

From preclinical study of chronic radiation cystitis to cell therapy treatment

Brossard Clément (PhD student)

Supervisor: Chapel Alain

Team head: Milliat Fabien

Service de recherche en radiobiologie et en médecine régénérative (SERAMED),
Laboratoire de Radiobiologie des expositions médicales (LRMED)

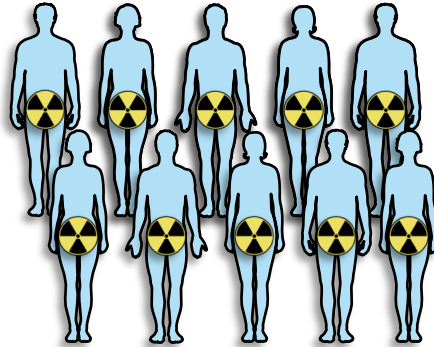
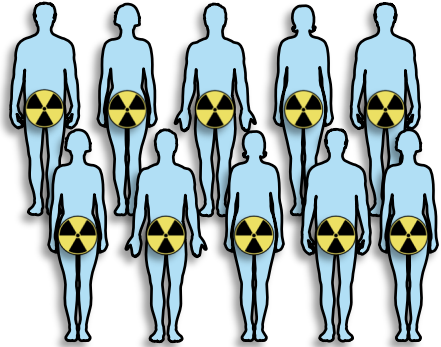
Mail contact: clement.brossard@irsn.fr

Clément Brossard* ^{1,2}, Anne-Charlotte Lefranc^{1,2}, Morgane Dos Santos ^{1,3}, Mohamedamine Benadjaoud¹, Christelle Demarquay^{1,2}, Valérie Buard^{1,2}, Georges Tarlet^{1,2}, Claire Squiban^{1,2}, Christine Linard^{1,2}, Noëlle Mathieu^{1,2}, Romain Granger⁴, Amandine Sache⁴, Delphine Denais Lalieve⁴, Jean-Marc Simon⁵, Marc Benderitter¹, Fabien Milliat^{1,2}, Alain Chapel^{1,2}

¹Service de recherche en radiobiologie et en médecine régénérative (SERAMED), ²Laboratoire de Radiobiologie des expositions médicales (LRMED), ³Laboratoire de radiobiologie des expositions accidentelles (LRAcc), ⁴Service de recherche sur les effets biologiques et sanitaires des rayonnements ionisants (SESANE), Groupe de support à la recherche et à l'éthique animale (GSEA), Institut de Radioprotection et de Sûreté Nucléaire, Fontenay-aux-roses, ⁵Département de radio-oncologie, Hôpital universitaire de la Pitié-Salpêtrière, Paris, France

Acute and chronic radiation cystitis

Population concerned



**Pelvic
Radiotherapy**



Days- months



6 months - 20 years

Acute complications
40% of patients

Acute radiation cystitis¹

Chronic complications
5 - 10 % of patients

Chronic radiation cystitis (CRC)^{1,2}

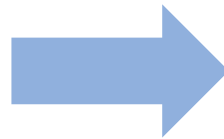
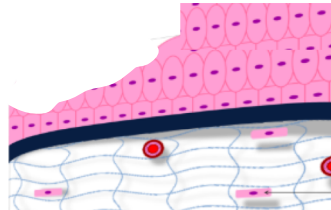
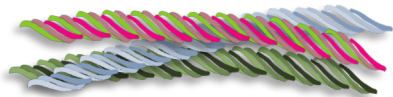


1 Rigaud et al., 2004

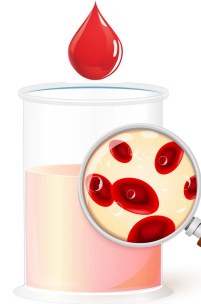
2 Martin et al., 2019

Clinical symptoms and major mechanisms

Majors mechanisms



Clinical symptoms



Vascular lesion

Fibrosis

Urothelium integrity

Hematuria

Incontinence

Pain

Decrease bladder functionality

Decrease the quality of life

What is the problem today?



