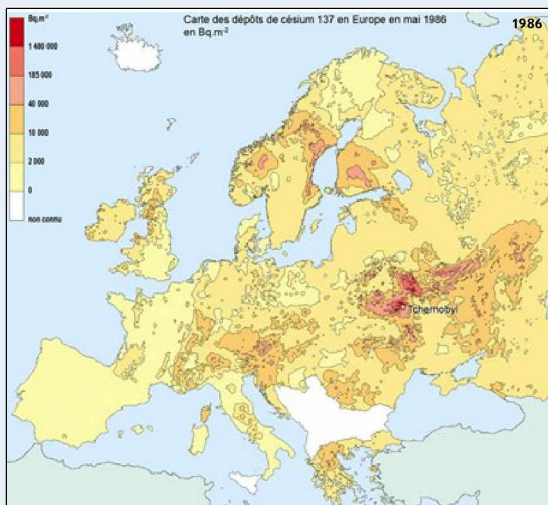
Chernobyl incident (1986)



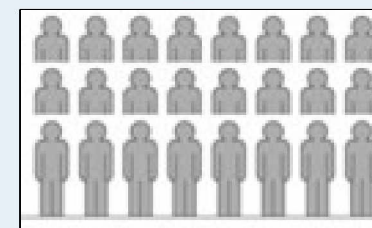
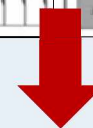
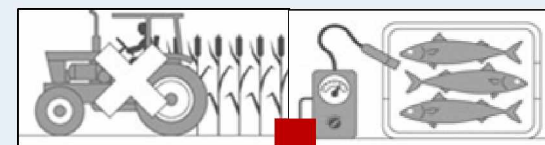
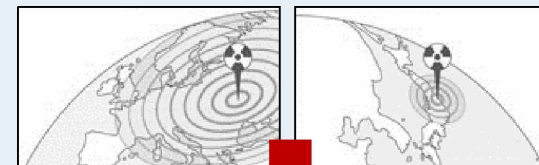
Fukushima daiichi incident (2011)



- Most abundant long-lasting radionucléid released
- 30 year half-life
- Ubiquitous in contaminated ecosystems



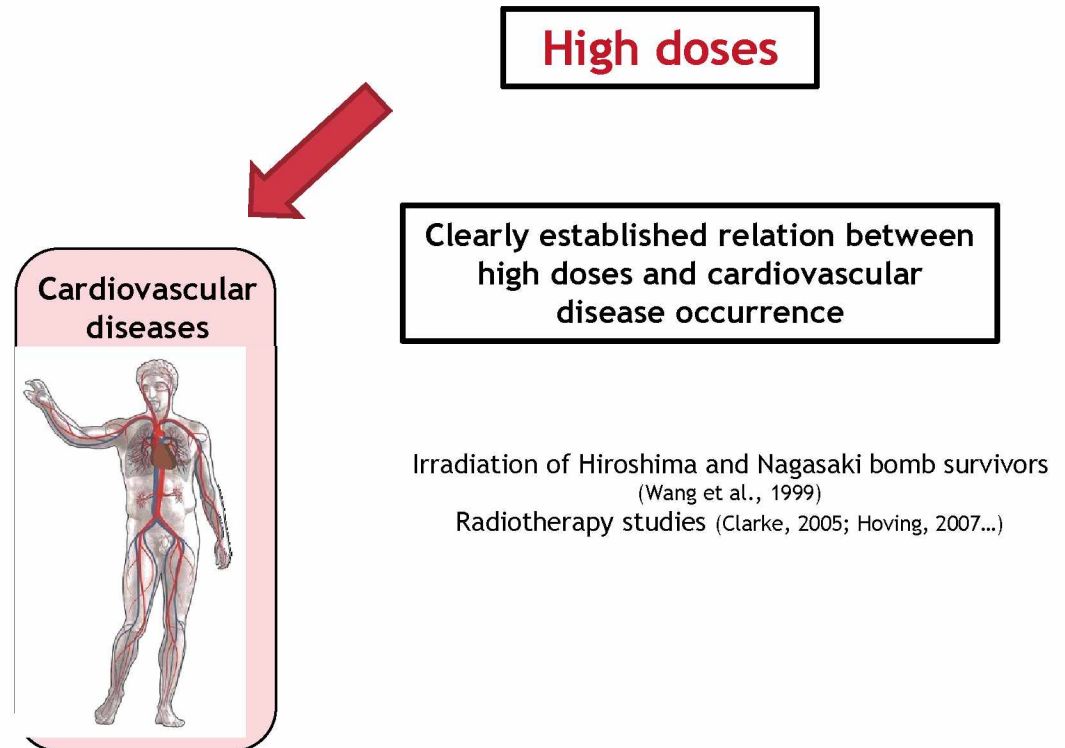
Maps of cesium 137 remanent deposits at the regional / continental scale for Tchernobyl (left) and Fukushima (right) incidents (source: www.IRSN.fr)



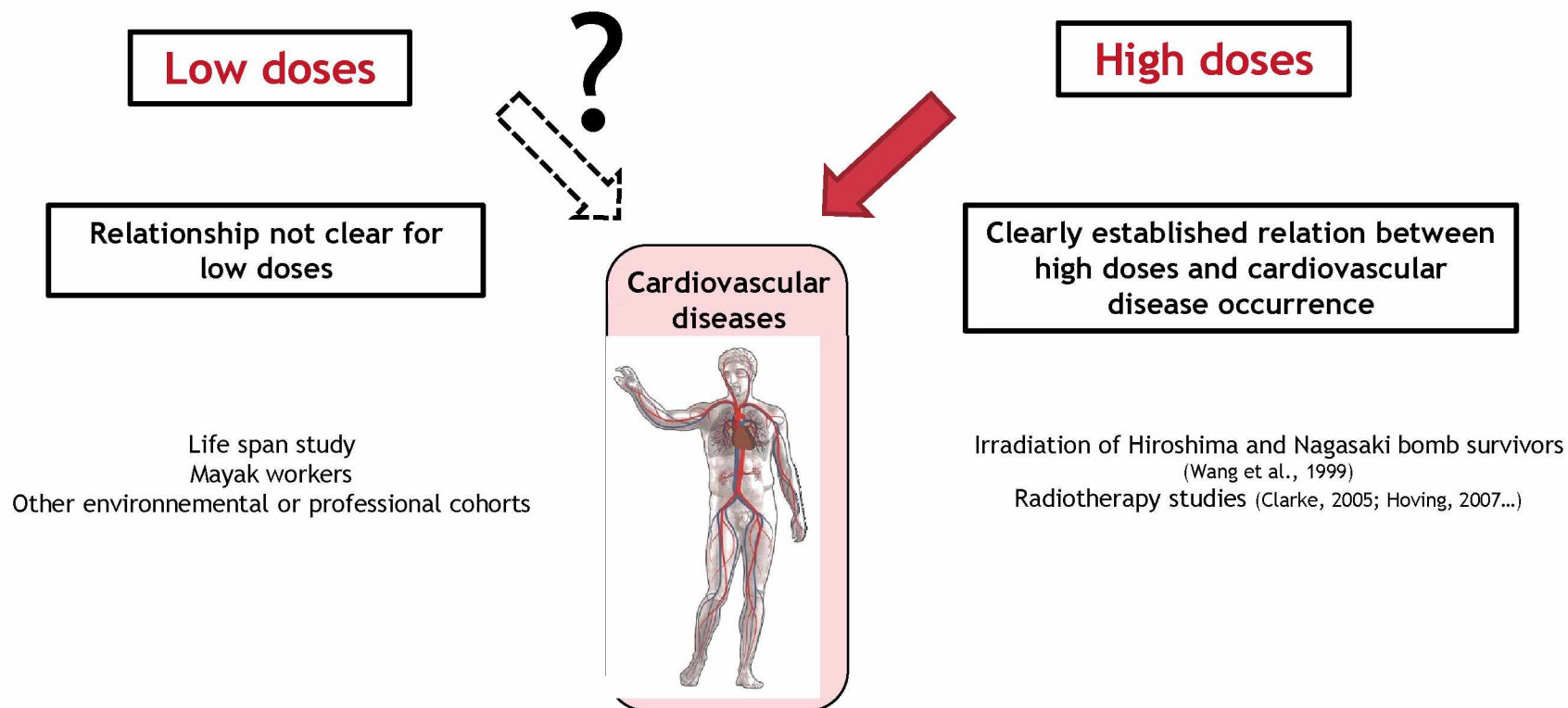
Chronic exposure to radionucléides by ingestion of contaminated food

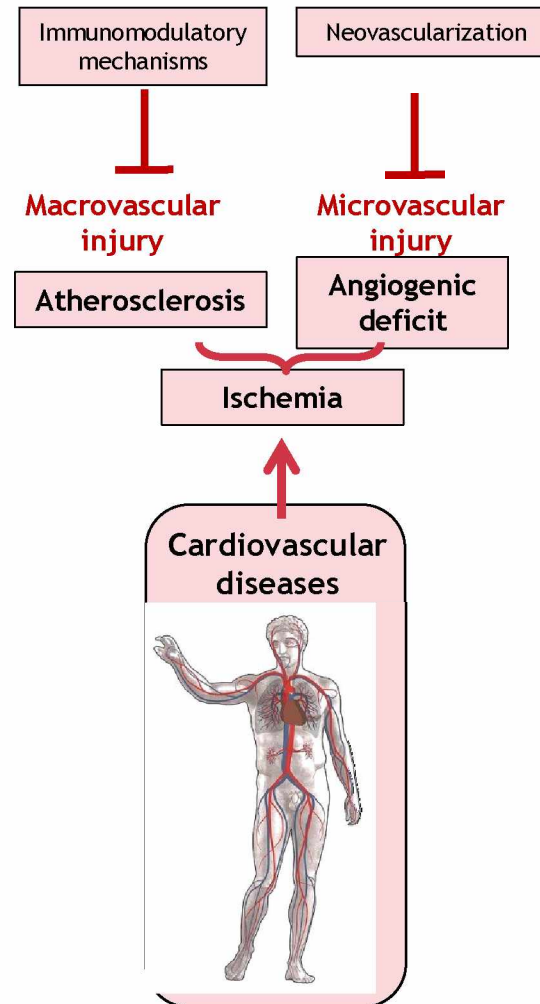
Effects of ionizing exposure on physiological functions in long term ?

