IRSN
INSTITUT
DE RADIOPROTECTION
ET DE SÛRETÉ NUCLÉAIRE

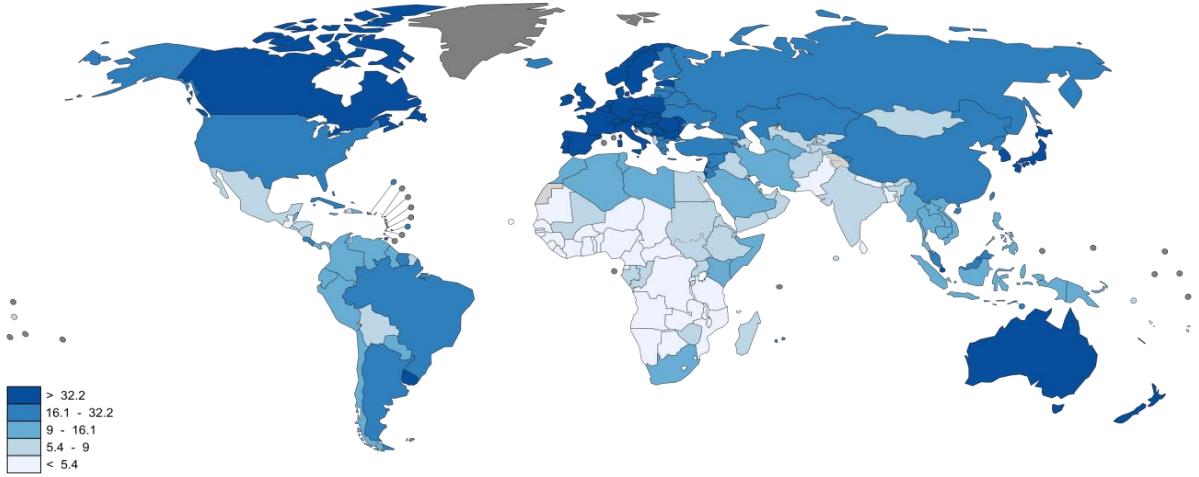
IRSN … enhancing nuclear safety

Bowel Radiation Injury :
Complexity of the Pathophysiology and promise of Cell and Tissue Engineering

Noëlle MATHIEU,

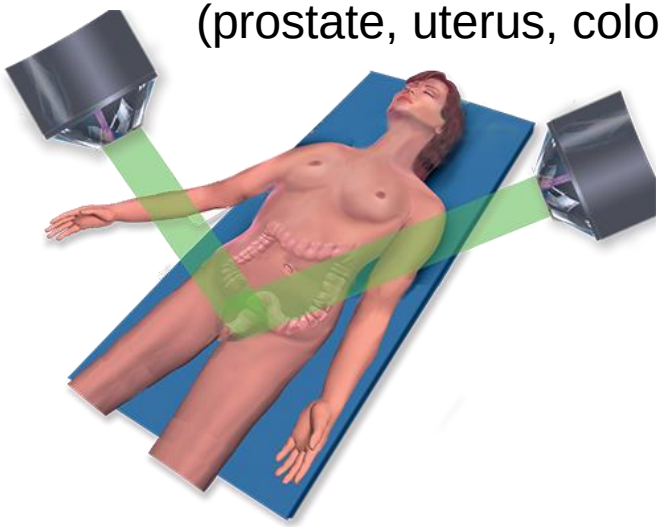
8th European Immunology Conference, Madrid, June 2017

In World: In 2012, **colorectal cancer** was the **4th** leading **cause of death by cancer**



Abdomino-pelvic cancers are treated by **radiotherapy**

(prostate, uterus, colon/rectum...)



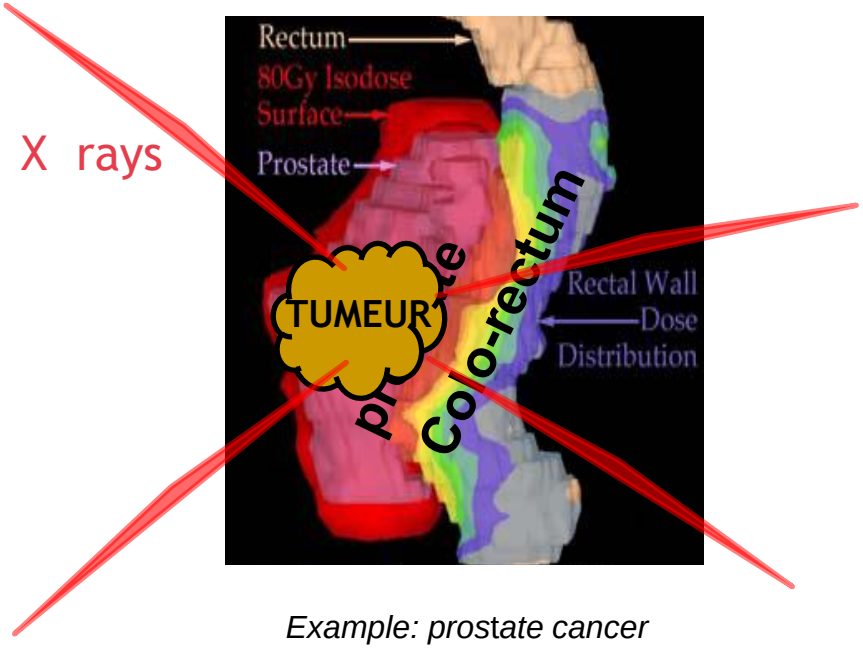
Abdomino-pelvic radiotherapy

RISK

Exposure of healthy tissues surrounding the tumor to ionising irradiation

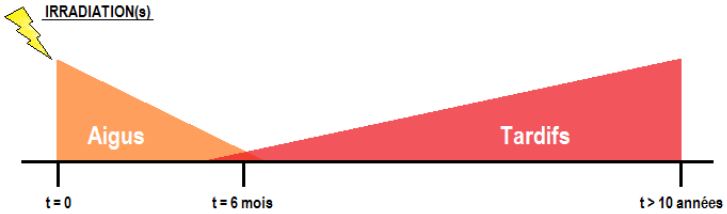
BENEFIT

Control of tumor



Example: prostate cancer

Abdomino-pelvic radiotherapy



Early complications
90 % of patients

Late complications
10% of patients 10 years after treatment

- Symptoms : 5 to 10% of patients
  - Chronic pain
  - Abdominal cramps
  - Diarrheas / Constipations
  - Bleeding
- Causes
  - Chronic Inflammation
  - Severe epithelium damages
  - Fibrosis > Stenosis/Occlusions
  - Atherosclerosis > Ischemia
  - Infections

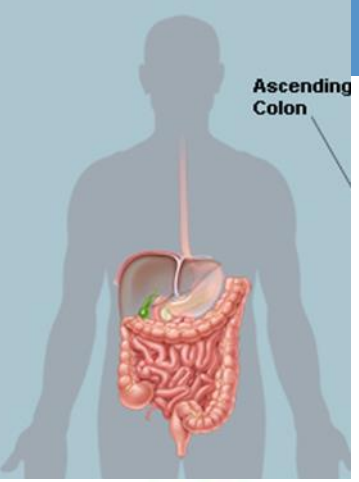
New disease

« Pelvic radiation disease »

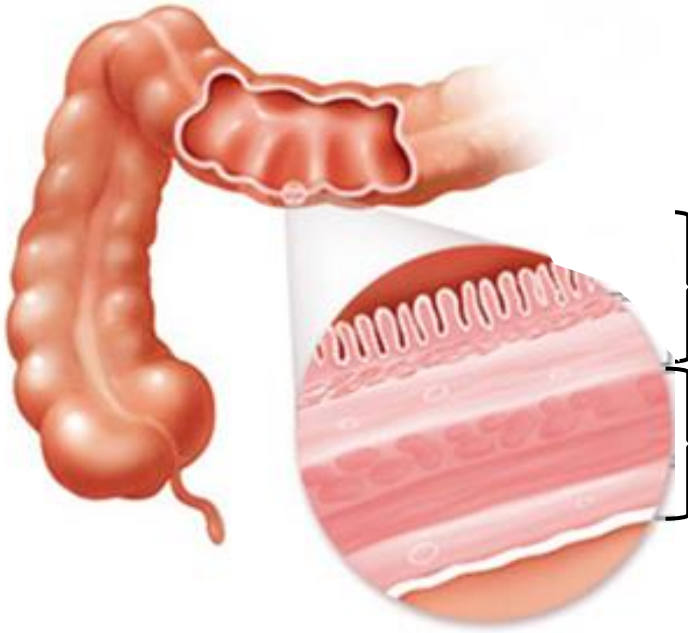
(Andreyev HJ, 2010)

Symptomes	Available Symptomatic Treatment
Abdominal Pain	Analgesic / muscle relaxers
Diarrhea	Anti-diarrhea / Antibiotic
Bleeding	Anti-inflammatory / Formaldehyd/ Formalin chemical cauterization/ surgery
Obstruction	Surgery resection

No curative treatment



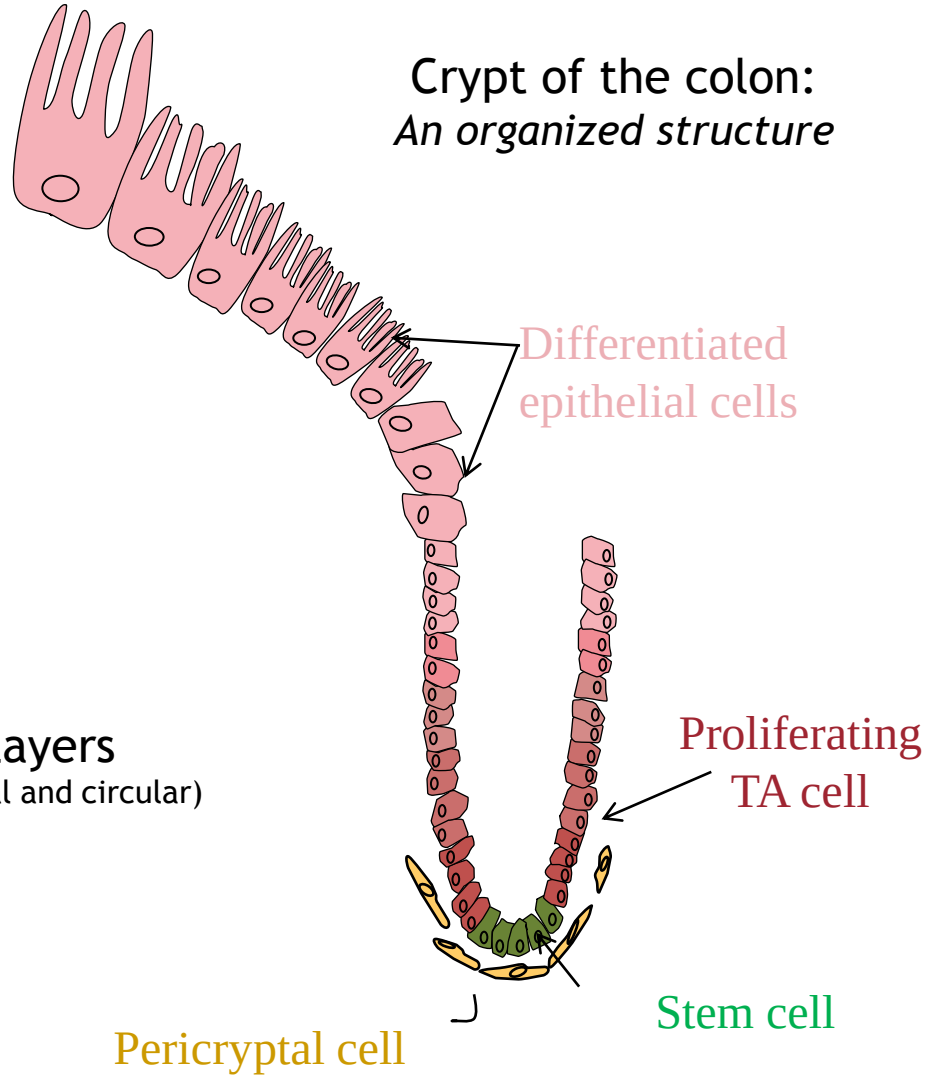
The colon



Mucosa

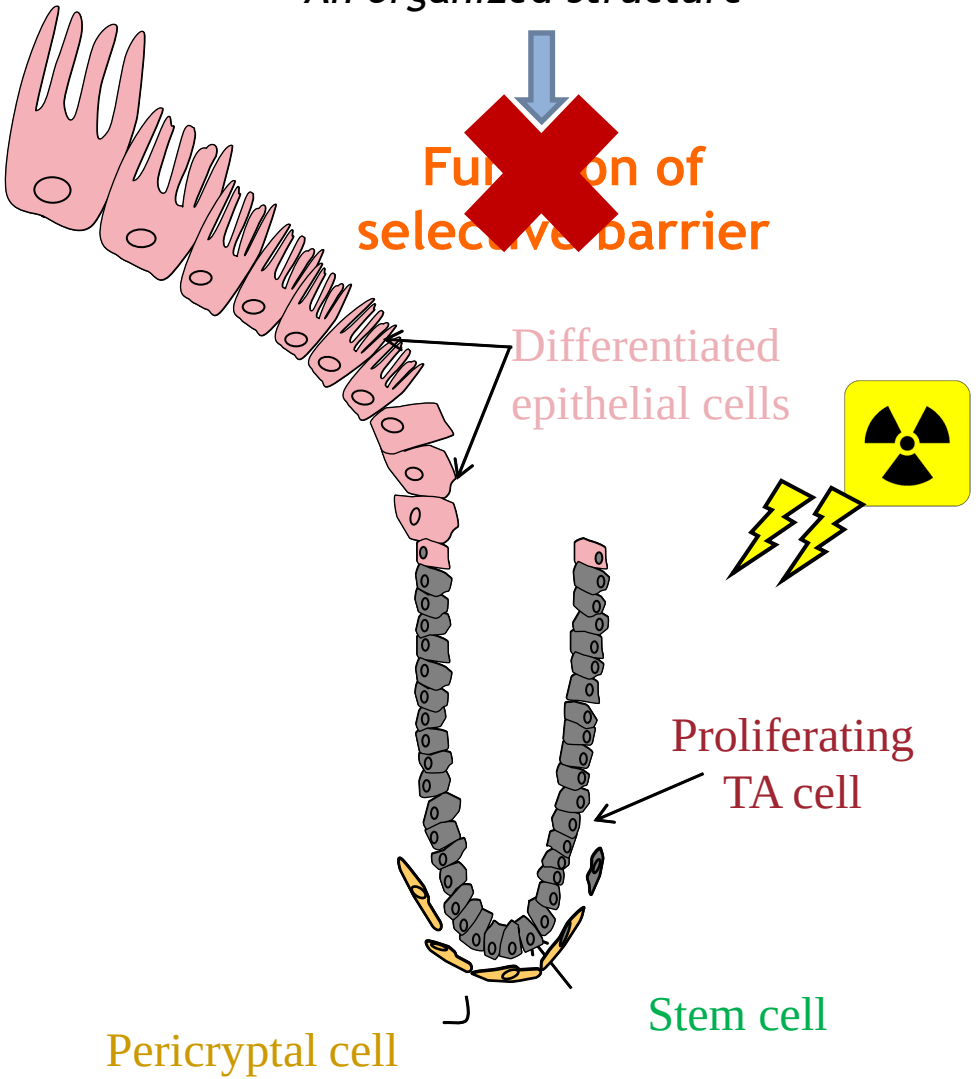
Muscle layers
(longitudinal and circular)