- Few studies on chronic radiation cystitis
- Molecular mechanisms partially known
- No curative treatment



Preclinical research to develop innovative treatments

How to choose a new treatment?

Clinical symptoms and majors biologicals mechanisms of Chronic radiation cystitis

Similar to

Interstitial cystitis³⁻⁵

(incidence 0,01 to 0,575%)

Hemorrhagic cystitis^{6,7}

Chemically induced by chemotherapy
(Incidence 20-25%)

Vascular lesion

Inflammation

Urothelium integrity

Fibrosis

Adipose-Mesenchymal Stem Cells (AdMSCs)⁷⁻¹¹

↑ Tissue regeneration

↓ Inflammation

↓ Fibrosis

3 Chung et al., 2019
4 Song et al., 2015
5 Furuta et al., 2018

6 Ringdén et al., 2007
7 Yang et al., 2021

Pre clinical Study:

7: Song et al., 2015 (IC)
8: Kim et al., 2016 (IC)

Clinical study:

9: Cesaro et al., 2003 (HC)
10: Hassan et al., 2011 (HC)
11: Ringdén et al., 2007 (HC)

Hypothesis: MSCs reduced inflammation, fibrosis and increase tissue regeneration in CRC?

Scientific Background

Objectives and experimental design

Results

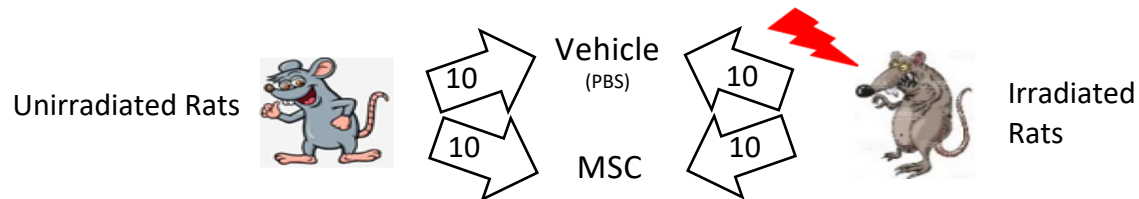
Conclusion &
perspective



Experimental design

Experimental conditions: 10 rats for each condition and for each euthanasia time.

Experimental conditions for each time of euthanasia



Local bladder irradiation in rats with SARRP*

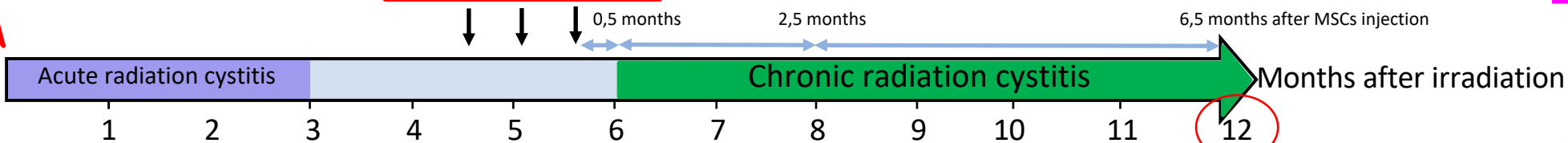
age: 12 weeks (permitted long studies) female (avoid prostate)
protocol ethics committee: P19-09



40 Gy

3 Injections of 5 millions of MSCs intravenously

Protocol use in radiation rectitis: Potentially enhances the MSC effect¹²



Euthanasia of rats and analysis of the effects of MSCs on CRC (vascular damage, urothelium dysfunction, chronic inflammation, fibrosis)