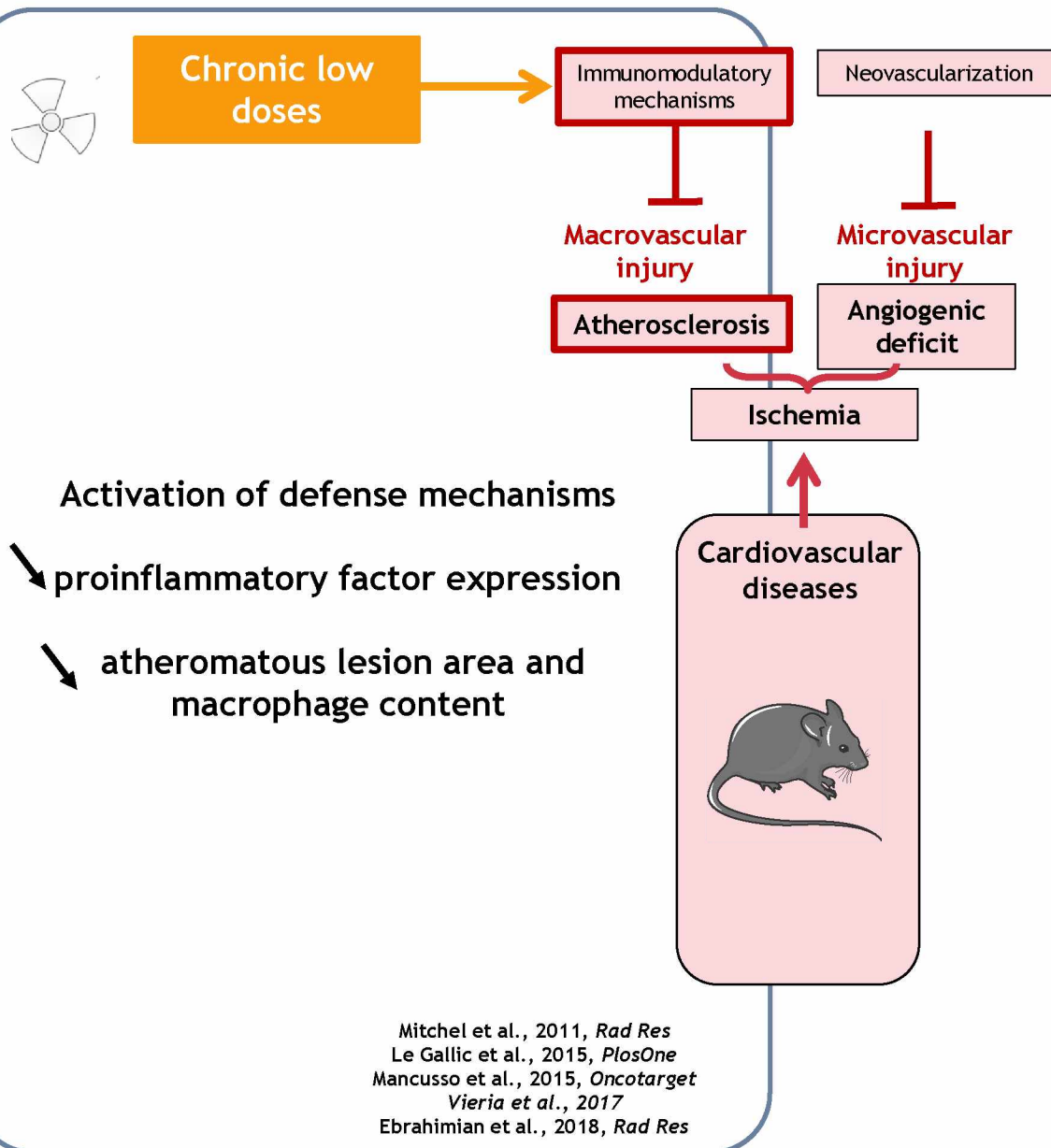
Epidemiological studies

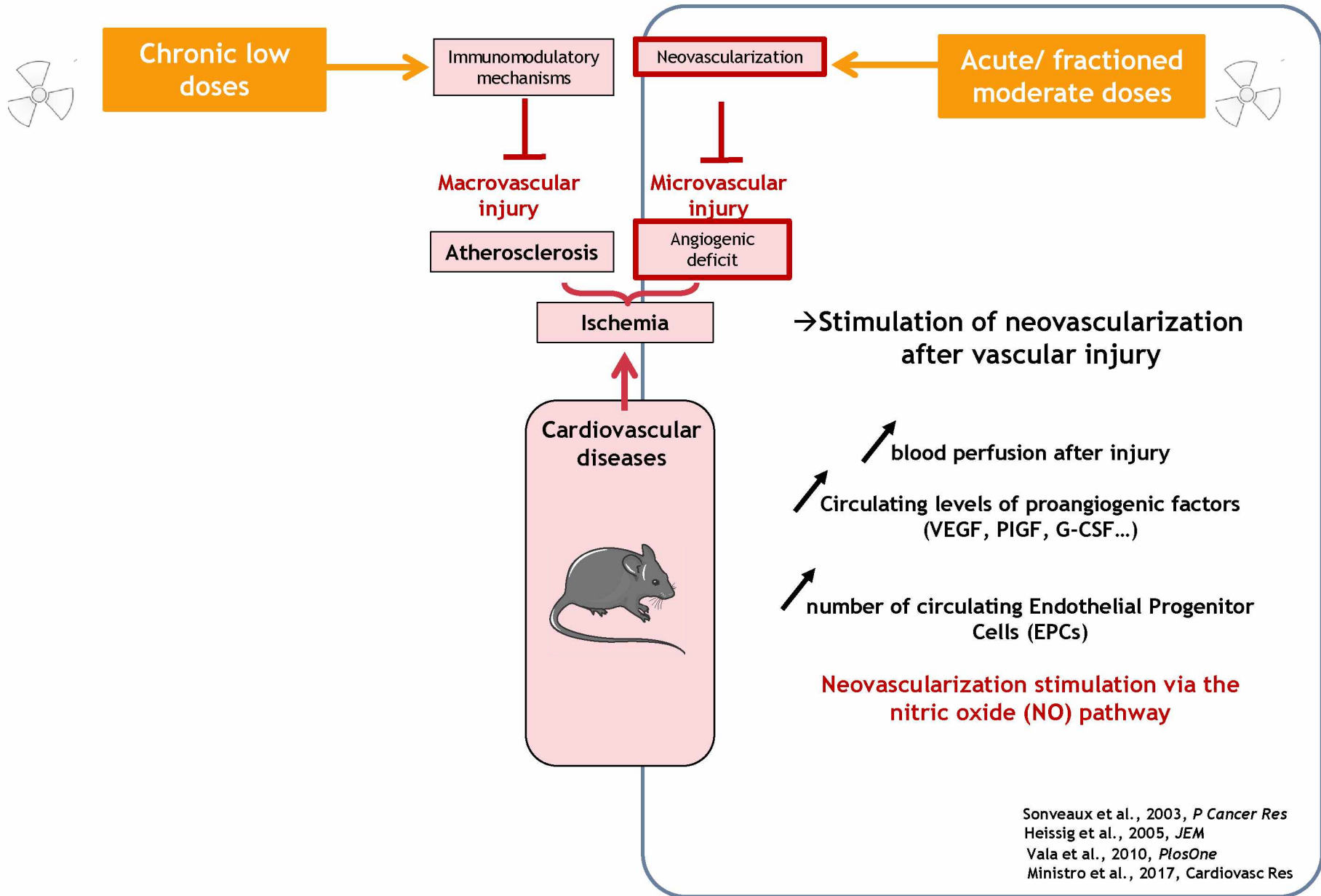


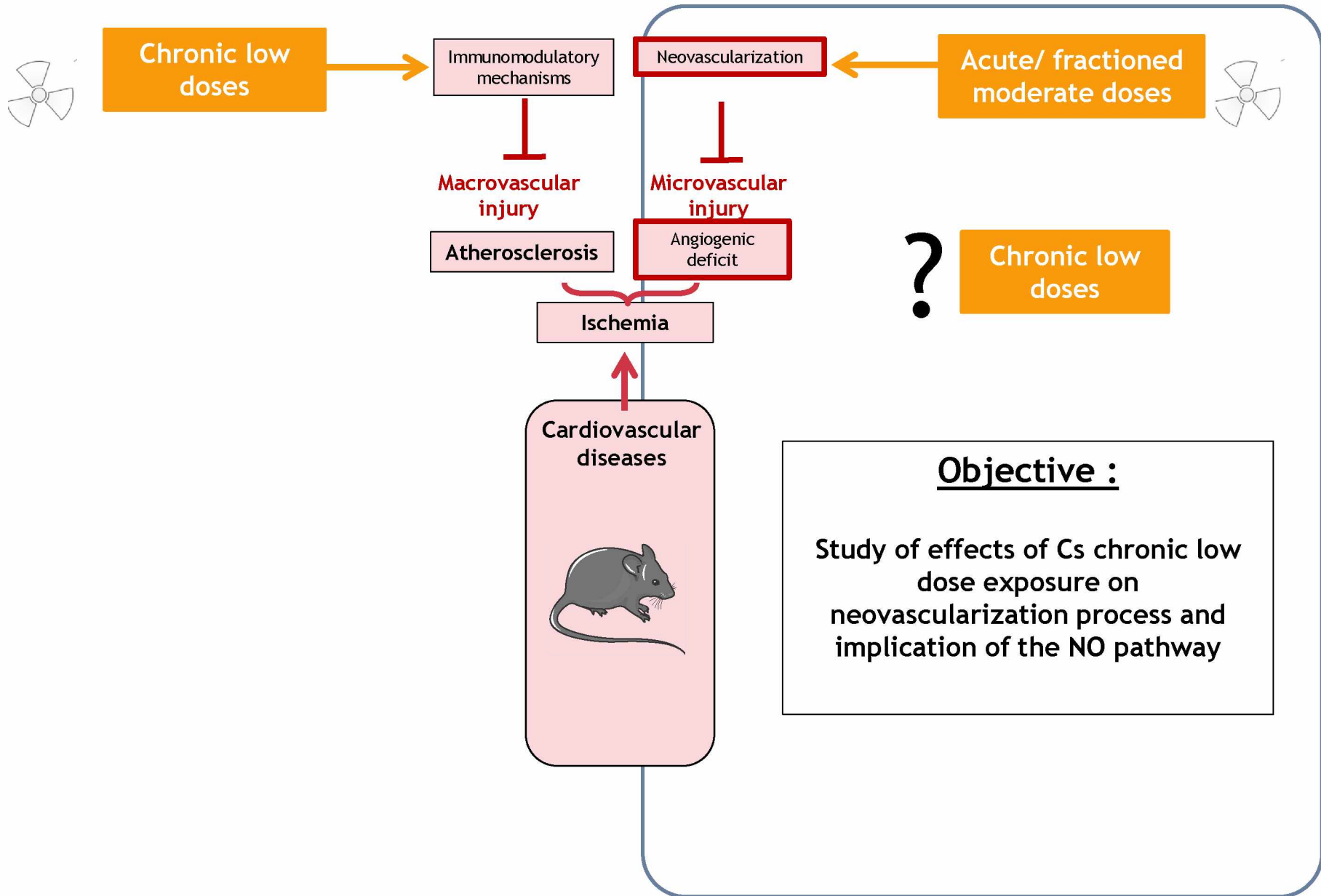
Epidemiological studies



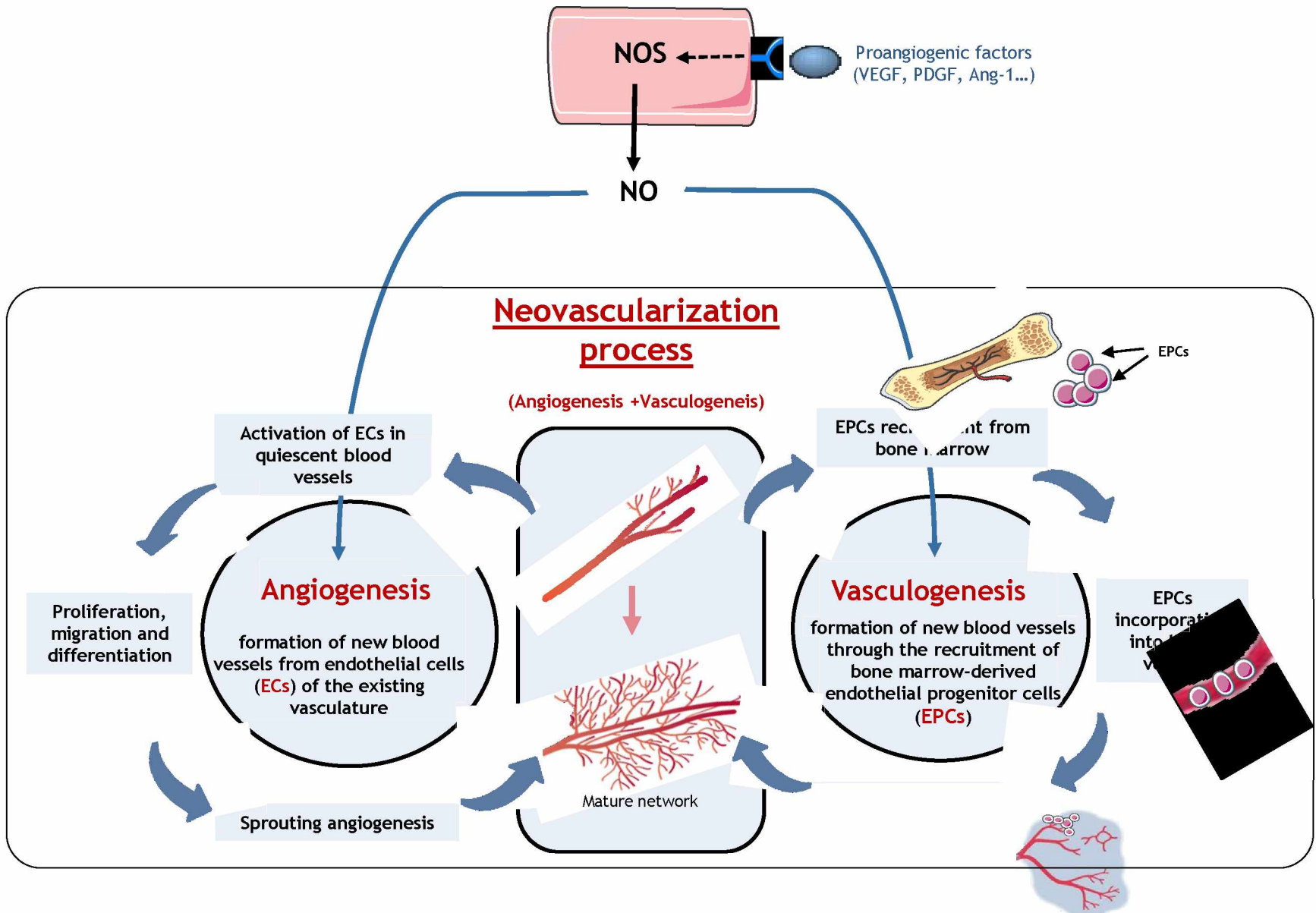




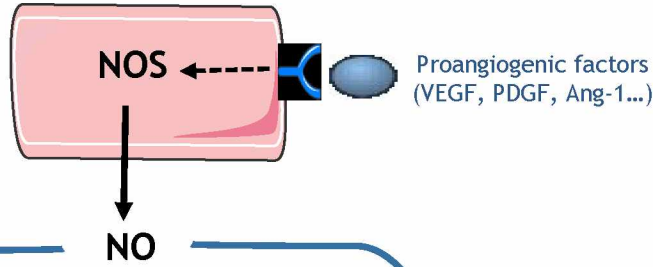
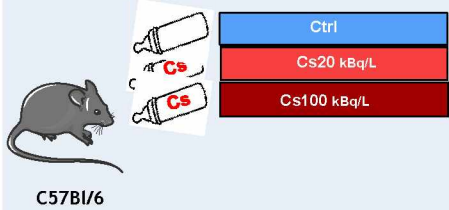




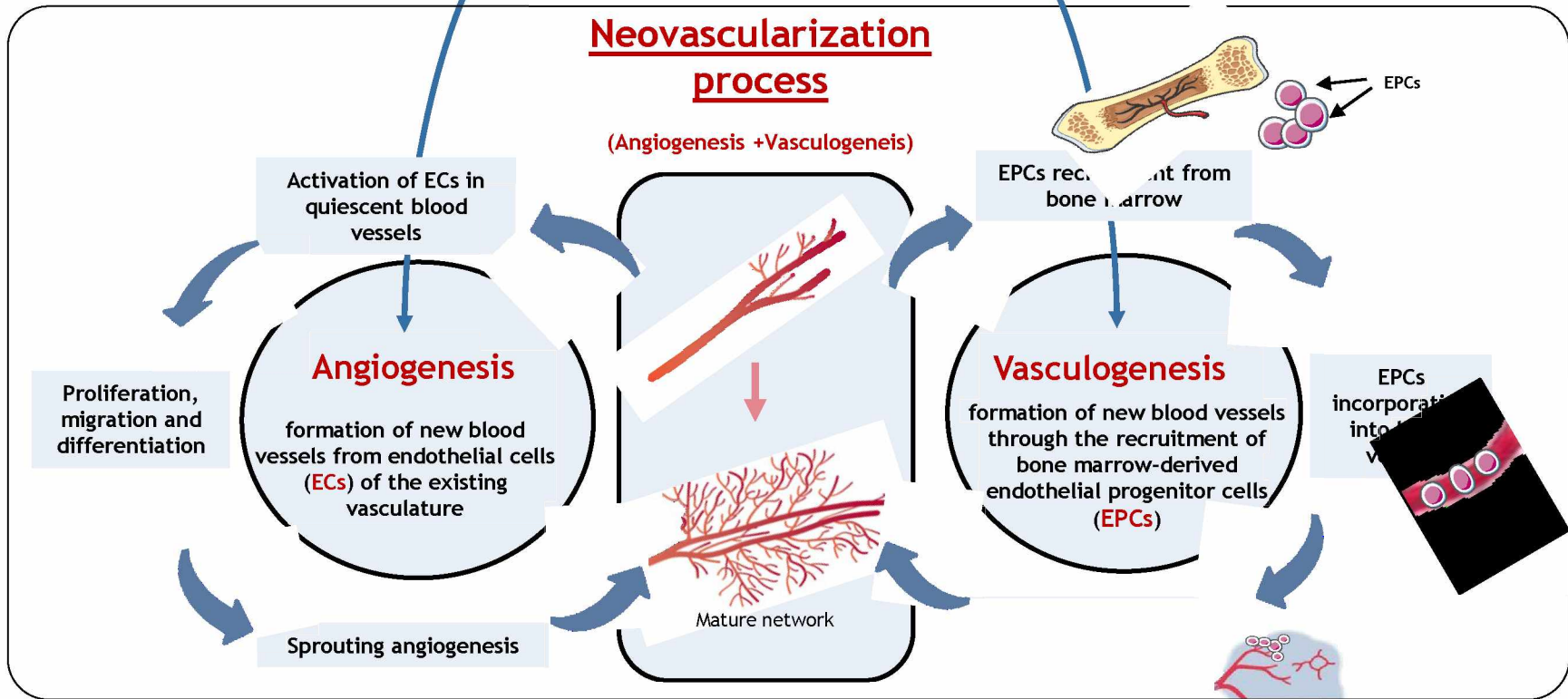
Objective :