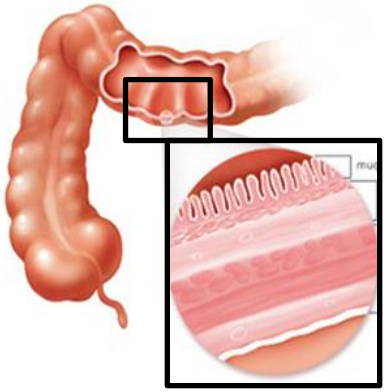
Crypt of the colon:
An organized structure



Crypt of the colon:

An organized structure





Radiosensitive

+++

Healthy colonic tissue

Cell death

Pro – inflammatory molecules

Epithelium

Vascular

Permeability increase

Immune system activation

Ulceration

Fibrosis

Mucosa

Muscularis mucosa

Submucosa

Muscularis propia

Bleeding, diarrhea, incontinence, chronic pain, occlusion (lethal)

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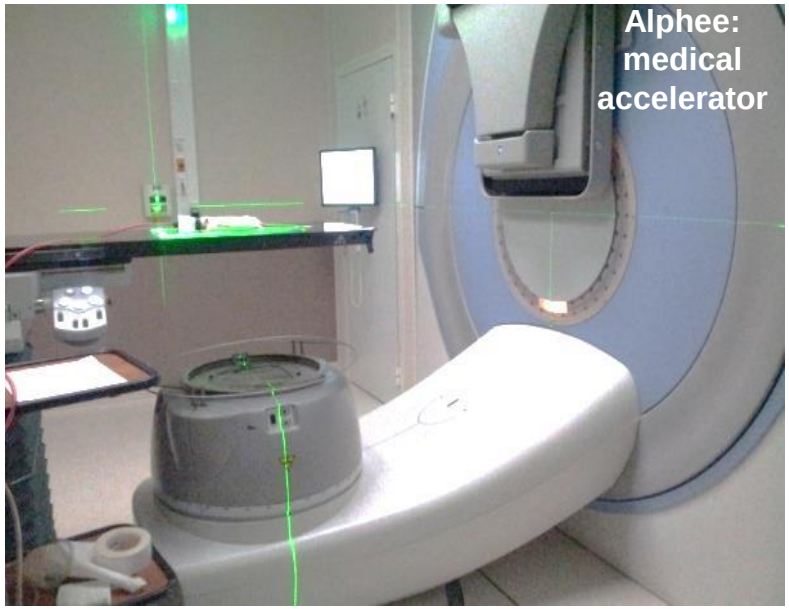
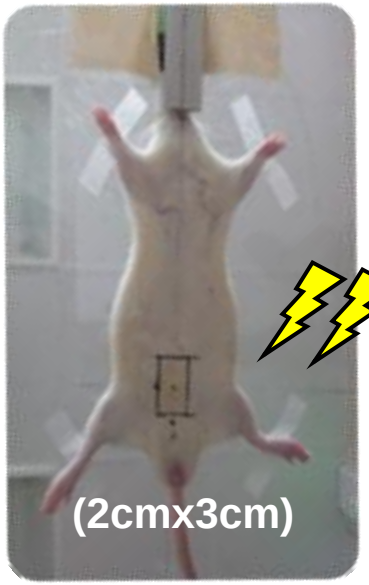
Animal model of late side effects of radiotherapy

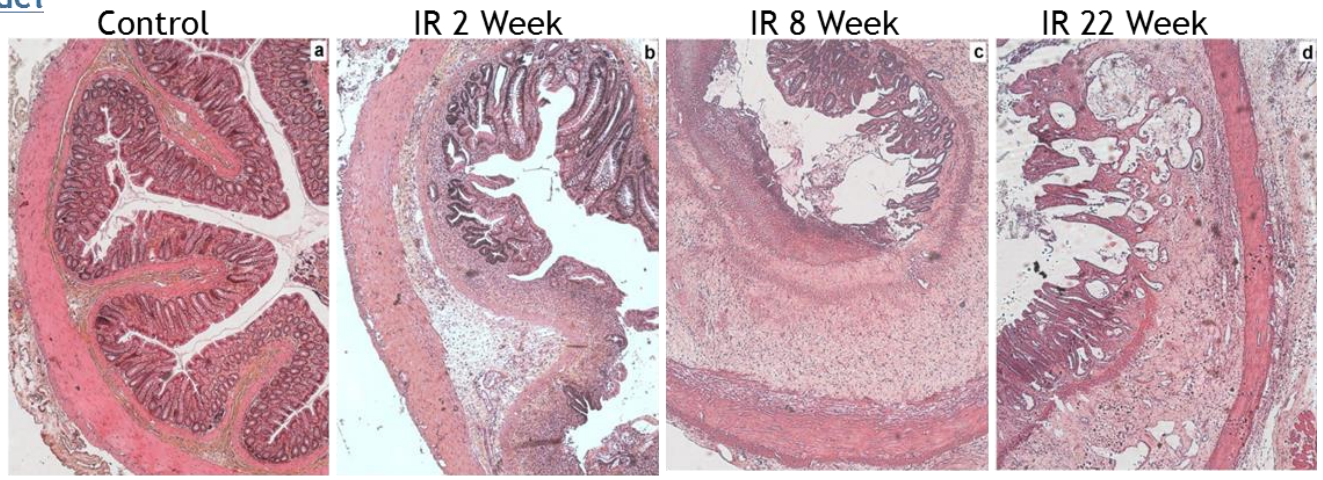


RAT

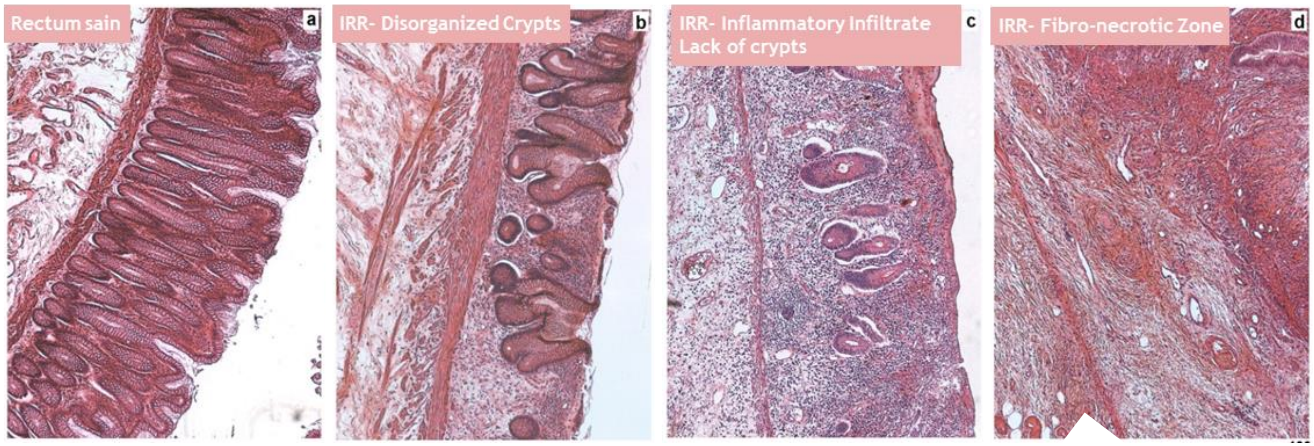


Localized irradiation 29Gy Colorectum

Rat model



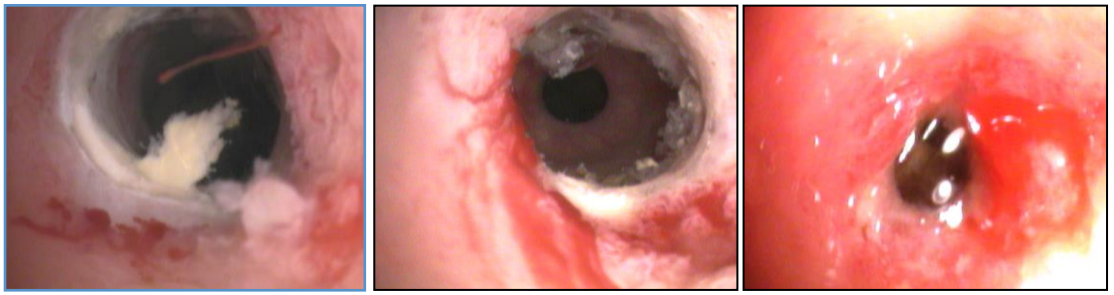
Patients



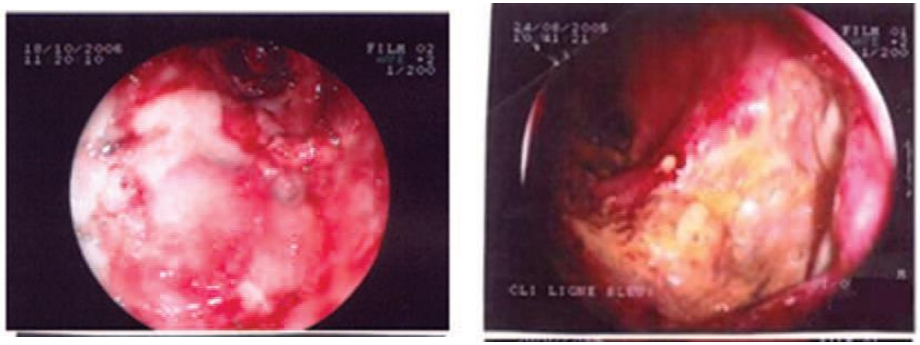
Histologic damages similar to those observed in patients

Endoscopy

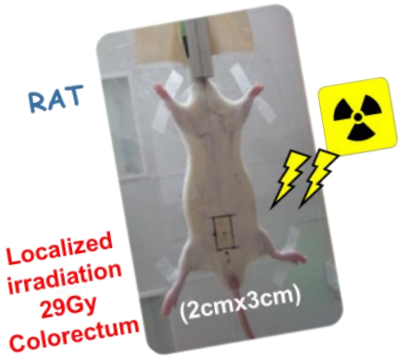
Rat



Human



Bleeding are observed similar to patients



Animal model to

Characterize radio induced damages
Investigate innovative treatment

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Characterization of Radio-induced damages

29Gy
Colorectal
irradiation

4 weeks
8 weeks
1 3 7 14 21 28 56
Days of taking after irradiation
Days

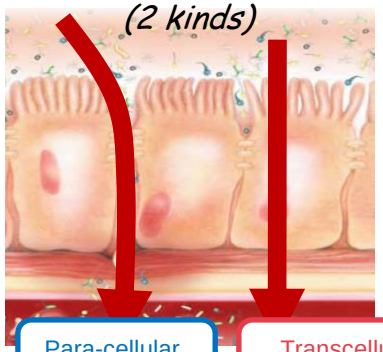
Mucosal damages

Structure

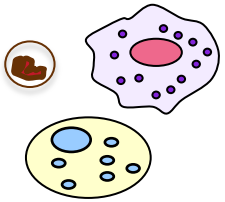
Histology

Function of barrier

Permeability
(2 kinds)