Urothelium regeneration
Urothelium thickness
Vascular damages

Tissue

Physiological:
Hematuria

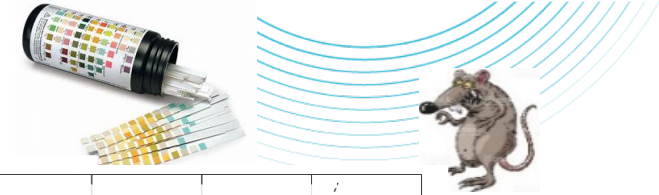
Transcriptomic

RT-qPCR (TLDA : Taqman low density array)

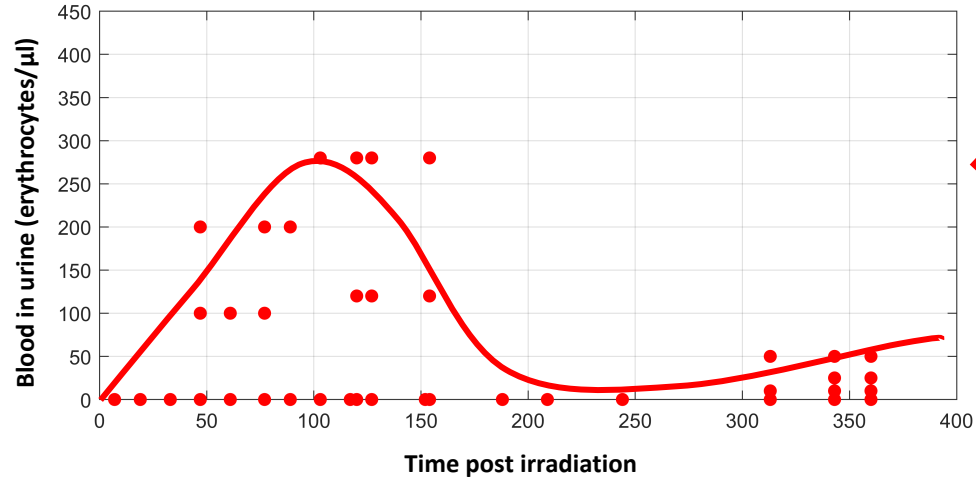
* Small Animal Radiation Research Platform