 Study of effects of Cs chronic low dose exposure on neovascularization process and implication of the NO pathway



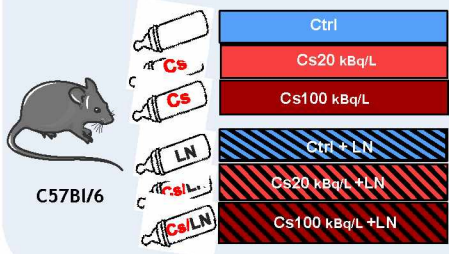
6-month Cs contamination



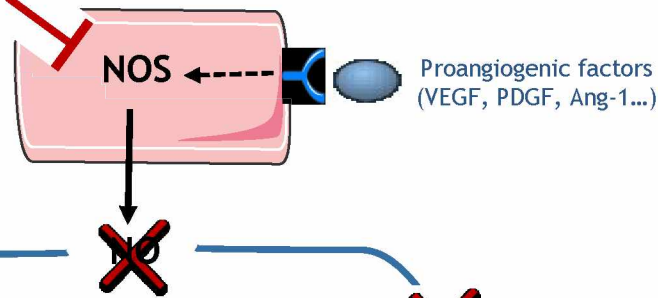
Neovascularization process
(Angiogenesis + Vasculogenesis)



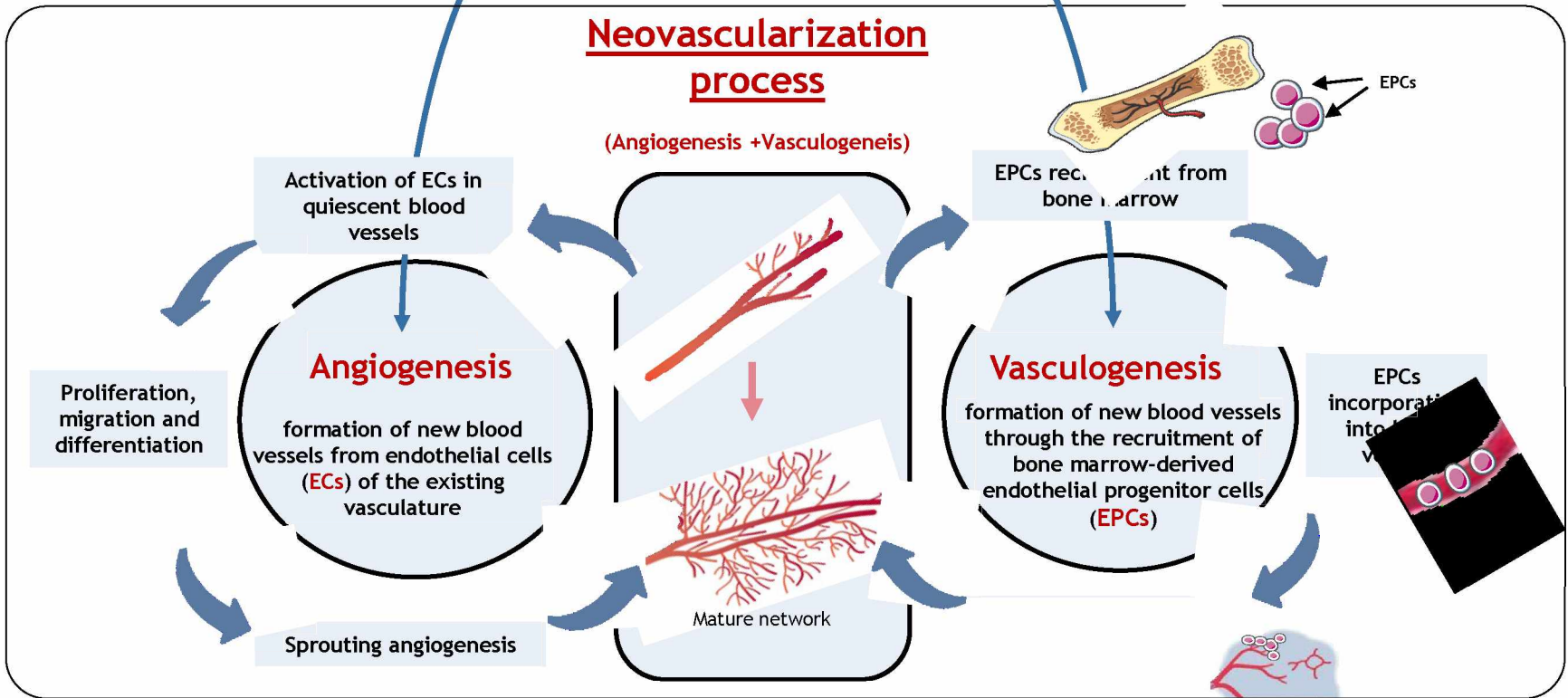
6-month Cs contamination +/- LN



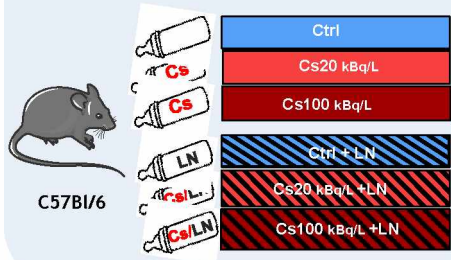
L-NAME (LN)