Inflammatory Process



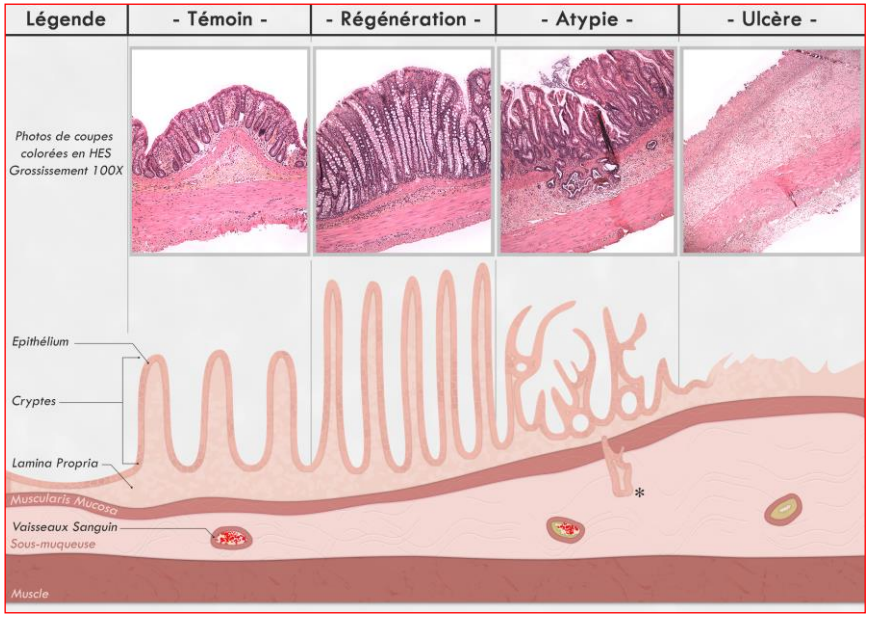
Innate cells

Adaptative cells

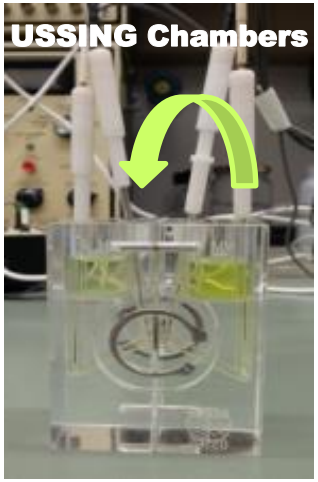
Inflammatory molecules

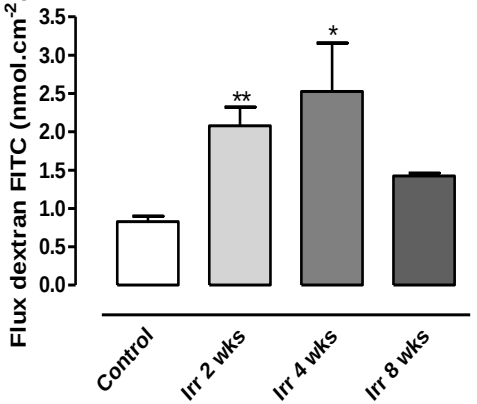
IRSN

Structure



Function of barrier





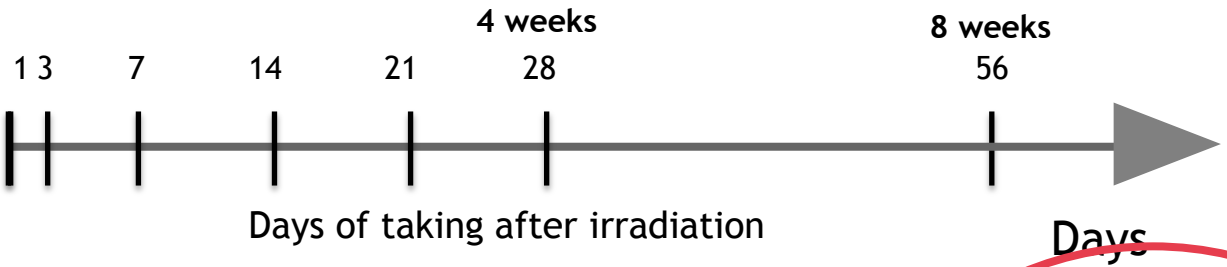
Summary on characterization of mucosal damages after irradiation

- The size of the ulcer is maximal **2 weeks** after irradiation
  Then, regenerative process takes place

- The severity of the damages increased involving all the compartment of the colon (mucosa, vessels and muscle layers)

Characterization of Radio-induced damages

29Gy
Colorectal
irradiation



Mucosal damages

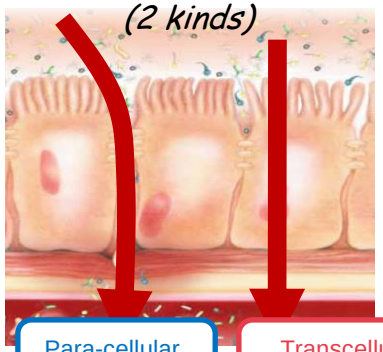
Inflammatory Process

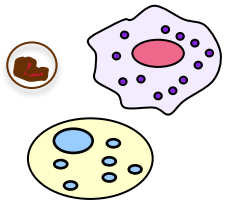
Structure

Histology

Function of barrier

Permeability
(2 kinds)





Innate cells

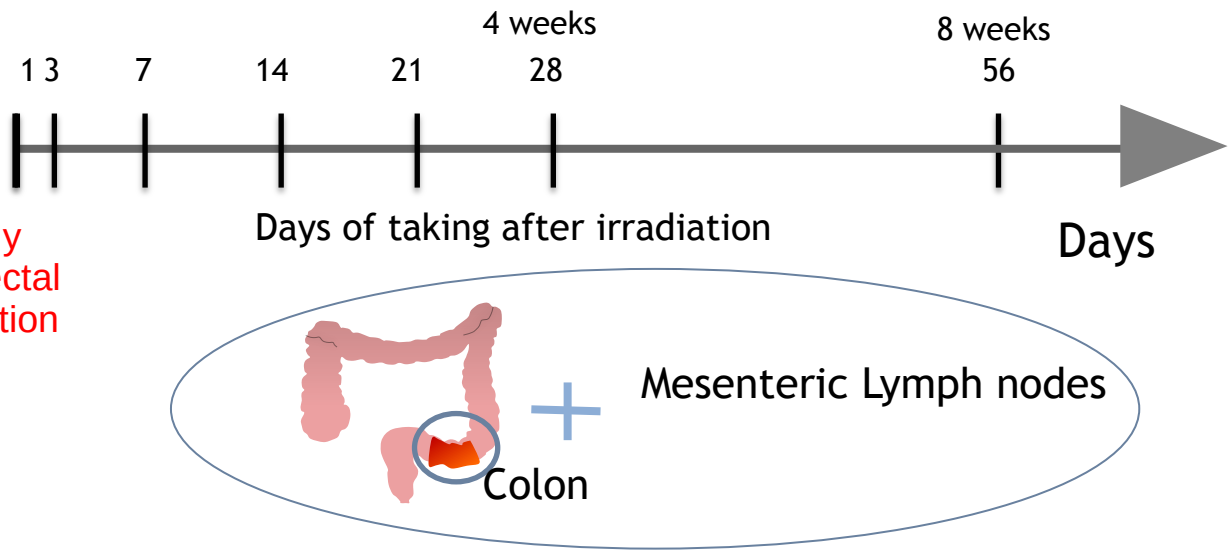
Adaptative cells

Inflammatory molecules

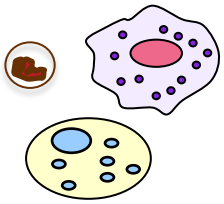
IRSN

Importance of inflammatory infiltrate after colorectal irradiation

Inflammatory Process



Quantity



Innate cells

Adaptative cells

Activation

Cytokine secretion

Proliferative capacity (BrDU assay)

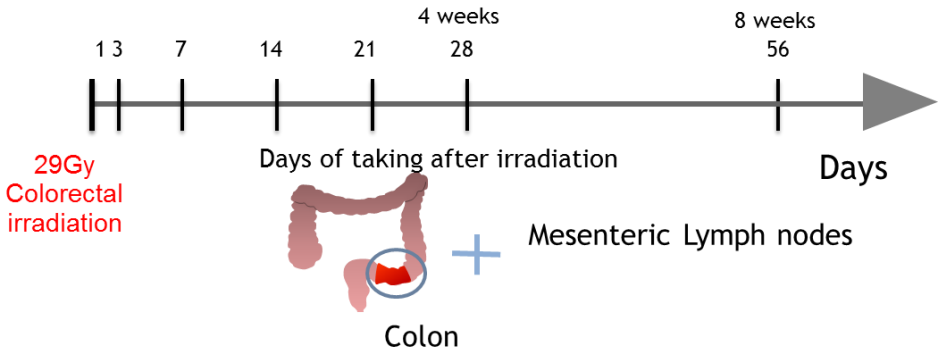
Adoptive transfert experiment (in Nude Rats)

Depletion

Is T cells depletion influence radio-induced damages ?

Importance of inflammatory infiltrate after colorectal irradiation

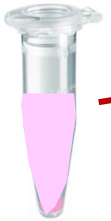
Inflammatory Process



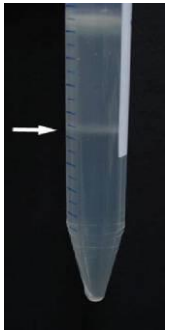
Flow cytometry analysis (colon)

Quantity

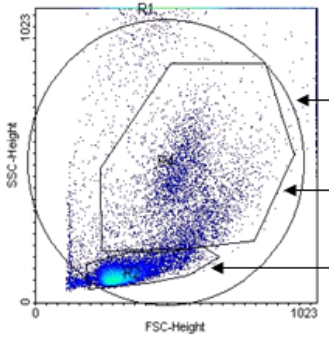
Mechanical and enzymatic digestion



Percoll purification



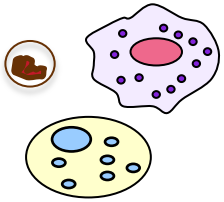
After



Population prise en compte

Monocytes et Macrophages

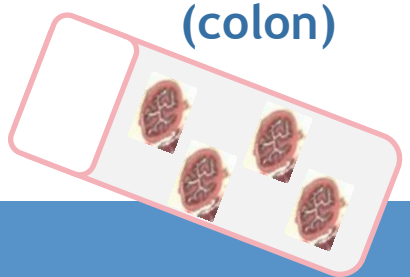
Lymphocytes et NK



Innate cells

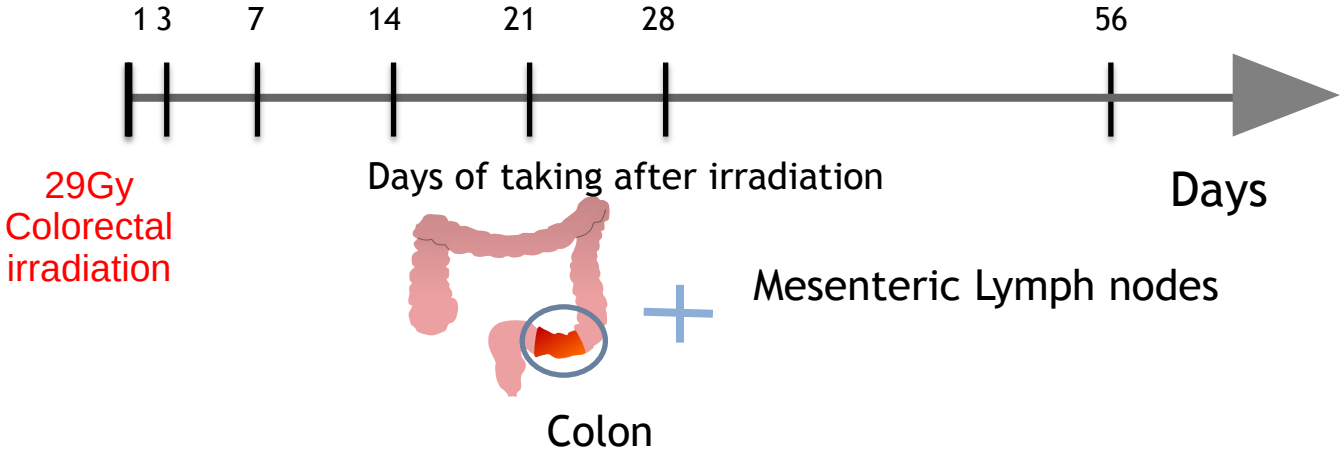
Adaptative cells

Immuno-histochemistry analysis (colon)

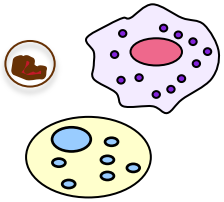


Importance of inflammatory infiltrate after colorectal irradiation

Inflammatory Process



Quantity



Innate cells

Adaptative cells

Activation

Cytokine secretion

Proliferative capacity (BrDU assay)

Adoptive transfert experiment (in Nude Rats)

Depletion

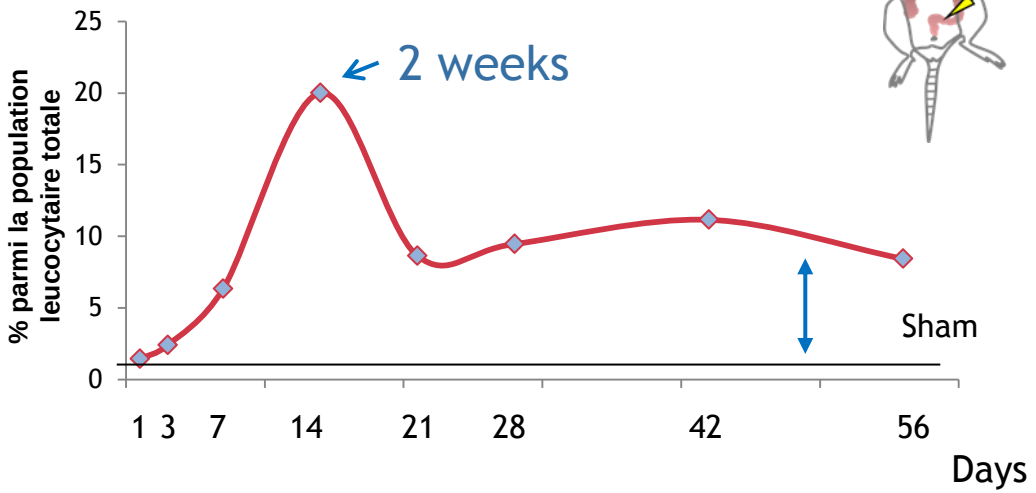
Is T cells depletion influence radio-induced damages ?