12: Usunier et al., 2015

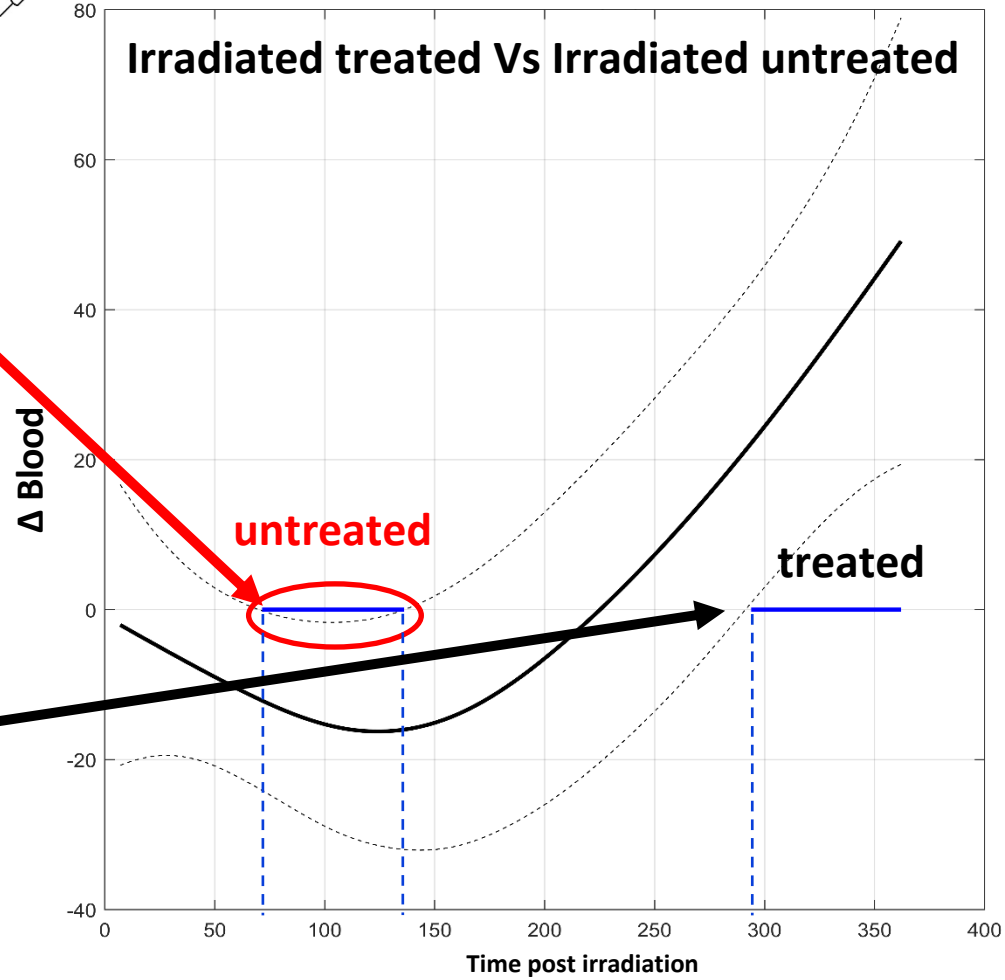
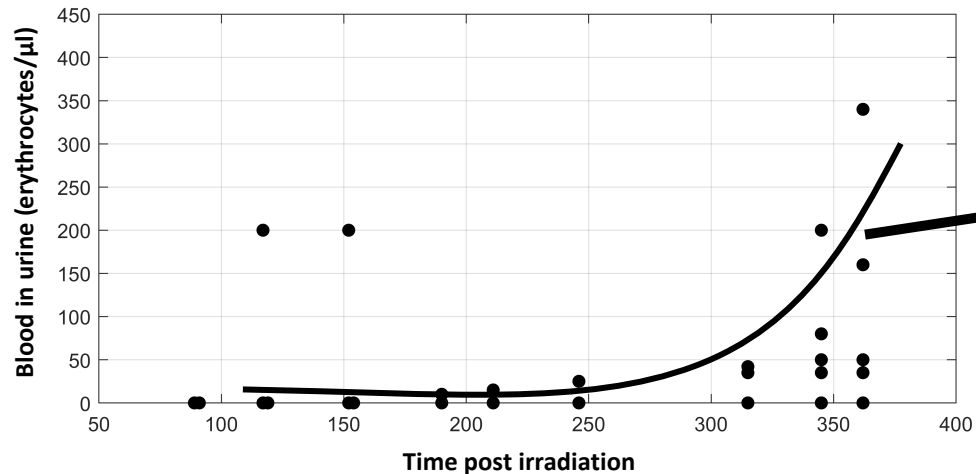
Hematuria in function of time after irradiation (40 Gy)



Irradiated untreated



Irradiated treated



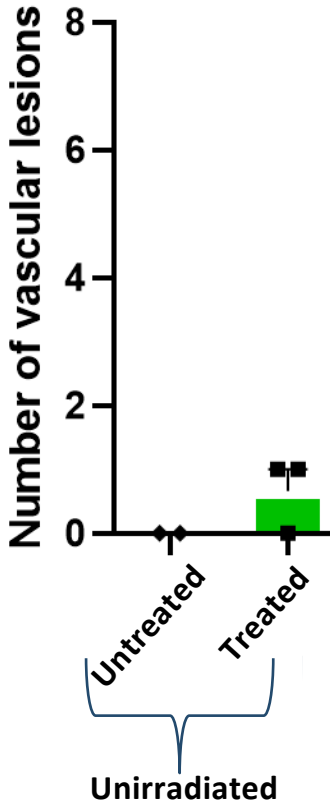
- Irradiated untreated = Early hematuria (75-130 days post irradiation)
- Irradiated treated = Late hematuria (>290 days post irradiation)