Neovascularization process
(Angiogenesis + Vasculogenesis)

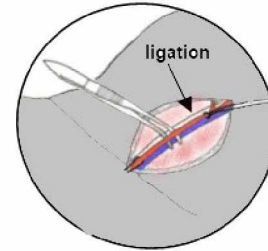
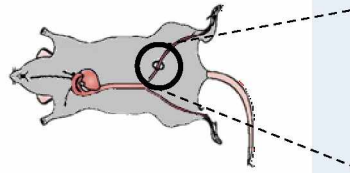


6-month Cs contamination +/- LN



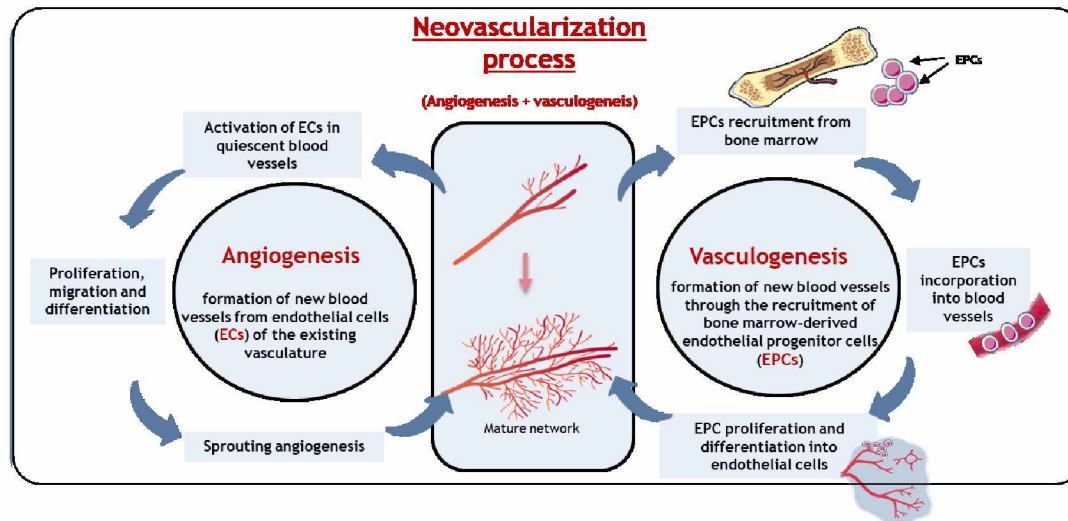
1/ Post-ischemic neovascularisation

Hindlimb femoral artery ligation → ISCHEMIA

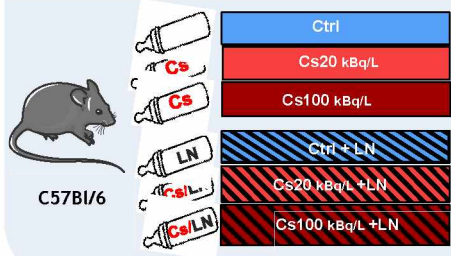


weeks

In vivo Cutaneous Blood flow measurement (Laser Doppler Imaging)



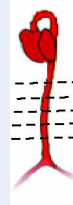
6-month Cs contamination +/- LN



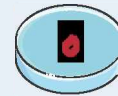
1/ Post-ischemic neovascularisation

2/ Angiogenesis

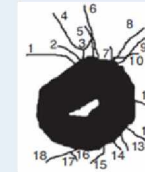
Aorta prelevement



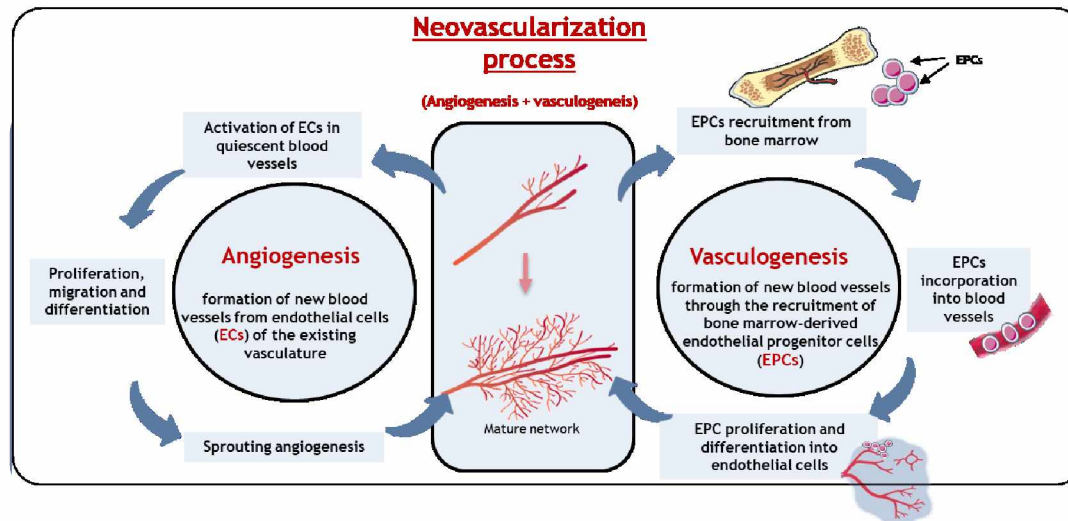
Aortic ring culture into matrigel



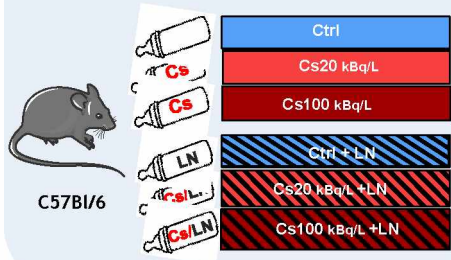
Sprouting analysis



8 days



6-month Cs contamination +/- LN

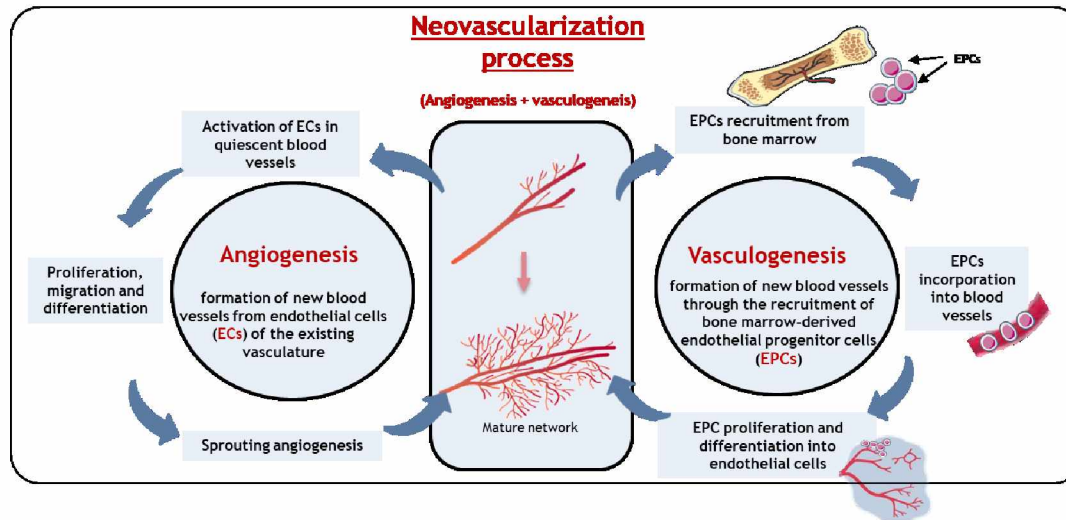
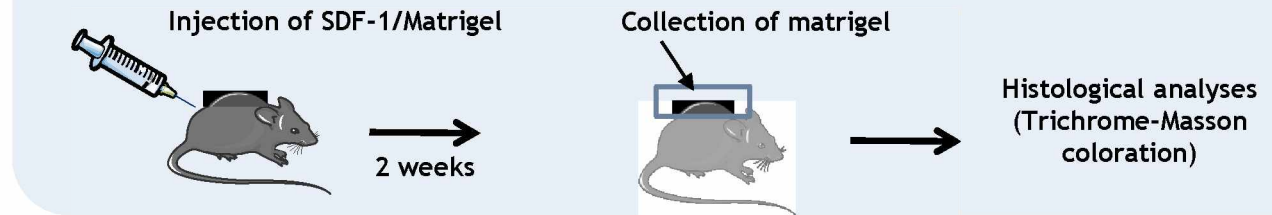


1/ Post-ischemic neovascularisation

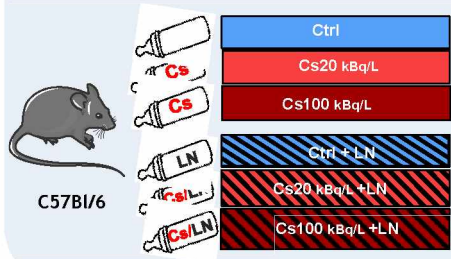
2/ Angiogenesis

3/ Vasculogenesis

3.a/ EPC migration & incorporation into matrigel



6-month Cs contamination +/- LN



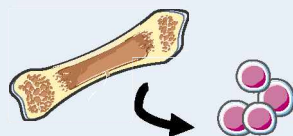
1/ Post-ischemic neovascularisation

2/ Angiogenesis

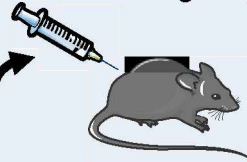
3/ Vasculogenesis

3.a/ EPC migration & incorporation into matrigel

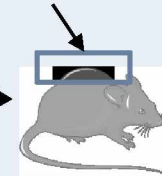
3.b/ EPC tubular formation into matrigel