Inflammatory Process

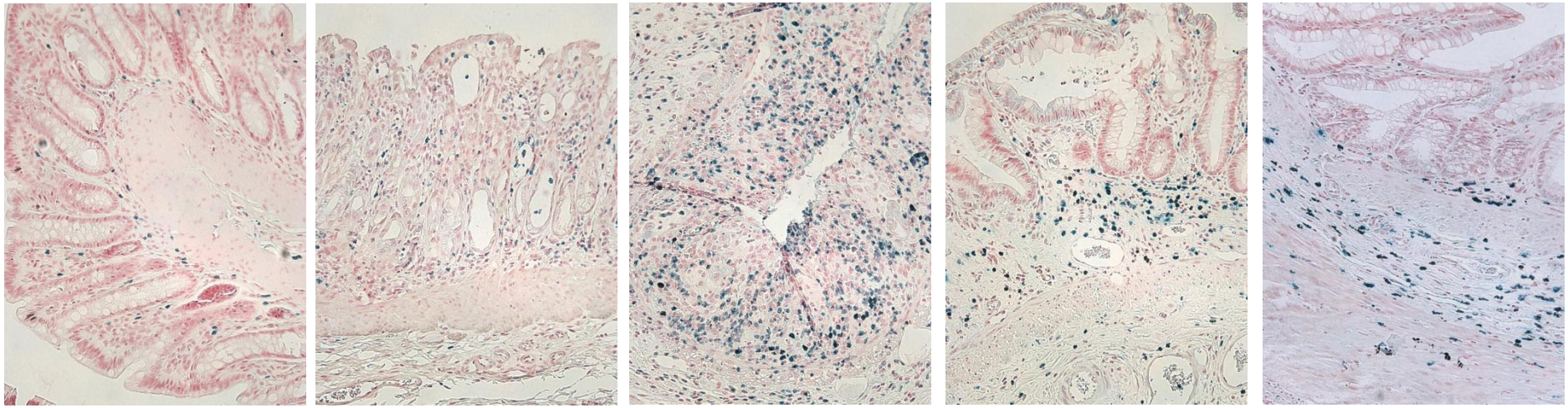
Innate immune cells

Neutrophils

Flow cytometry (colonic mucosa)



Control D7 D14 D28 D56



MyeloPerOxidase Immunostaining

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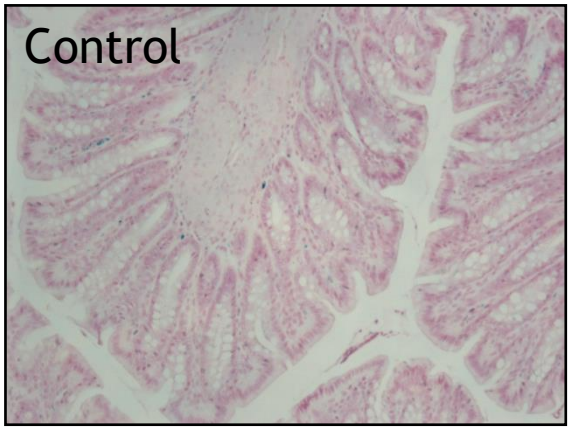
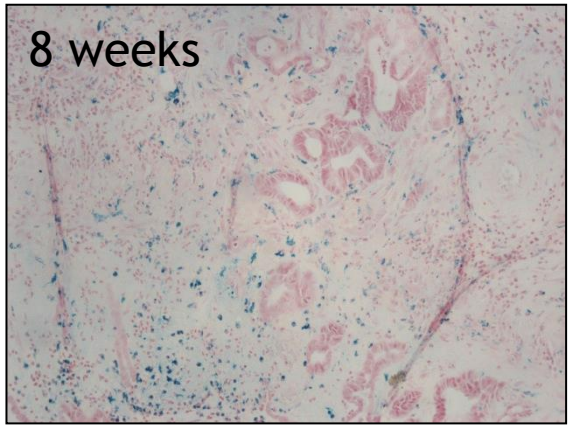
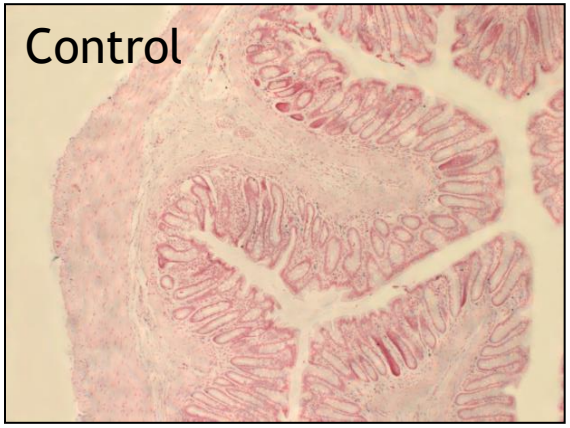
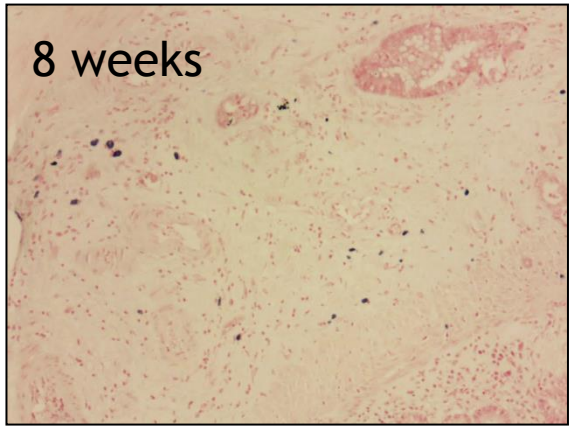
Macrophages





Mastocytes





Inflammatory molecules

IL1b

MIP2

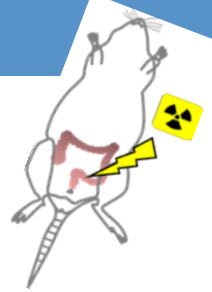
IL8

TGFb

Inflammatory Process

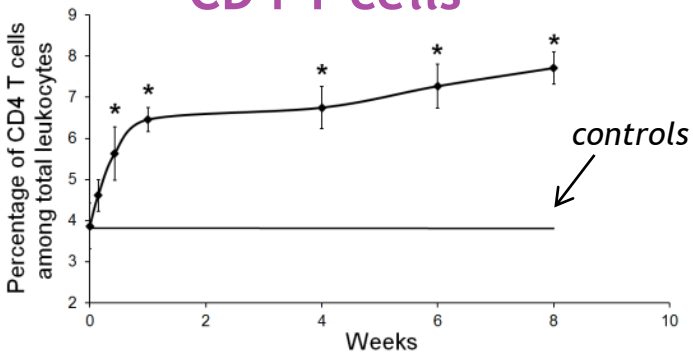
Adaptative immune cells

T cells

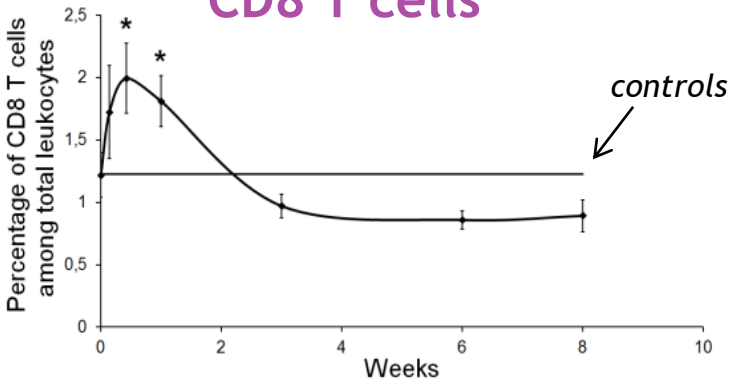


Quantity

CD4 T cells



CD8 T cells



Inflammatory Process

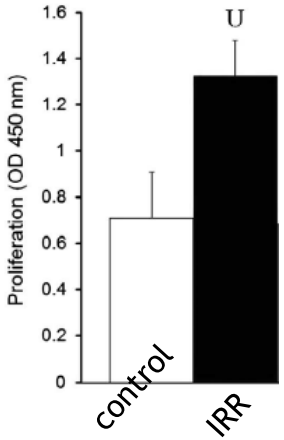
Activation

Adaptative immune cells

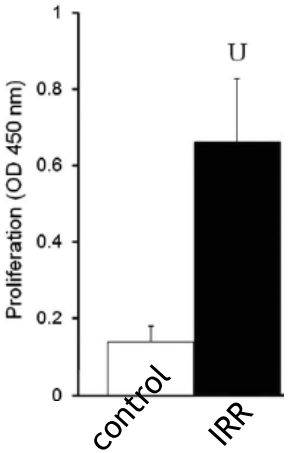
T cells

Analyses of proliferation abilities
on purified T cell population (4 weeks)

CD4



CD8



Analyses of activation markers
on purified T cell population

IL2R CD69

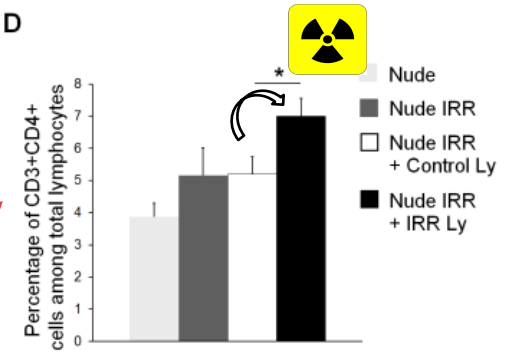
Analyses of T cell colonization
into MLN of NUDE rats

Taking of Mesenteric Lymph Nodes (4 weeks)

Adoptive transfert *in*

NUDE rats

D



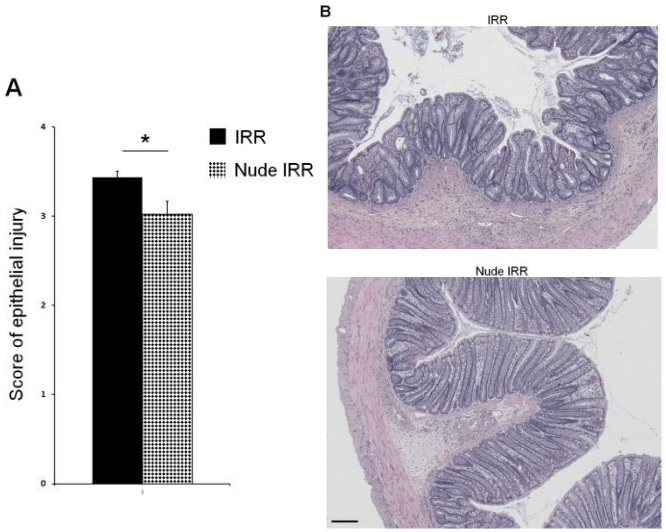
Depletion

Adaptative immune cells

T cells influence radio-induced damages ?