Bone marrow derived
mononuclear cell isolement

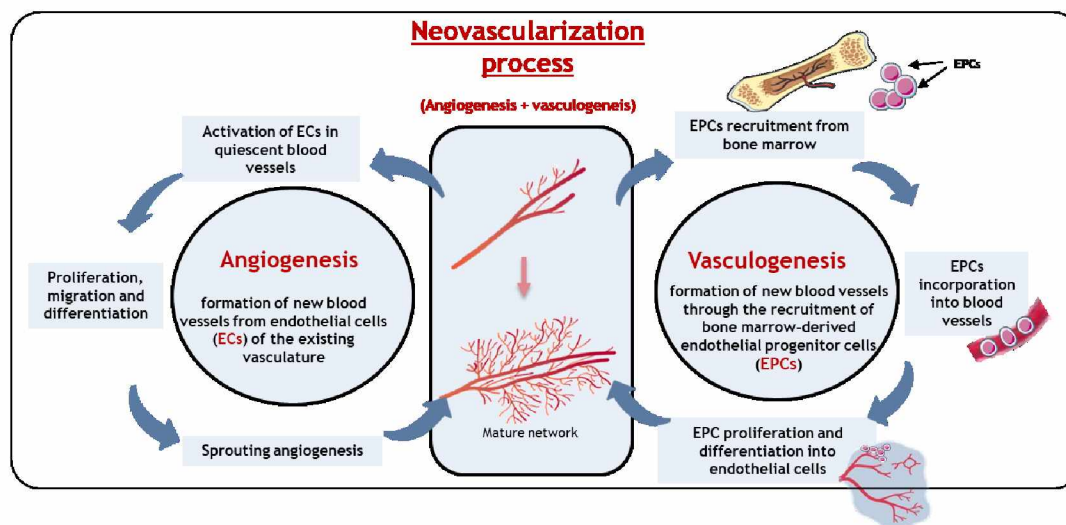
Cell/Matrigel injection

Non-contaminated
receiver mice

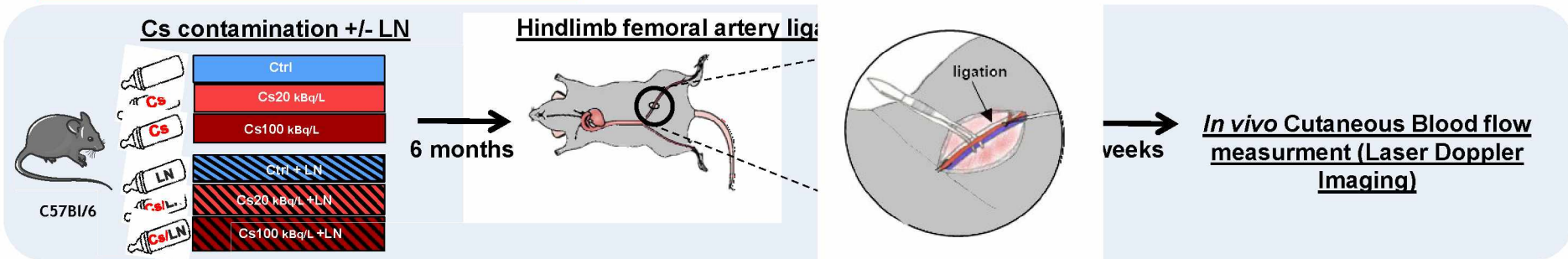
Collection of matrigel



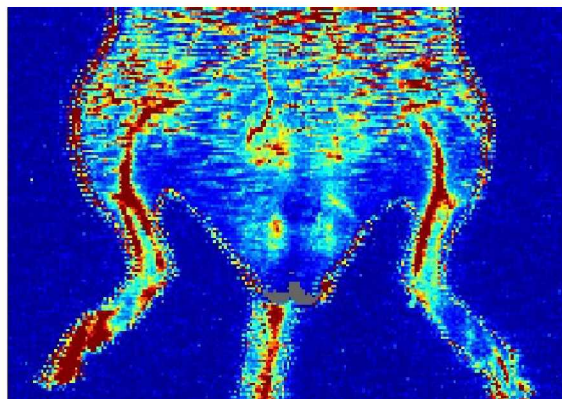
2 weeks

Histological analyses
(Trichrome-Masson
coloration)

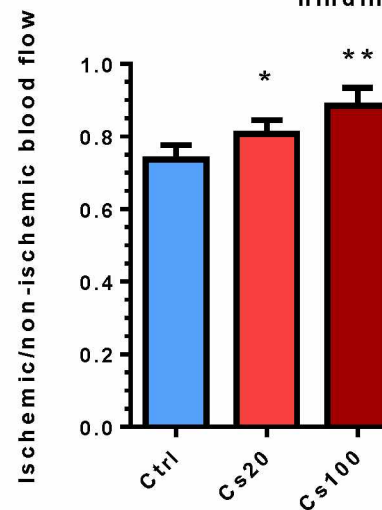
1/ Post-ischemic neovascularisation



Measurement of cutaneous blood flow of ischemic/non ischemic hindlimb ratio



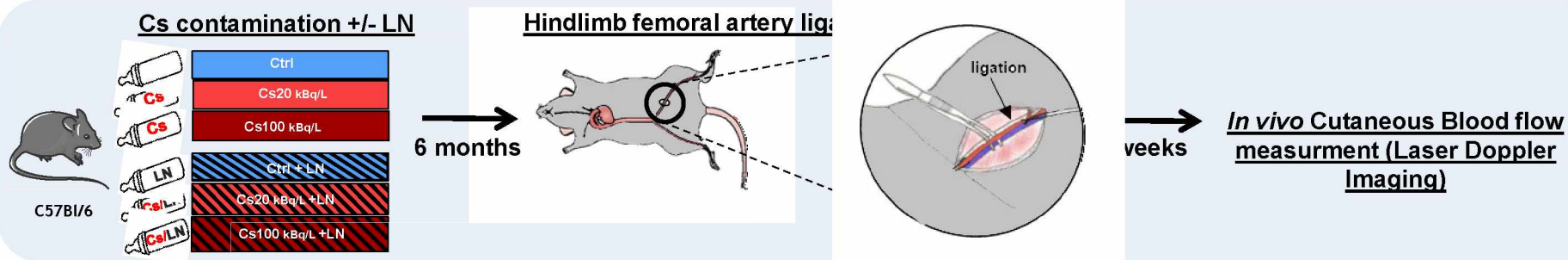
In vivo Cutaneous Blood flow measurement after unilateral hindlimb ligation



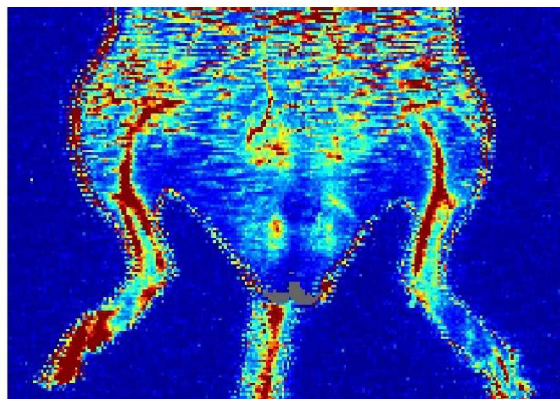
n=15 mice/group
*p<0.05 vs Ctrl; **p<0.005 vs Ctrl

➔ Increased hindlimb perfusion after Cs chronic exposure for Cs20 and Cs100 groups

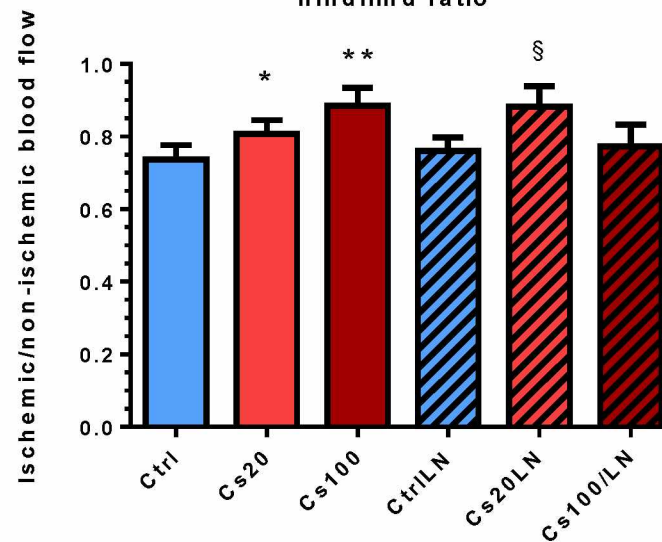
1/ Post-ischemic neovascularisation



Measurement of cutaneous blood flow of ischemic/non ischemic hindlimb ratio



In vivo Cutaneous Blood flow measurement after unilateral hindlimb ligation

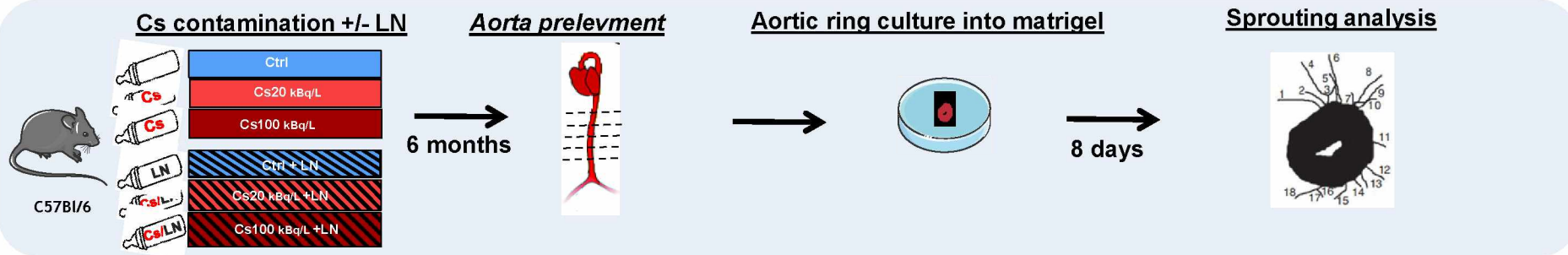


n=15 mice/group

*p<0.05 vs Ctrl; **p<0.005 vs Ctrl; §p<0.05 vs CtrlLN

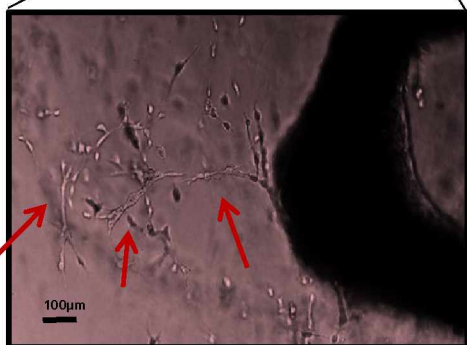
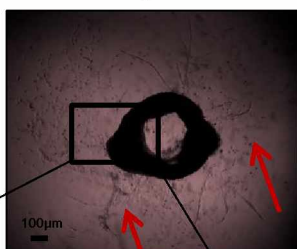
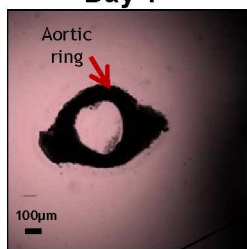
- ➡ Increased hindlimb perfusion after Cs chronic exposure for Cs20 and Cs100 groups
- ➡ LN traitement abolished the increase in ischemia hindlimb flow for the Cs100LN group.