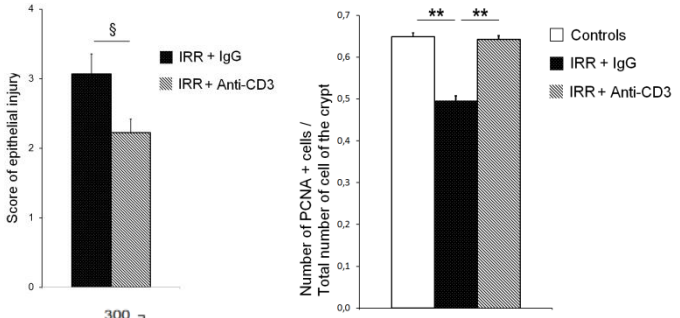
Use of depleted T cells animals models

Nude Rats

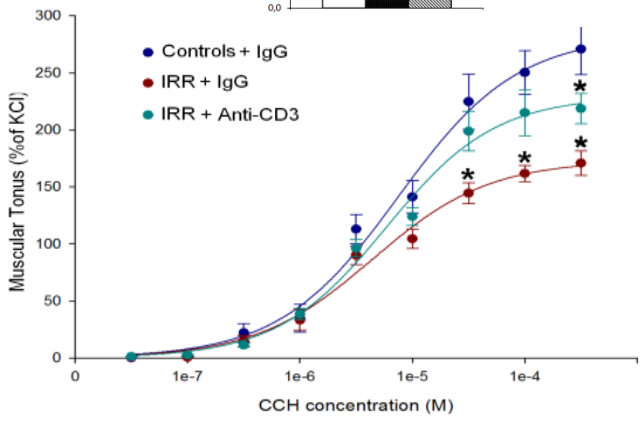


CD3 Blocking antibody

Histology



Function of muscle contractibility



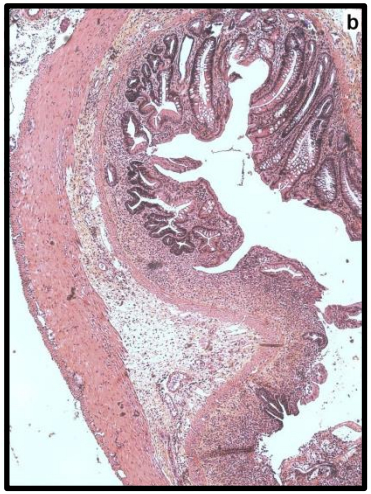
IRSN

Characterization of Radio-induced damages

Endoscopy

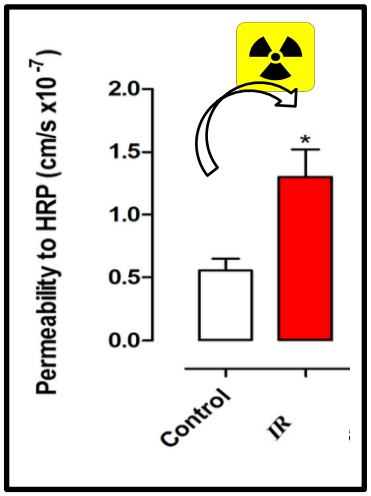
Haemorraghe observed by colonoscopy

Structure



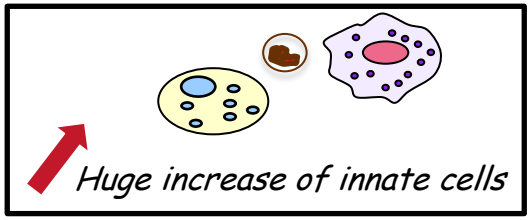
Structural damages : Epithelium/muscle

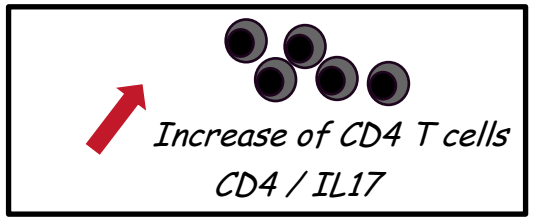
Function of barrier



Increase of mucosal permeability

Inflammatory Process



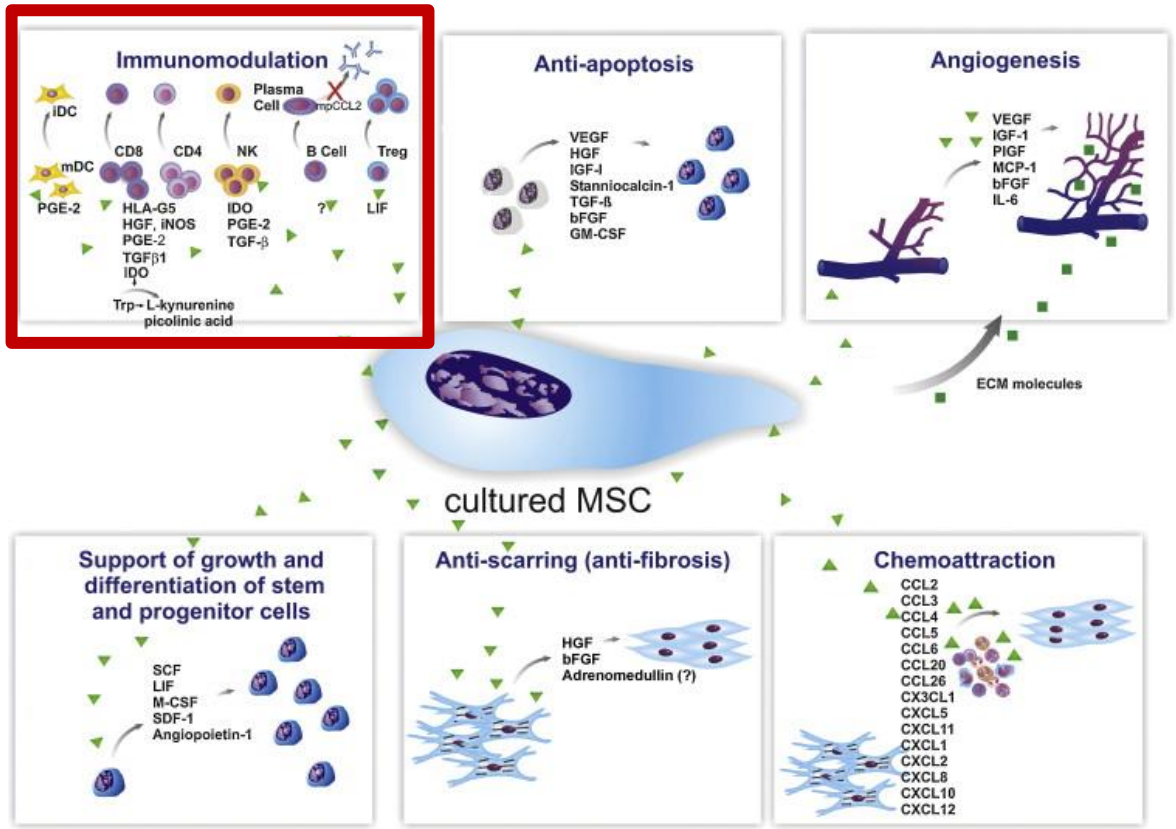


Depletion of T cells improve structure and function of irradiated colon

➡ ***Treatment that modulate inflammation could reverse radio-induced damages ?***

Cell therapy using Mesenchymal Stromal Cells

The secretion of a broad range of bioactive molecules
is now believed to be the main mechanism by which MSCs achieve their therapeutic effect

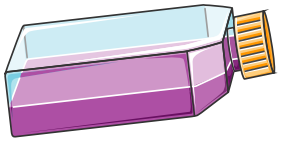


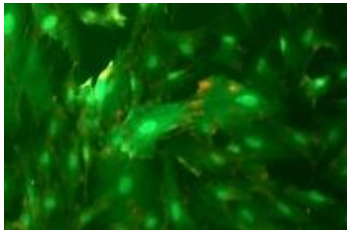
From Meirelles *et al*, 2009

➡ Use in clinical trials in various pathologies (Phase III in Crohn's disease)

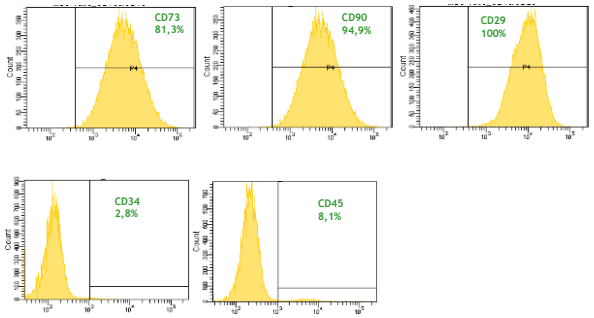
Protocols of treatment

MSC amplification

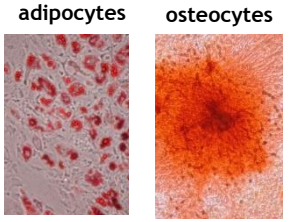


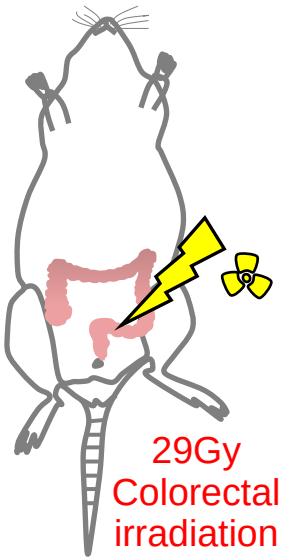


Flow cytometry characterization

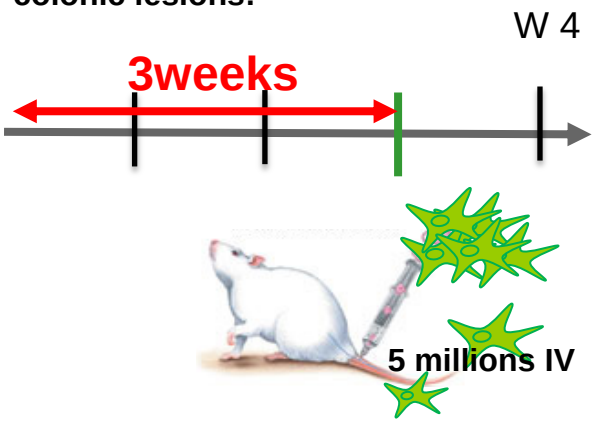


Differenciation potential





Establishment of radio-induced colonic lesions:

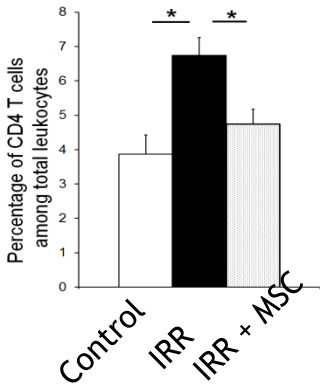


MSC treatment reduces radio-induced inflammation

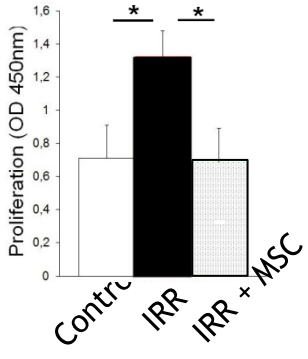
Anti-inflammatory action of MSC

➡ CD4 T cells

Quantity

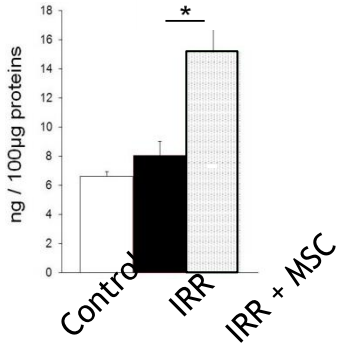


Activation
(Proliferation; BrDU)



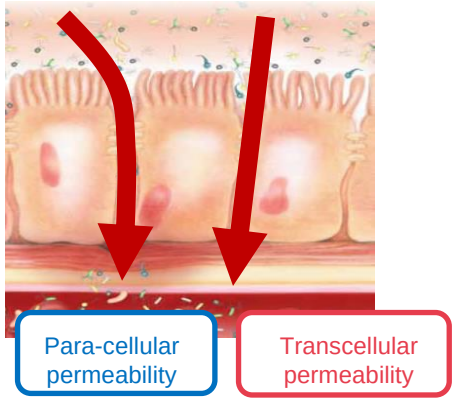
➡ Anti-inflammatory molecule

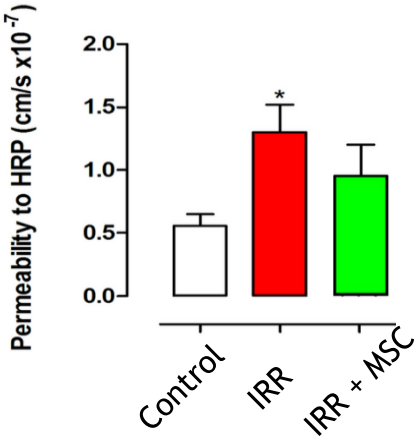
Corticosterone
(ELISA on colonic tissue)



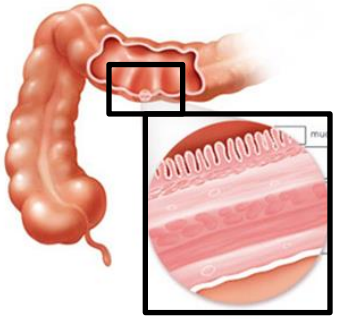
➡ Barrier function

Permeability is increased in inflammatory conditions



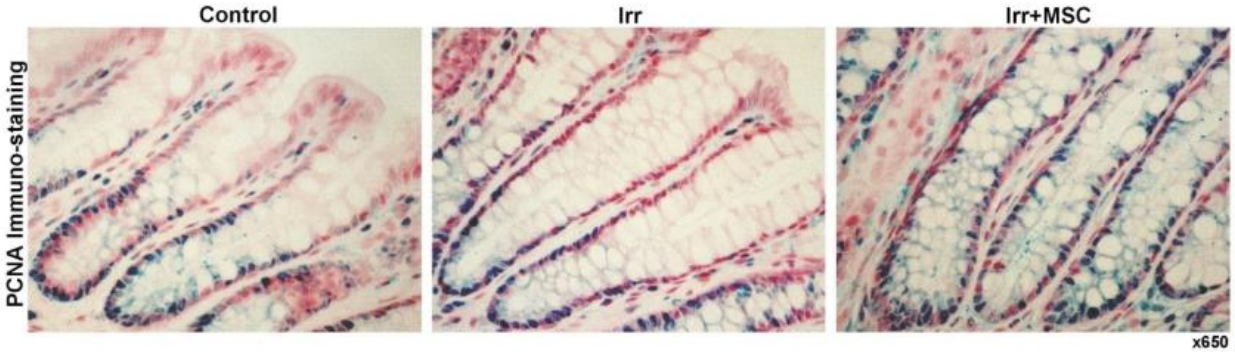


MSC treatment reduces colorectal damages

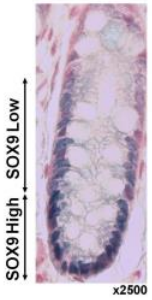


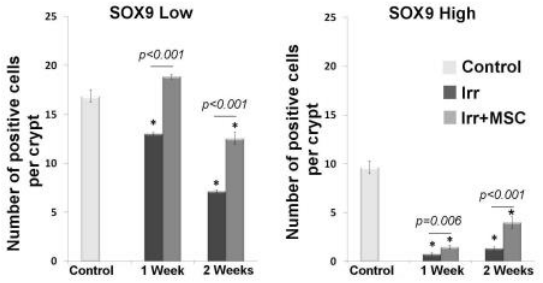
Sémont et al, Plos One 2013

Pro-proliferative action of MSC on epithelial compartment



Effect on Sox9 positive cells





Summary

Cell therapy using MSC reduce radio-induced colonic damages

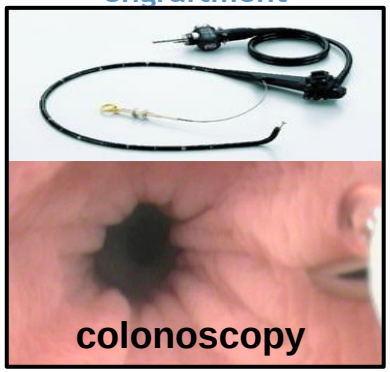
➡ improve epithelial regeneration and reduce inflammation by **paracrine factor secretion**

However

- Healing is not complete
- Repeated injections are necessary (intravenous injections)
- Low cell engraftment
- Benefit is temporary

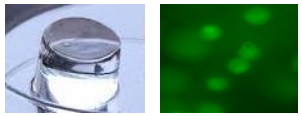
Strategy **to improve the effectiveness of MSC treatment for colonic radio-induced damages**

Local injection of MSC to improve colonic engraftment



Protection of the cells in an hydrogel

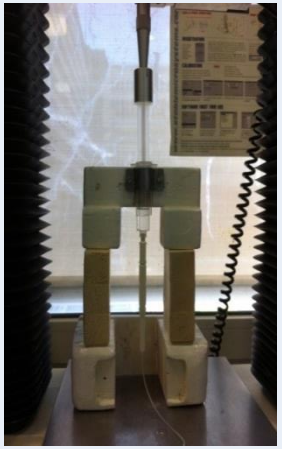
Si-HPMC
(Silanised Hydroxy Propyl Methyl Cellulose)



« ANTHOS »

Experimental procedure

Rheological parameters

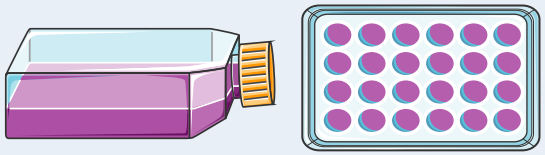


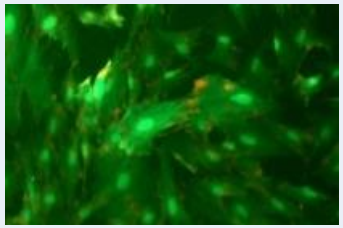
Injectability

Si-HPMC
1% and 1,5%
can be used

Gel point

In vitro

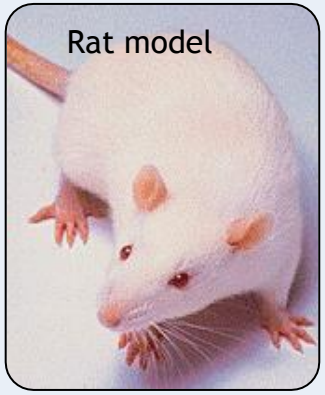




Viability

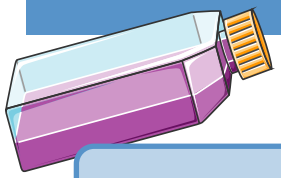
Secretome

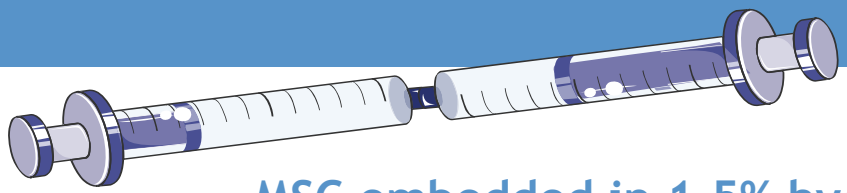
In vivo



Therapeutic benefits

Engraftment



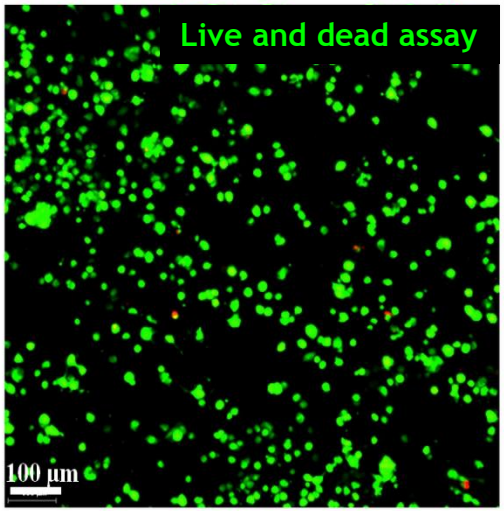


Girish Pattappa

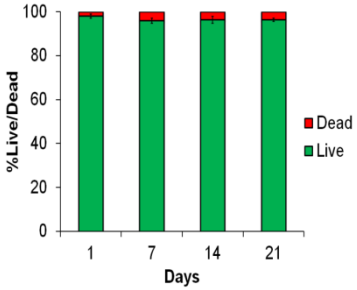
In Vitro

MSC embedded in 1,5% hydrogel are:

➡ Viable

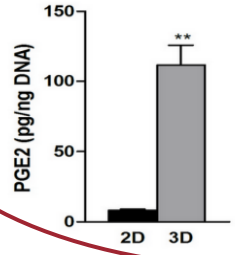


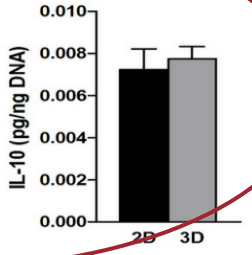
Up to 95% until 21 days



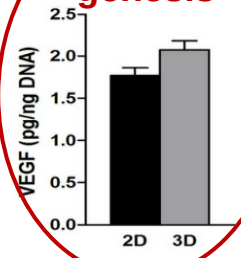
➡ Able to secrete factors

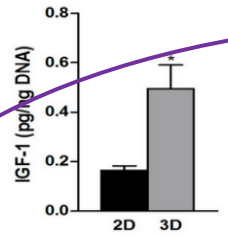
Anti-inflammatory

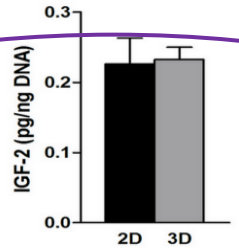


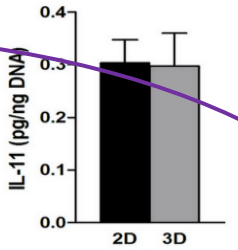


Angio-genesis

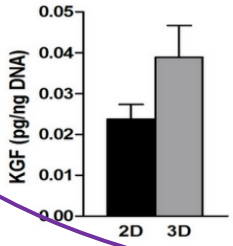


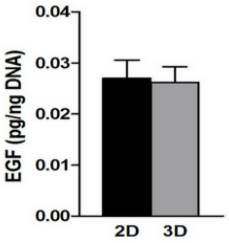


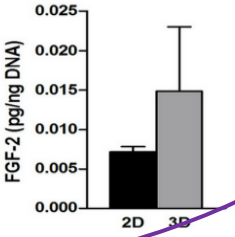




Epithelial regeneration



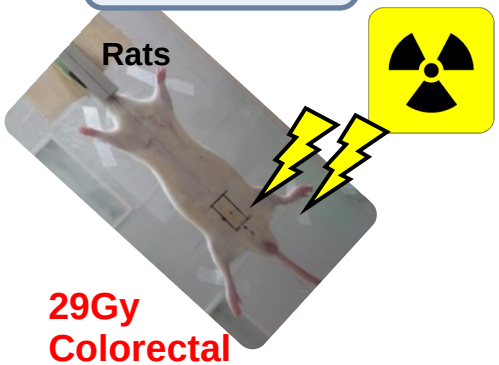




Protocol

Lara Moussa

In Vivo



29Gy Colorectal irradiation

Engraftment of MSC

D7

Time (weeks)

Establishment of radio-induced colonic lesions: 3weeks

IV or Local injection of MSC

+/- Si-HPMC

Analyzes

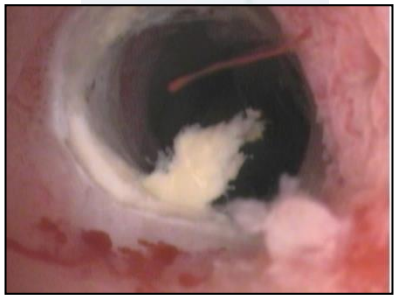
Structural, Functional and Molecular

MSC amplification

Rats SD-GFP

IV injection of 5.10^6 or 1.10^6 of cells

Injected volume 500µl

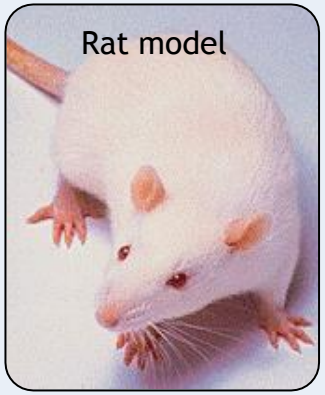


Local injection of 1.10^6 of cells

2 injection points upstream and downstream of the lesion

Injected volume 300µl

In vivo



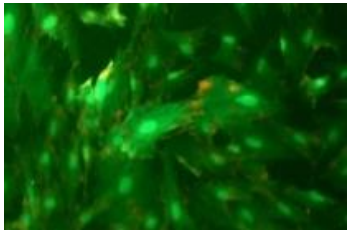
Therapeutic benefits

Engraftment

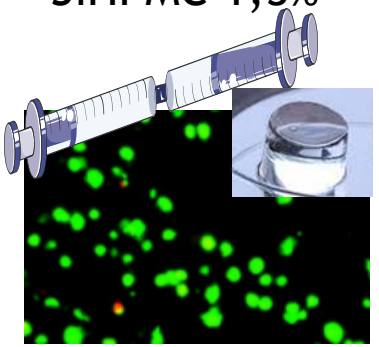
- ✓ In vivo proliferation
- ✓ In vivo apoptosis

Local injection of MSC using colonoscope

+PBS

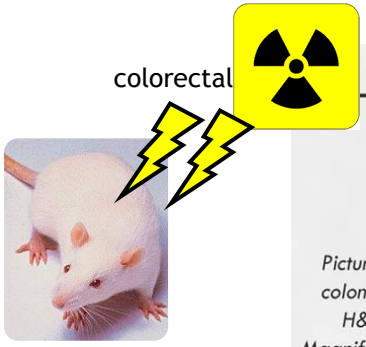


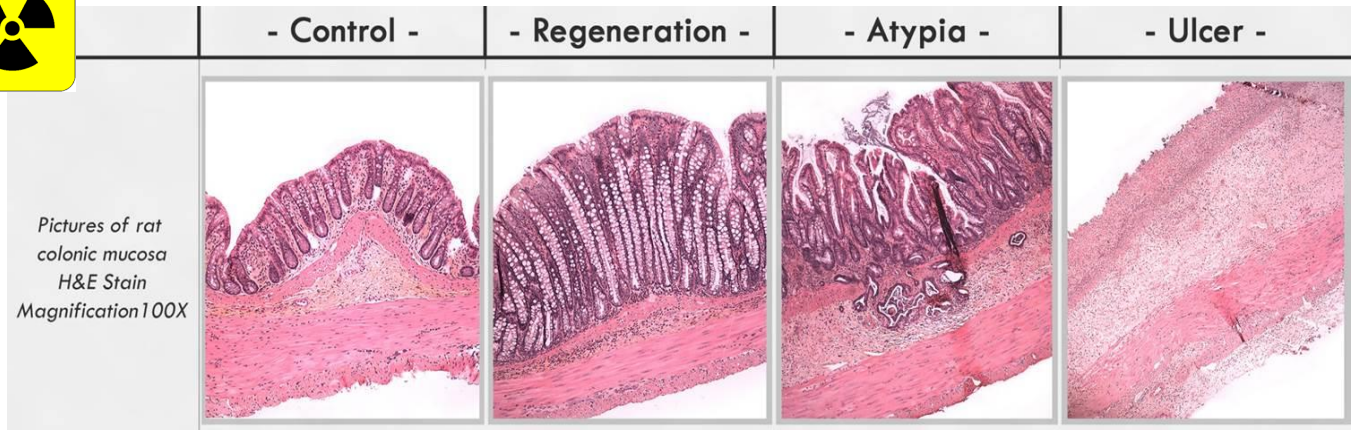
+SiHPMC 1,5%

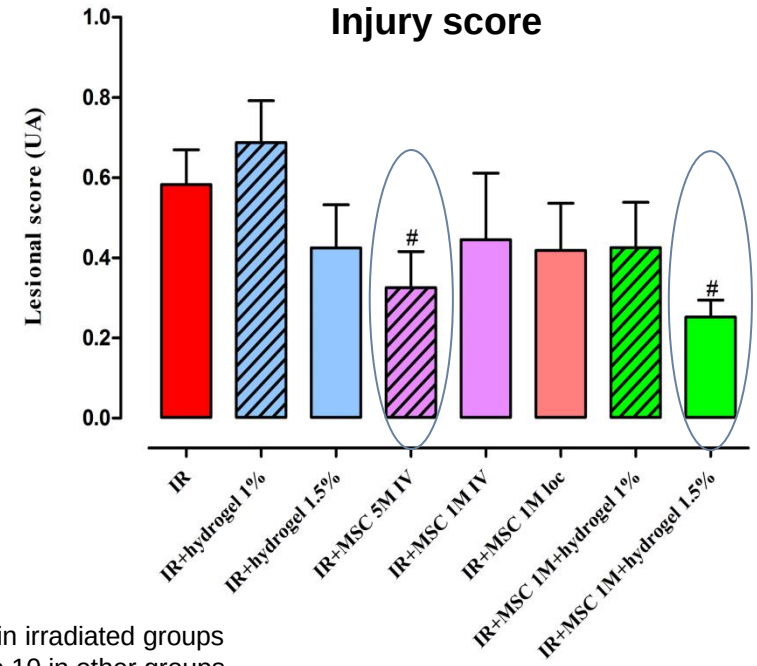


(No MSC in the colon after IV injection 5 Millions in this model)

Effects on injury score







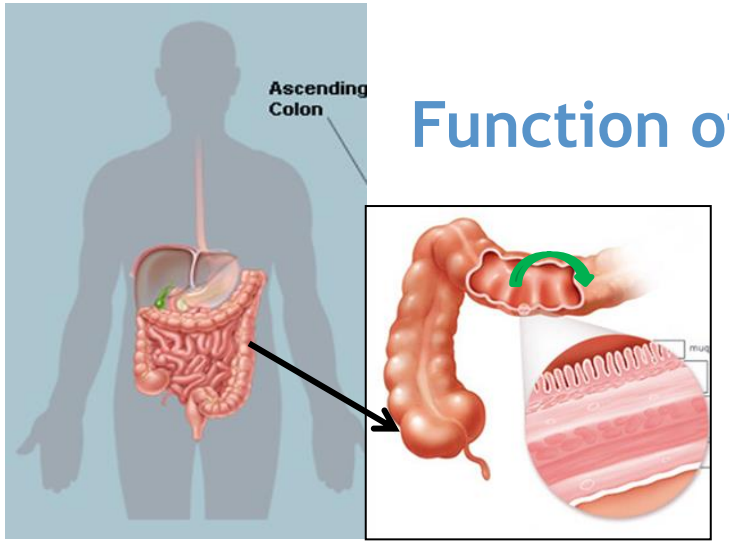
- **IV** injection of **5M MSC**

and

- **local** injection of **1 M of MSC + 1,5% Si-HPMC**

reduce the injury score

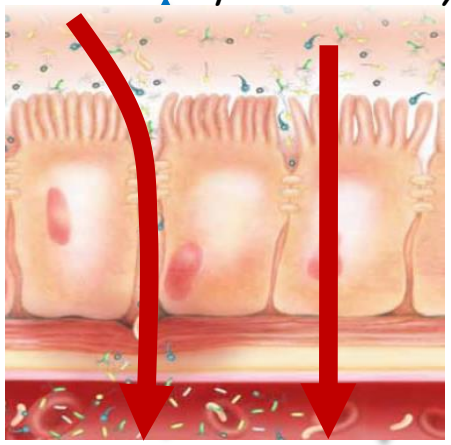
n= 13 in irradiated groups
n= 5 to 10 in other groups
#p<0.05 in comparison to IR group



Function of barrier of colonic epithelium

- selective barrier
- prevents the penetration of luminal contents

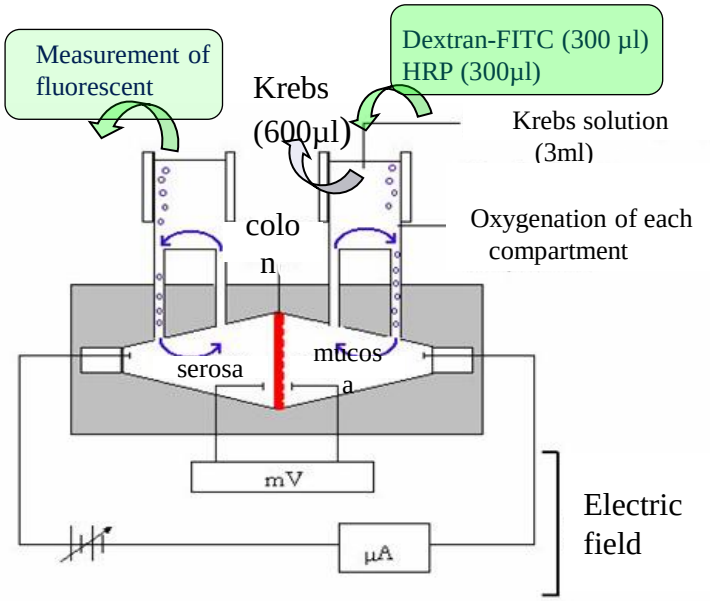
2 kinds of permeability



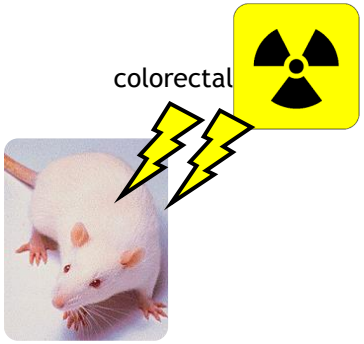
Para-cellular permeability

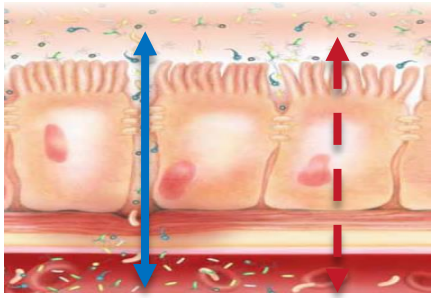
Transcellular permeability

*Measurment with **Ussing Chambers***

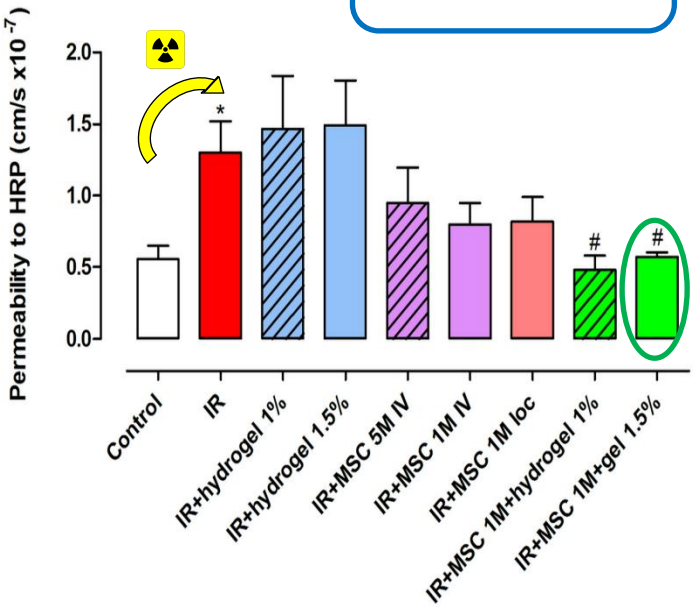


Therapeuric benefit on epithelial barrier

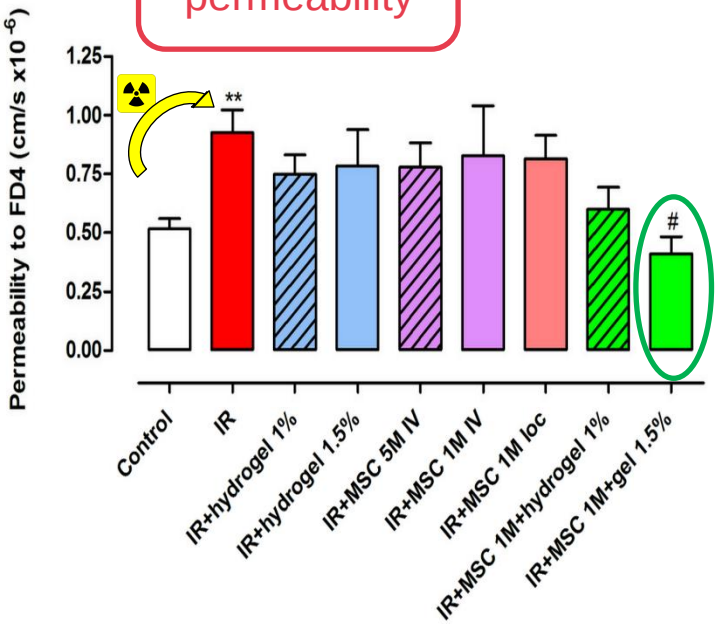




Para-cellular permeability



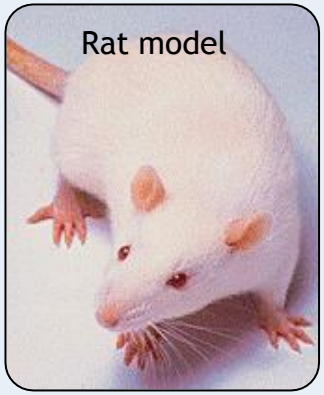
Transcellular permeability



N= 30-38 in controls and irradiated groups
N= 8 to 16 in other groups
**p<0.01 in comparison to controls
#p<0.05 in comparison to IR group

Benefit of
local injection of 1M of **MSC+ 1,5 %hydrogel**

In vivo



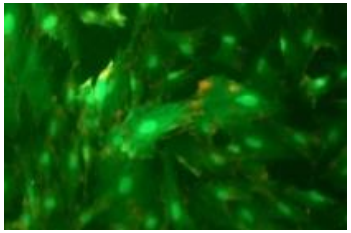
Therapeutic benefits

Engraftment

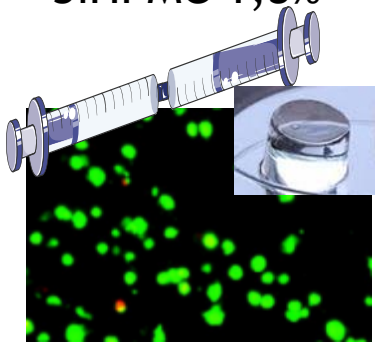
- ✓ **In vivo proliferation**
- ✓ **In vivo apoptosis**

Local injection of MSC using colonoscope

+PBS



+SiHPMC 1,5%

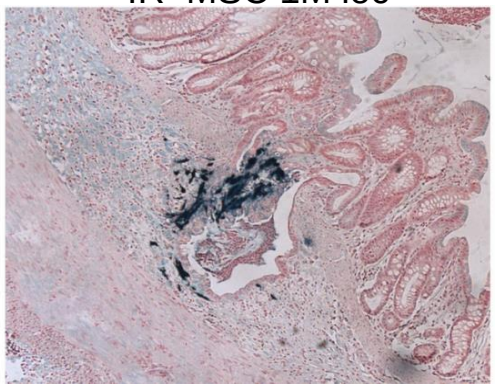


(No MSC in the colon after IV injection 5 Millions in this model)

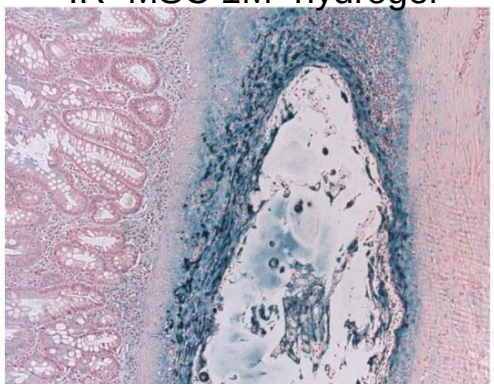
Engraftment of MSC

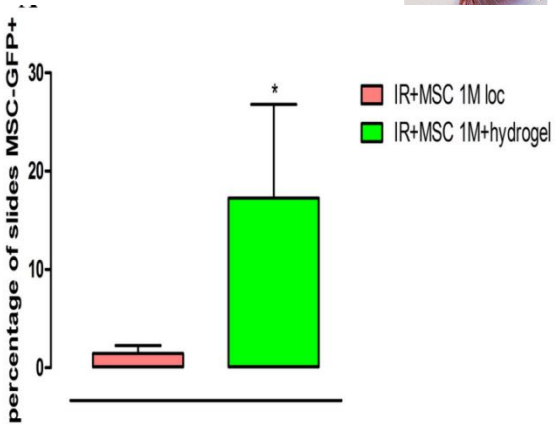
MSC from GFP rats

IR+MSC 1M loc



IR+MSC 1M+hydrogel

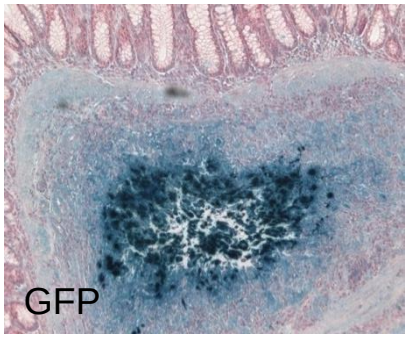


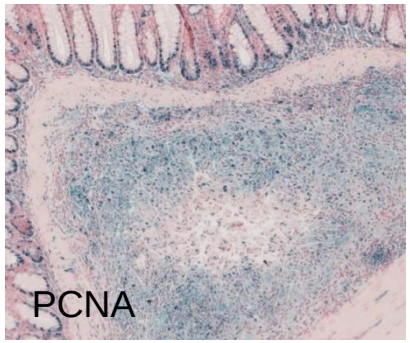


Day 7

In vivo proliferation?

PCNA immunostaining

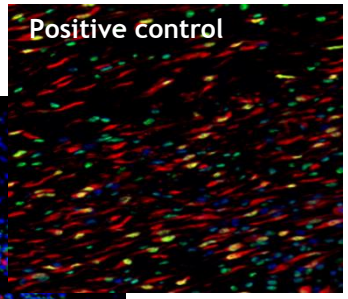


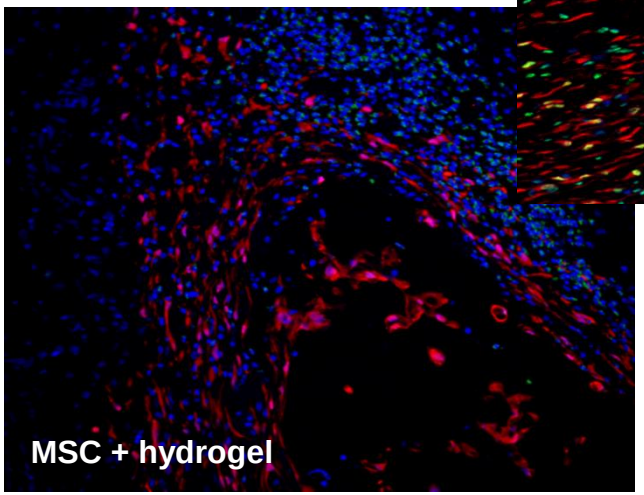


Few MSC are proliferating

In vivo Viability?

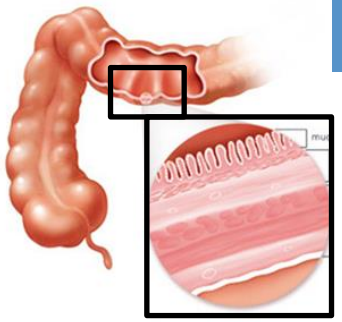
Tunel immunostaining



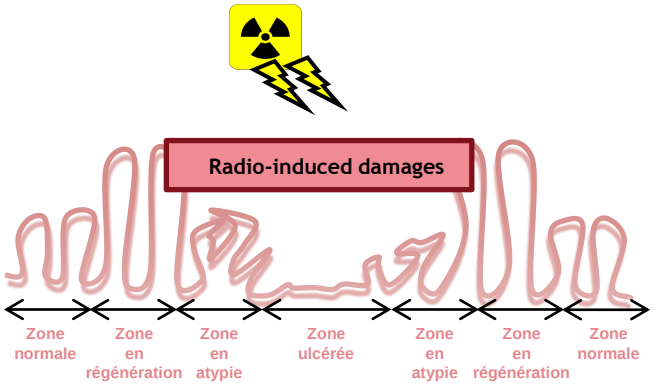


MSC were not apoptotic

IRSN



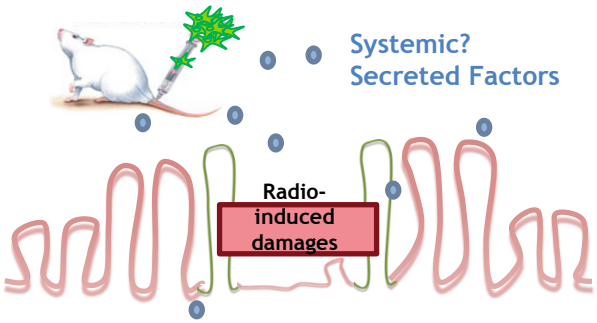
IRR



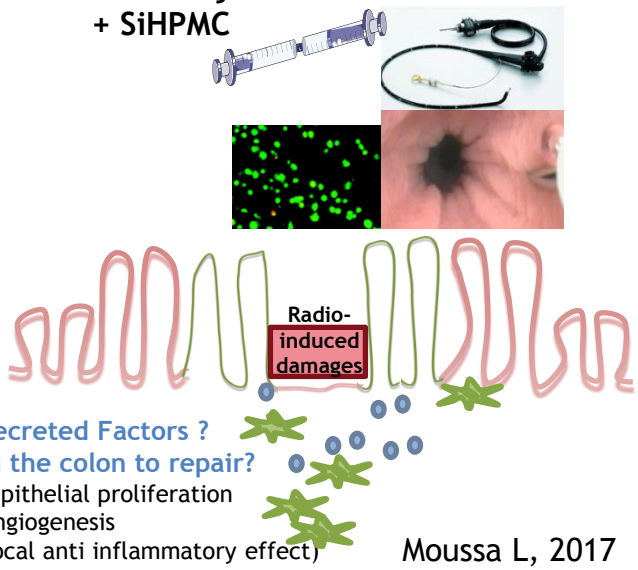
MSC traitement

Increasing the therapeutic benefit

Intravenous injection
5 millions of cells



Intra-colic injection 1 millions of cells
+ SiHPMC



Perspectives

Co-injection of

Colonic Stem Cells

+

MSC

Moussa L, 2017

Thank You!!