2/ Angiogenesis

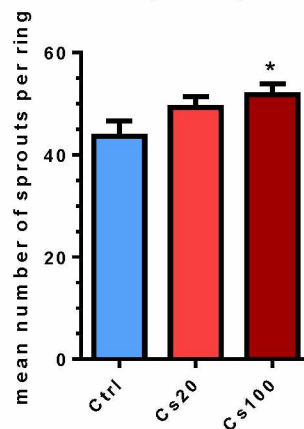


Day 1

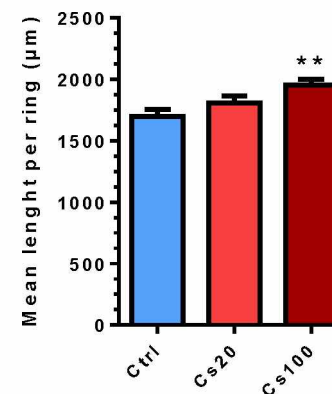
Day 8



Measurement of mean number of sprouts per aortic ring



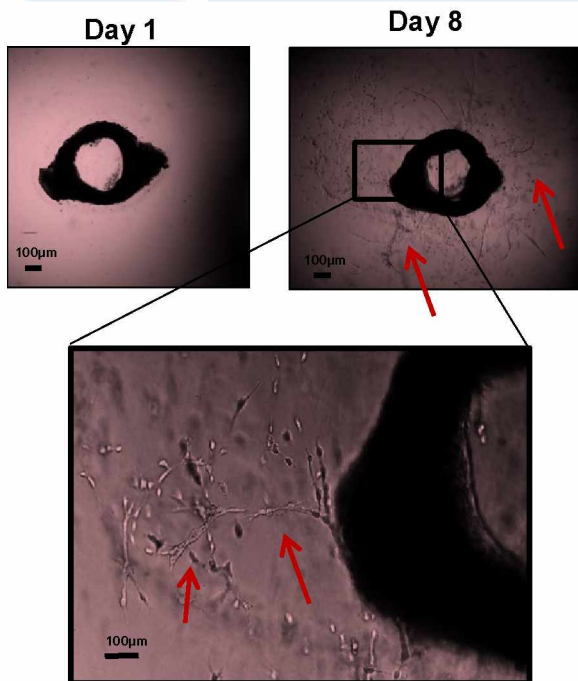
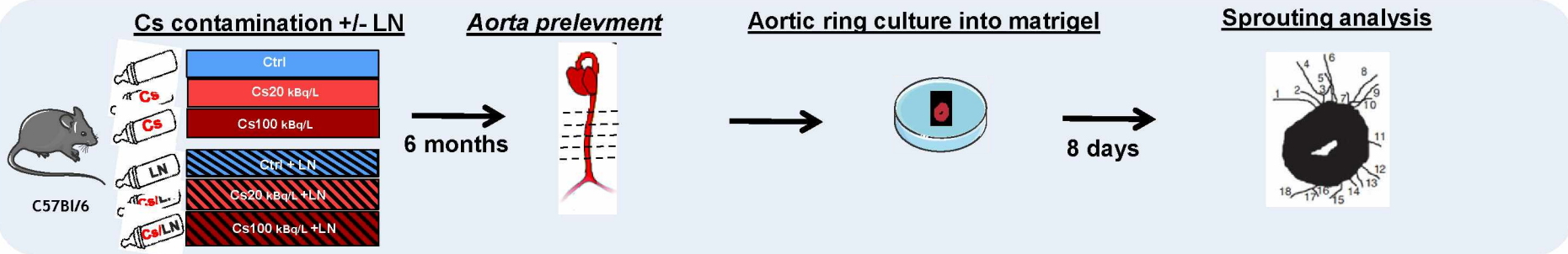
Measurement of mean length of sprouts per aortic ring



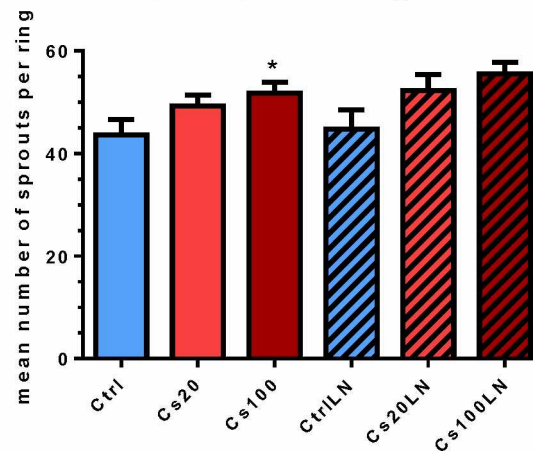
N=10rings/mouse, 2-3mice/group
*p<0.05 vs Ctrl; **p<0.005 vs Ctrl

➔ Increased sprouting angiogenesis after chronic exposure with Cs100

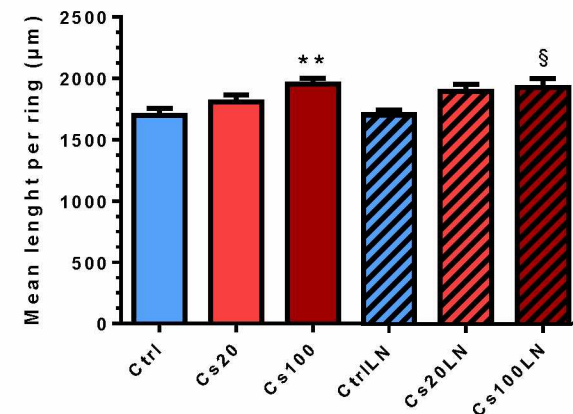
2/ Angiogenesis



Measurement of mean number of sprouts per aortic ring



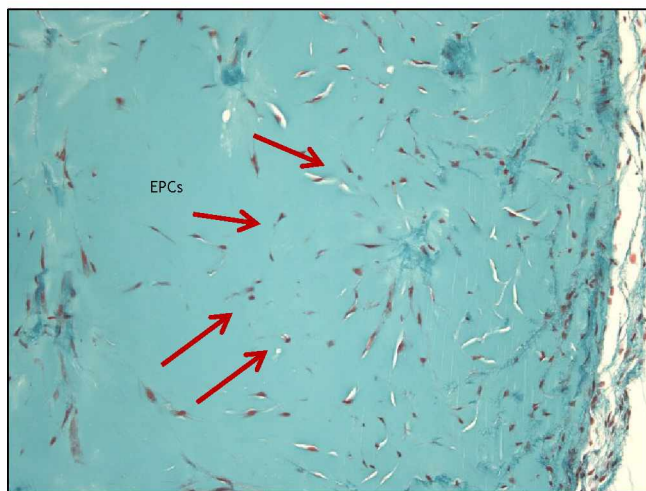
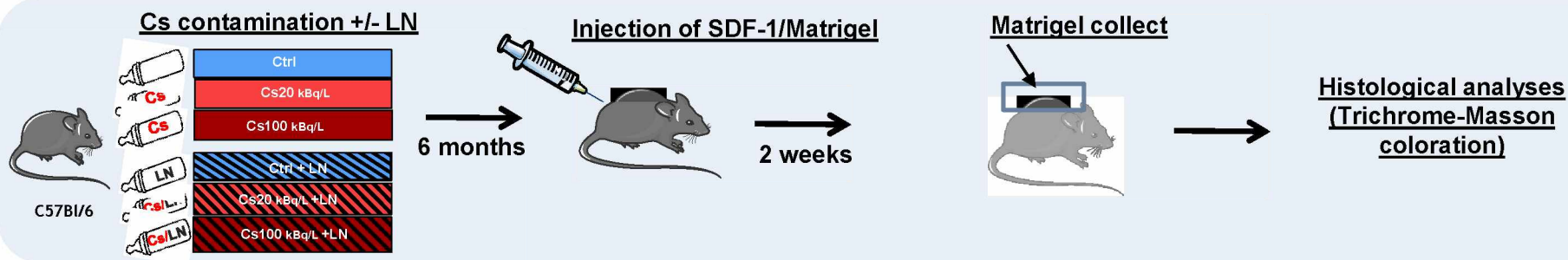
Measurement of mean length of sprouts per aortic ring



N=10rings/mouse, 2-3mice/group
 *p<0.05 vs Ctrl; **p<0.005 vs Ctrl; §p<0.05 vs CtrlLN

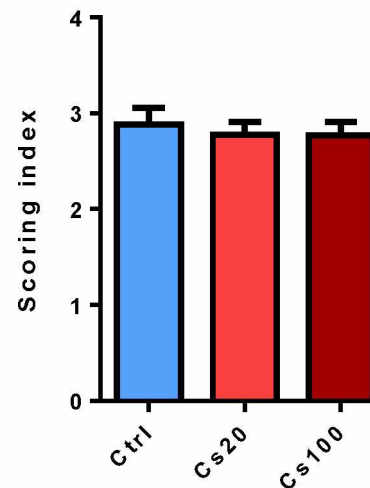
➔ Increased sprouting angiogenesis after chronic exposure with Cs100

3.a/ Vasculogenesis : EPC migration & incorporation into matrigel



Histological section of matrigel/SDF-1 injected subcutaneously. Trichrome-Masson coloration

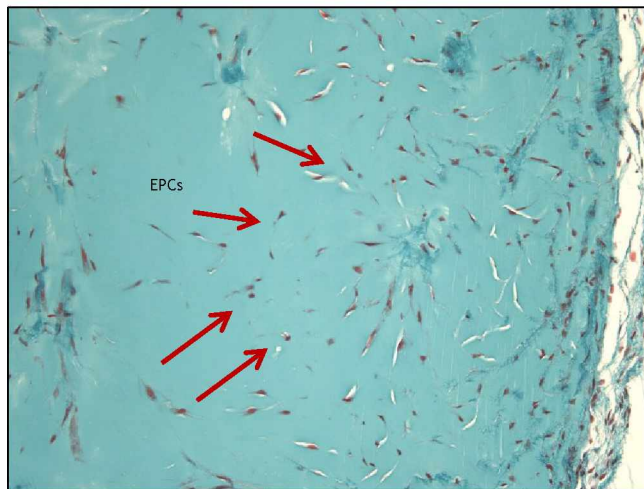
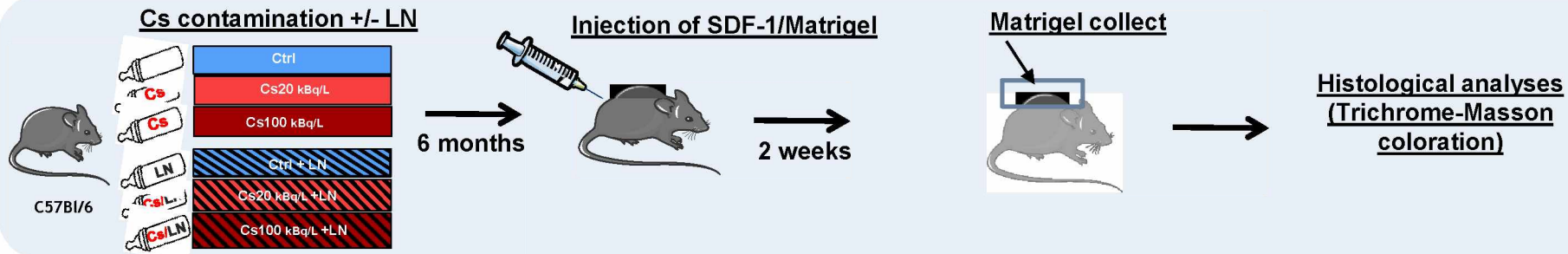
Evaluation of EPC ability to migrate and incorporate into matrigel



n=10 mice/group

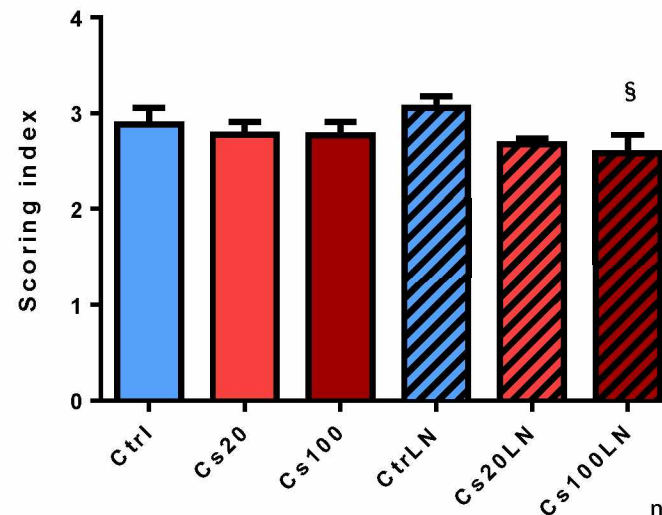
➔ No effect of Cs exposure on the EPC capacity to migrate from bone marrow and incorporate vessels