IRSN

Alexia Lapière

Alison Acarie

Virginie Monceau

Alexandra Sémont

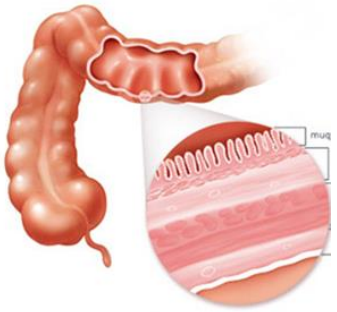
Christelle Demarquay

Lara Moussa

ANR

OTR³ Denis Barritault

LIOAD Laboratoire d'Ingénierie Ostéo-Articulaire et Dentaire — Pierre Weiss

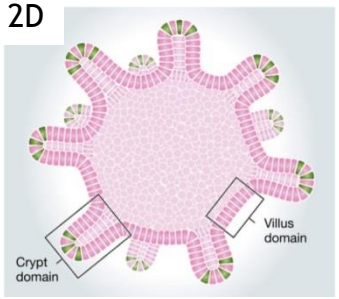


Crypt isolation

Culture

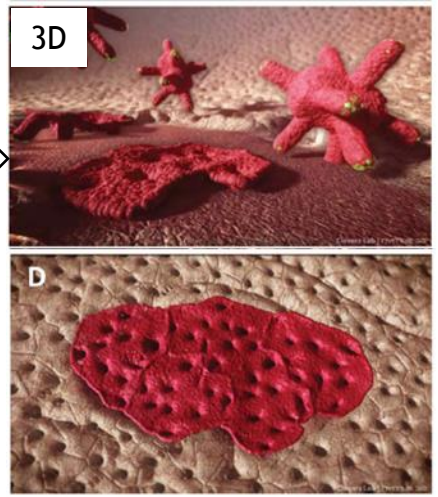
specific factors

« mini gut »



Clevers H

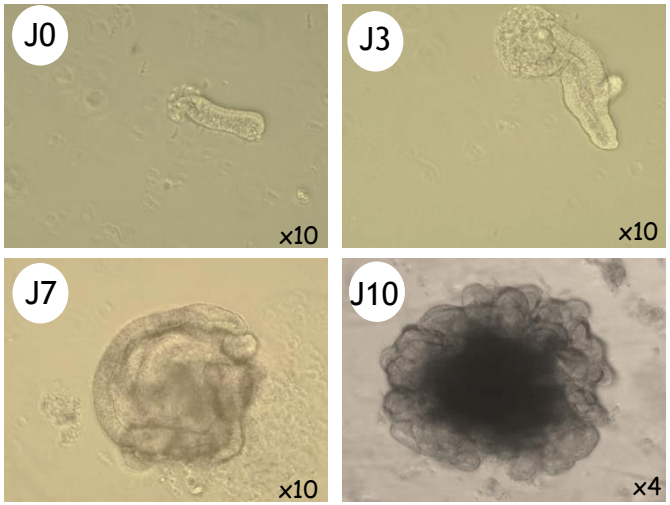
Cell therapy



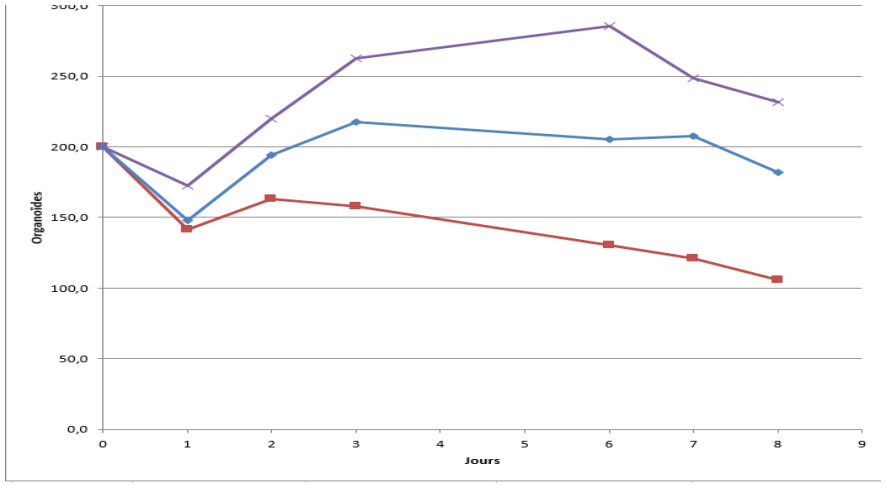
J. Schuijers and H. Clevers (2012)

Préliminary results

✓ Amplification of « mini gut » in culture

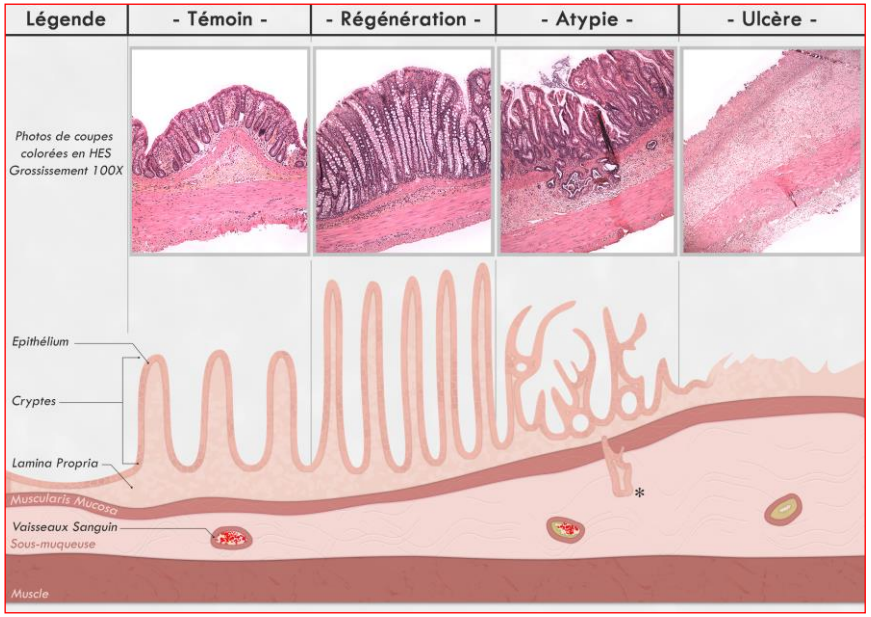


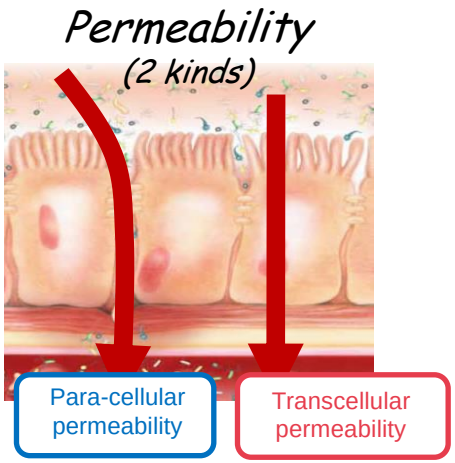
✓ MSC increase « mini gut » number

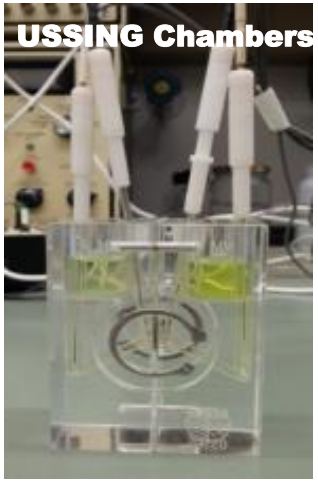


Structure

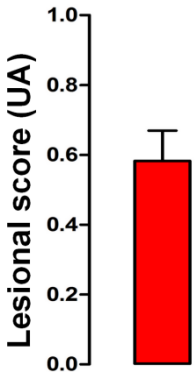
Function of barrier



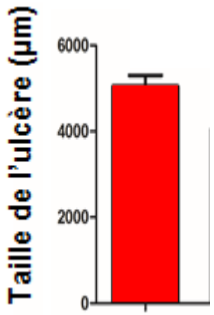


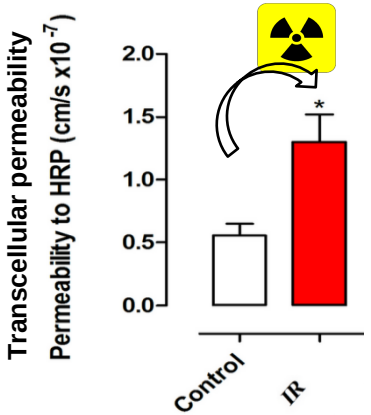


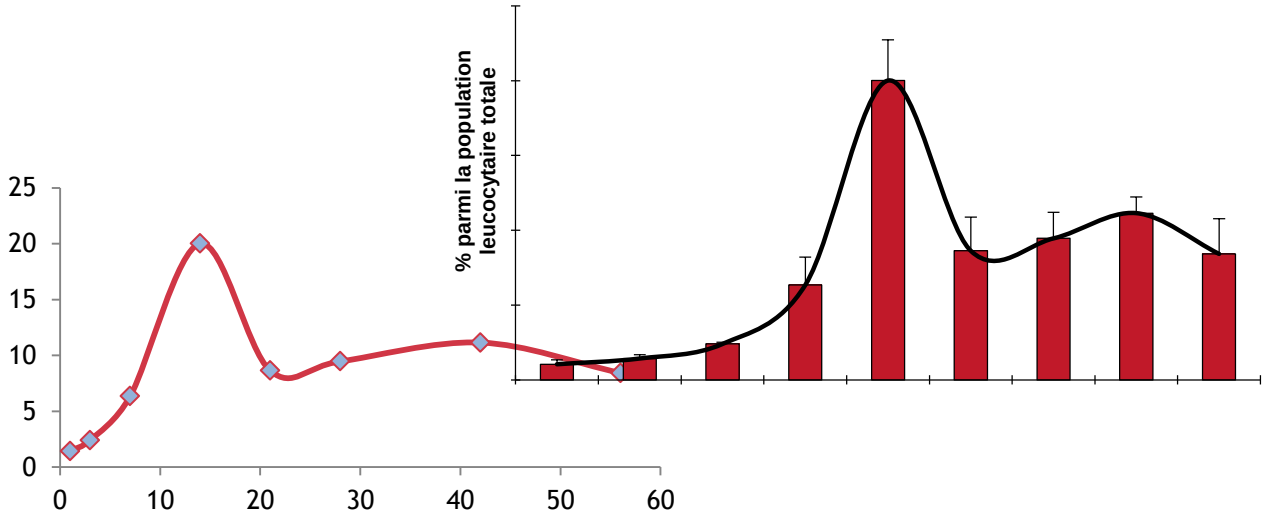
Score



Ulcer size

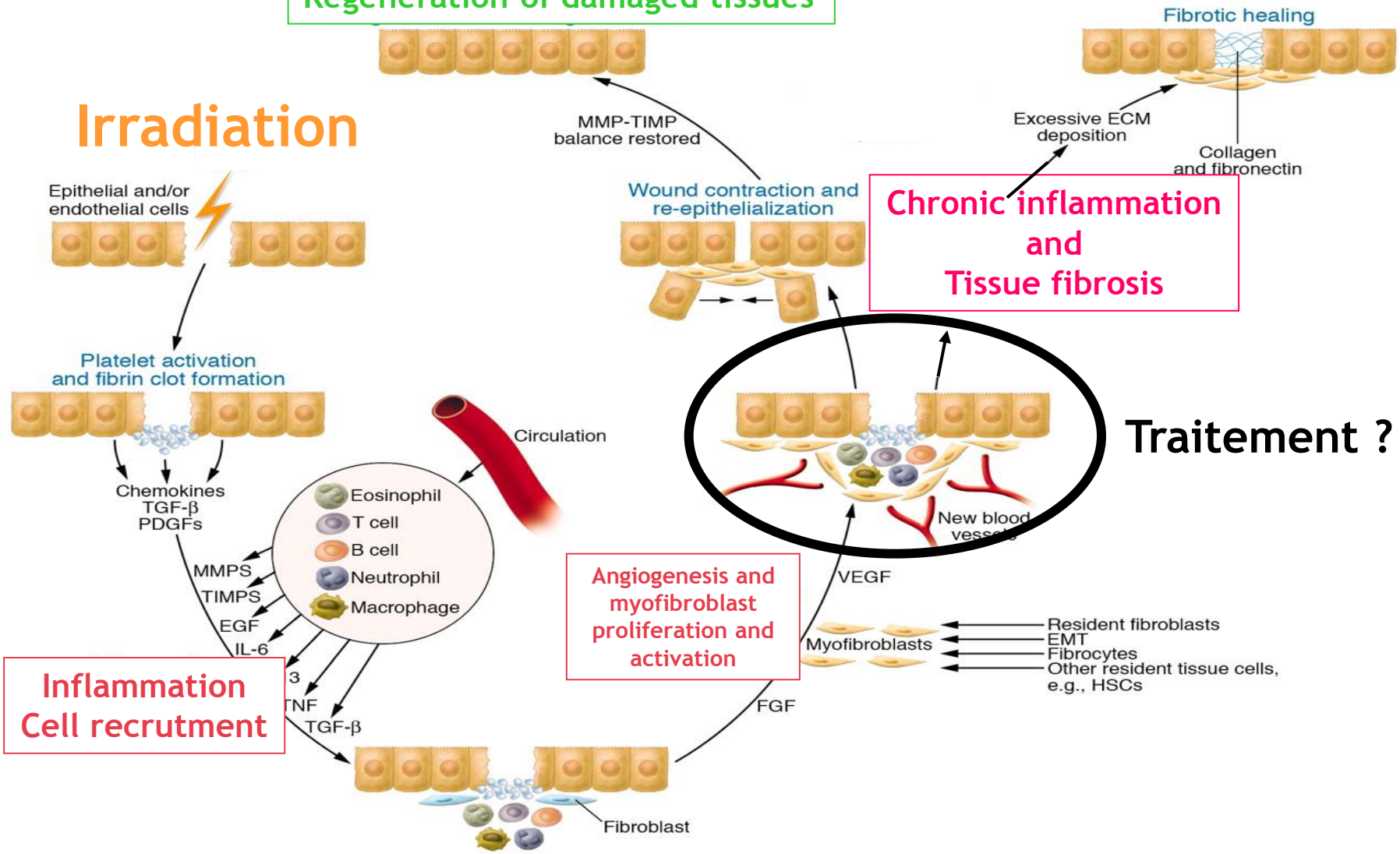





% parmi la population leucocytaire totale
25
20
15
10
5
0
0
10
20
30
40
50
60

Regeneration of damaged tissues

Irradiation



Chronic inflammation and Tissue fibrosis

Traitement ?

Angiogenesis and myofibroblast proliferation and activation

Inflammation Cell recrutment

CELL TRANSPLANTATION
The Regenerative Medicine Journal

From Moussa L, 2016