3.a/ Vasculogenesis : EPC migration & incorporation into matrigel



Histological section of matrigel/SDF-1 injected subcutaneously. Trichrome-Masson coloration

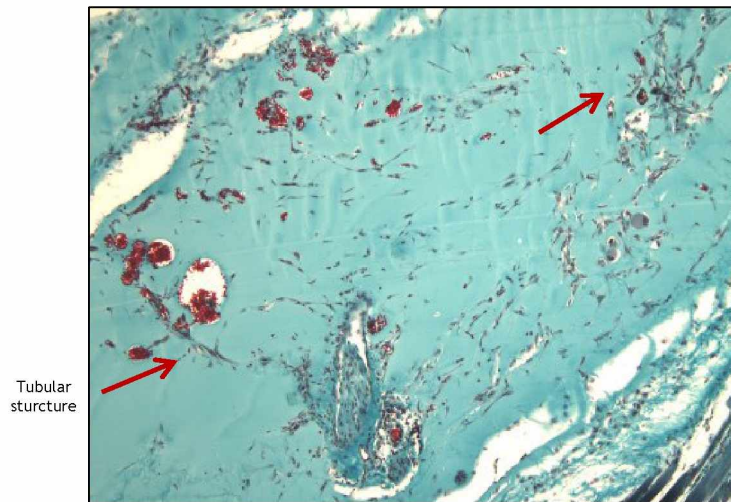
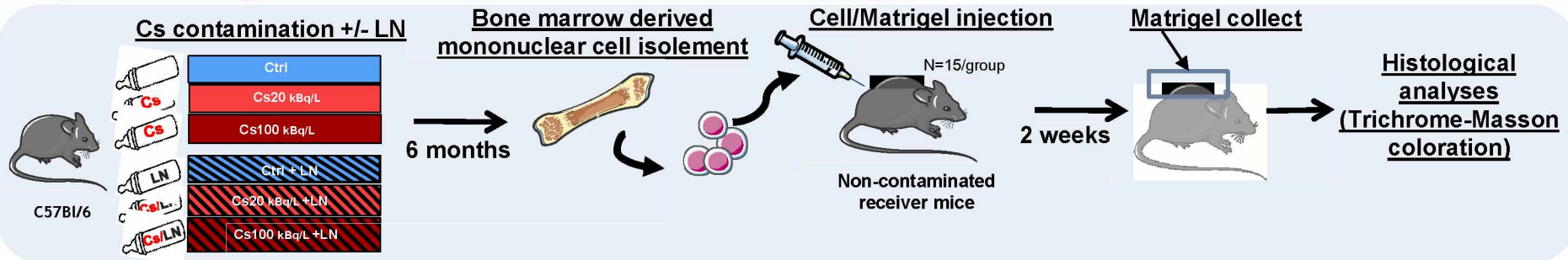
Evaluation of EPC ability to migrate and incorporate into matrigel



n=10 mice/group
§ p<0.05 vs CtrlLN

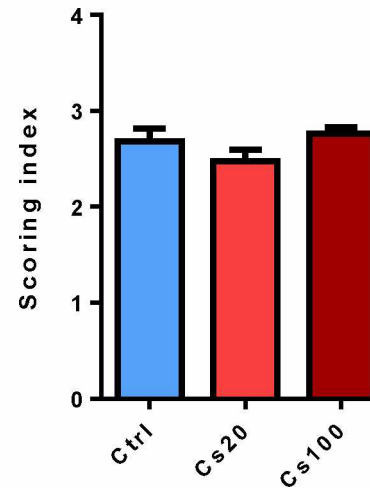
- ➔ No effect of Cs exposure on the EPC capacity to migrate from bone marrow and incorporate vessels
- ➔ Reduction of EPC potency for Cs100LN group

3.b/ Vasculogenesis : EPC tubular formation into matrigel



Histological section of matrigel injected subcutaneously and containing bone marrow derived mononuclear cell. Trichrome-Masson coloration

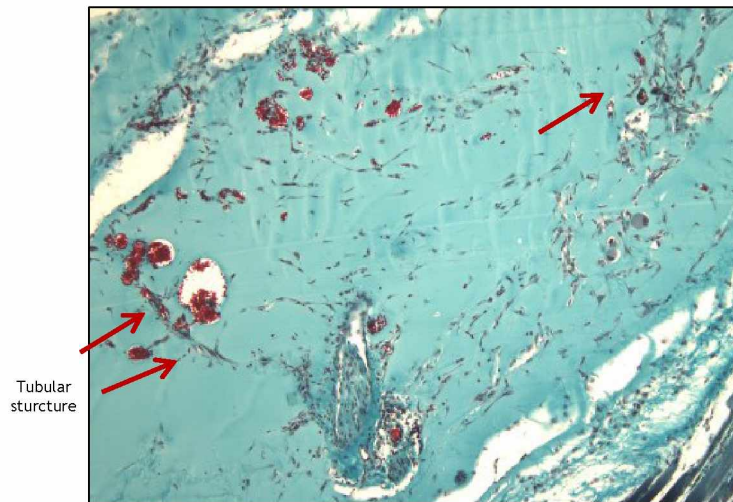
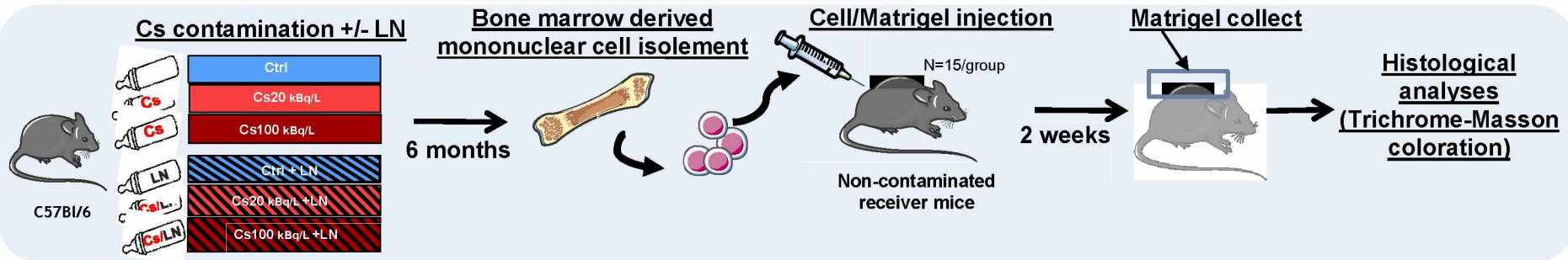
Evaluation of EPC ability to elaborate tubular structure into matrigel



n=15 mice/group

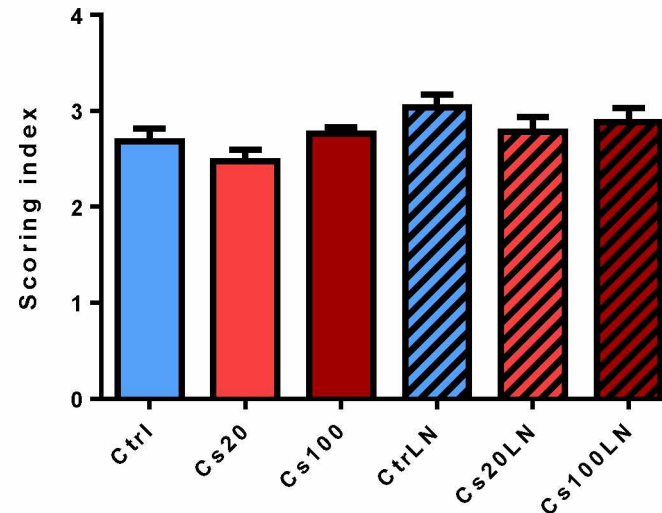
➔ No effect of Cs exposure on EPC capacity to form tubular structure

3.b/ Vasculogenesis : EPC tubular formation into matrigel



Histological section of matrigel injected subcutaneously and containing bone marrow derived mononuclear cell. Trichrome-Masson coloration

Evaluation of EPC ability to elaborate tubular structure into matrigel



- ➔ No effect of Cs exposure on EPC capacity to form tubular structure
- ➔ No effect of LN traitement

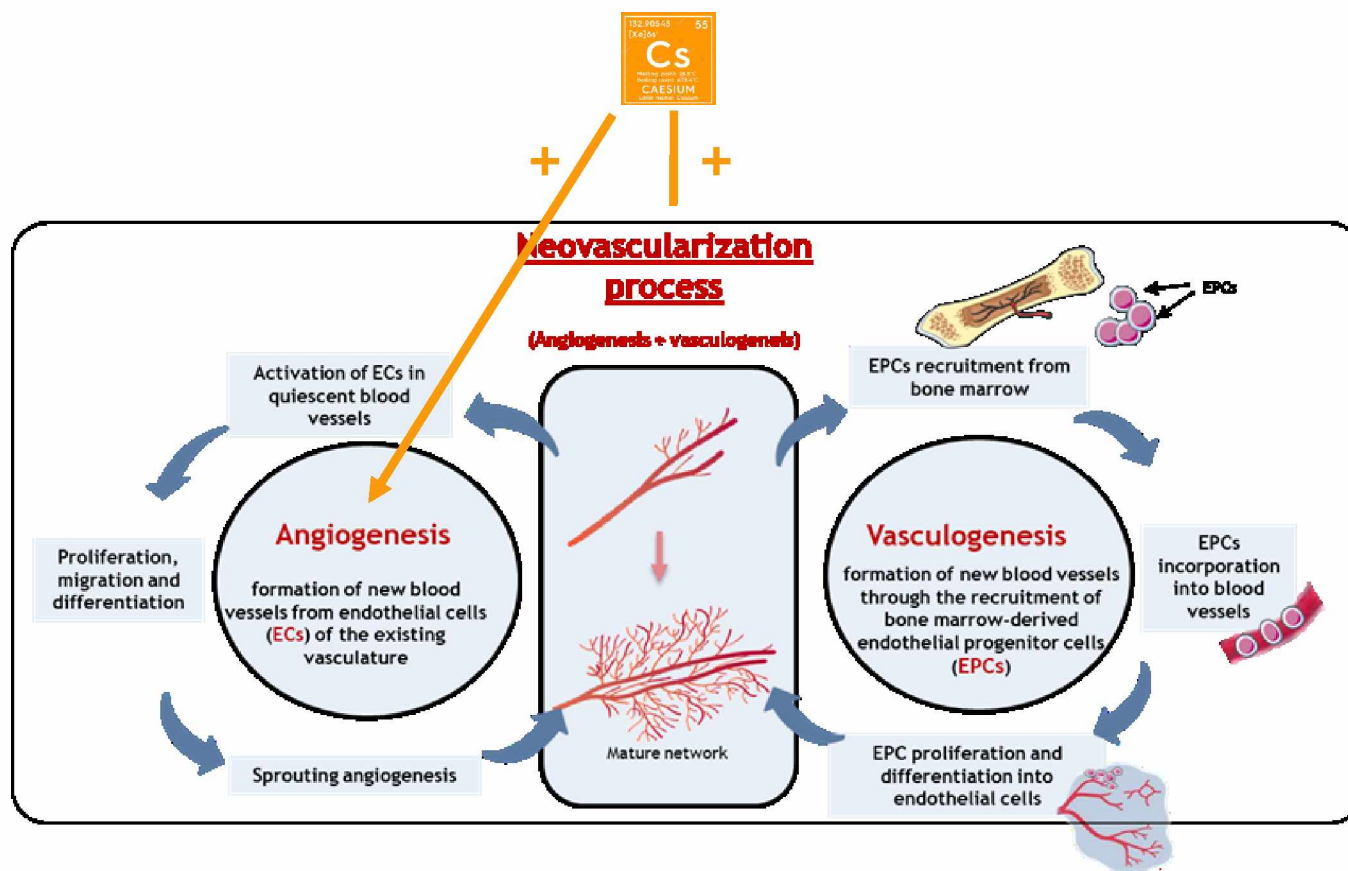
Cs chronic low dose contamination



Cs100 kBq/L

6 months

- ➔ Stimulation of post-ischemic neovascularization
- ➔ Enhancement of sprouting angiogenesis



Cs chronic low dose contamination



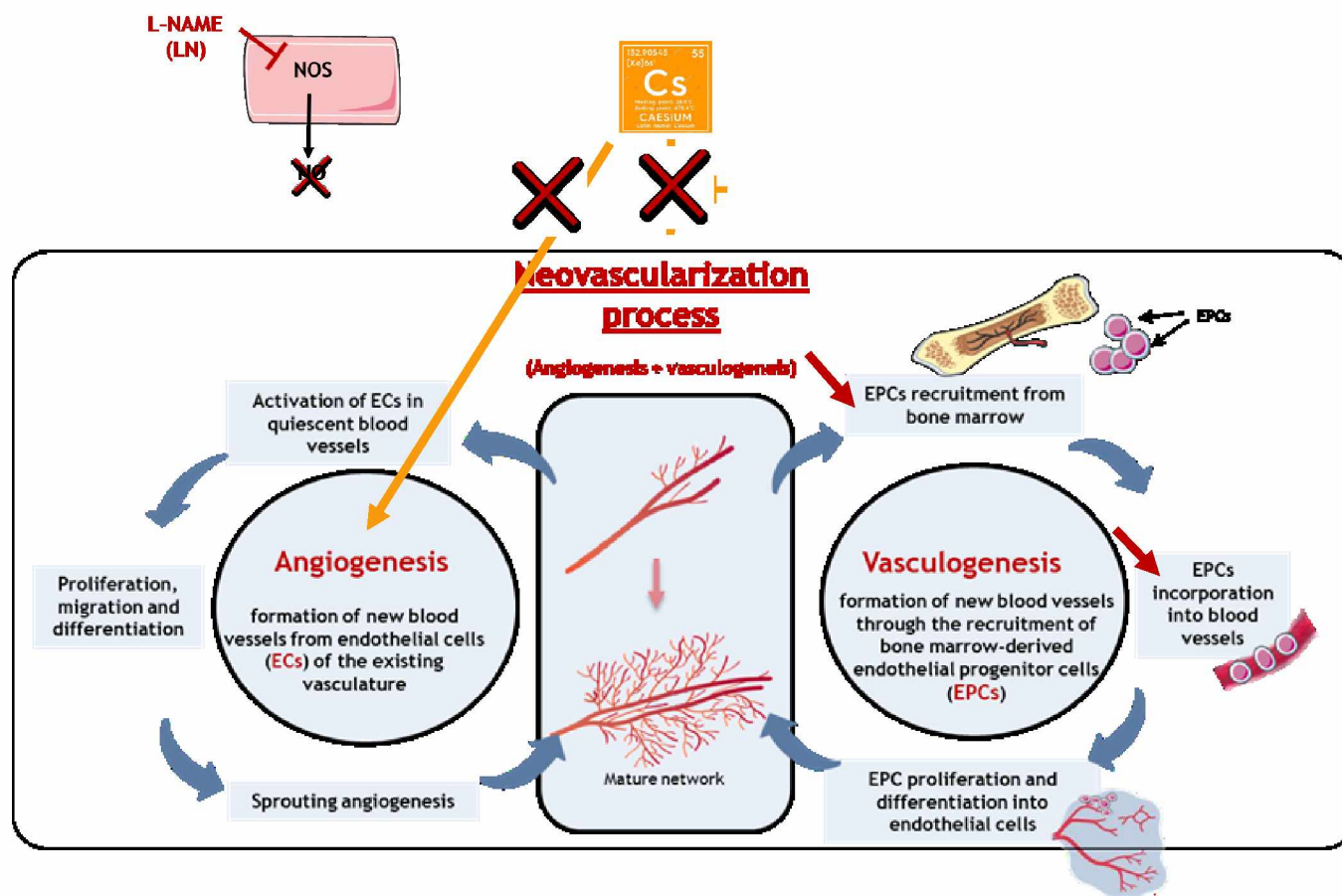
Cs100 kBq/L

6 months

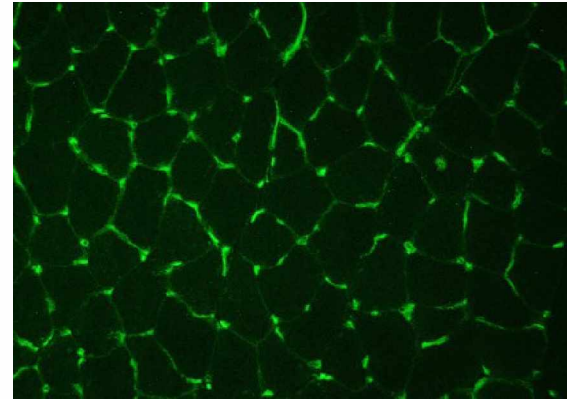
- ➔ Stimulation of post-ischemic neovascularization
- ➔ Enhancement of sprouting angiogenesis

} Implication of the NO pathway

Effects on vasculogenesis via the NO pathway ?



→ Evaluation of capillary density of hindlimb skeletal muscles (immunohistochemistry)

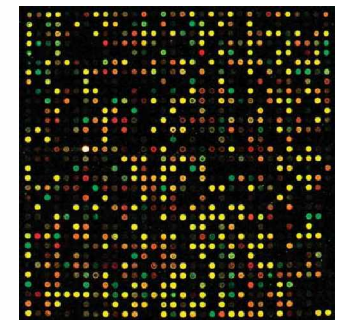
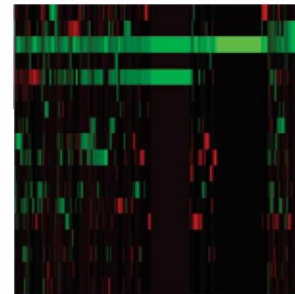


Immunostaining of isolectin on skeletal muscle sections

→ Analyse of molecular expression pattern of principal factors implicated in neovascularization process



Gene expression array



Proteom array

Acknowledgments

LRTOX laboratory

CHRONEO Team

Ebrahimian Teni G

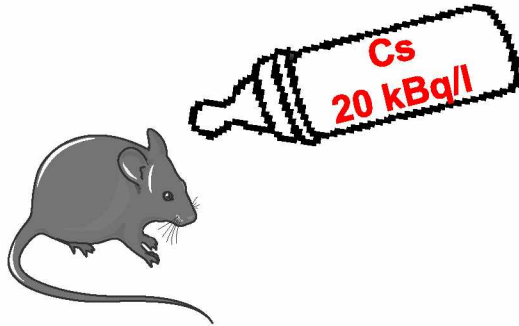
Kereselidze Dimitri

Magneron Victor

Beugnies Louison

Sache Amandine

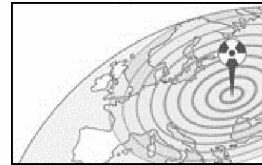
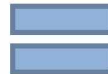




Daily chronic intake :

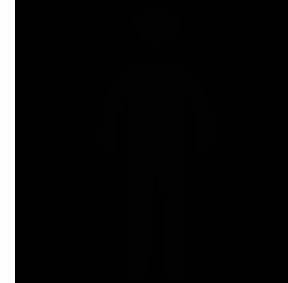
75-90 Bq/day/animal
3.2 kBq/kg/day

Bertho et al., 2010
Synhaeve et al., 2011
Le Gallic et al., 2015



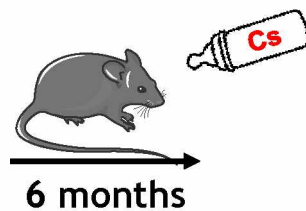
Chernobyl incident

12 to 15 years following
Chernobyl incident, in
zone II (near Narodici,
75km west Chernobyl)



Daily chronic intake of
populations living in
countries contaminated by
the Chernobyl accident

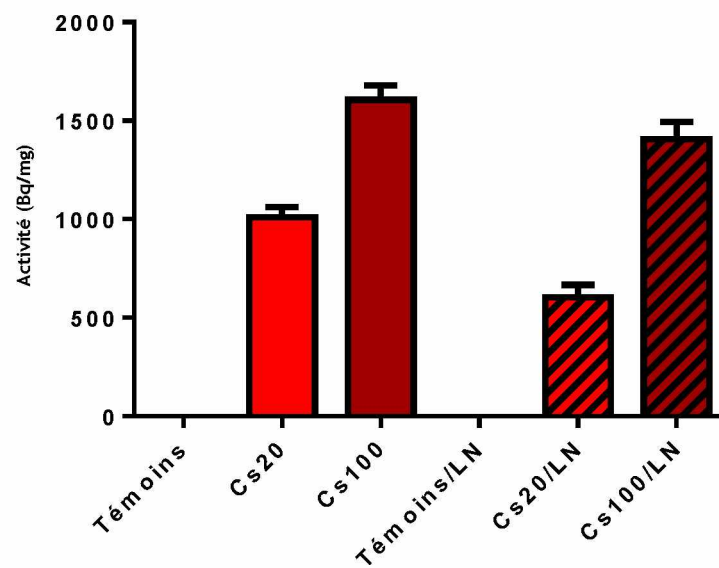
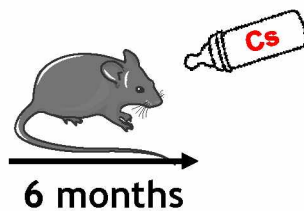
Handl et al., 2003



Cs concentration	Cumulative absorbed dose
20 kBq/L	15 mGy
100 kBq/L	75 mGy

Dose rate : 6 μ Gy/h

In vivo zooradiometric measurement



Energie (Kev): 661,66

Arterial pressure measurement