Hypothesis: MSCs treatment delays hematuria

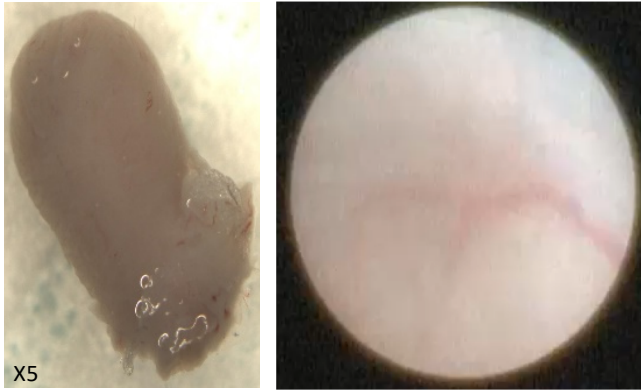
Visualization of the bladder wall by cystoscopy 12 months

Cystoscopy at 12 months post-irradiation

Number of vascular lesions in cystoscopy (12 months)



Unirradiated, untreated

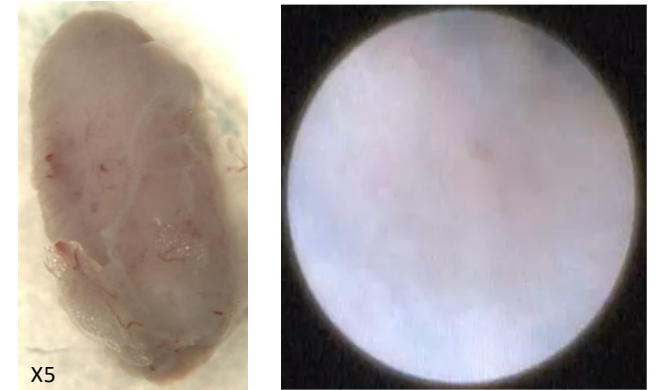


X5

3 rats tested by cystoscopy on 10 rats for this condition

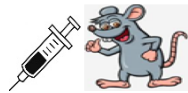


Irradiated, untreated

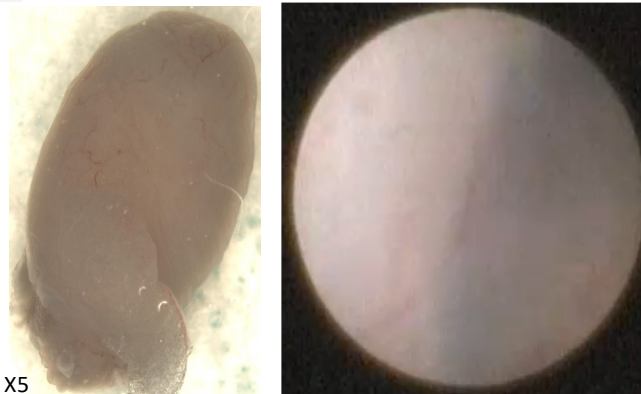


X5

3 rats tested by cystoscopy on 10 rats for this condition



Unirradiated, treated

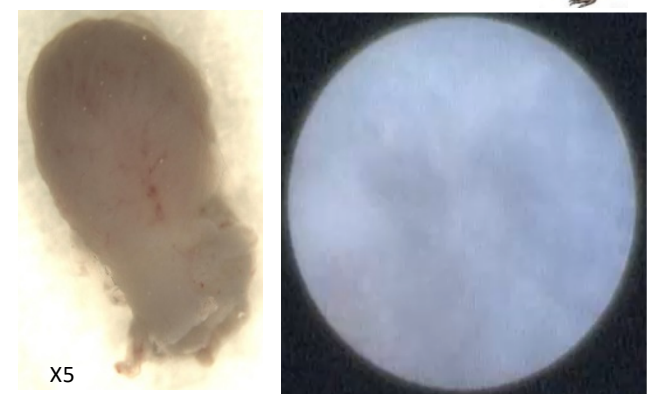


X5

3 rats tested by cystoscopy on 10 rats for this condition



Irradiated, treated

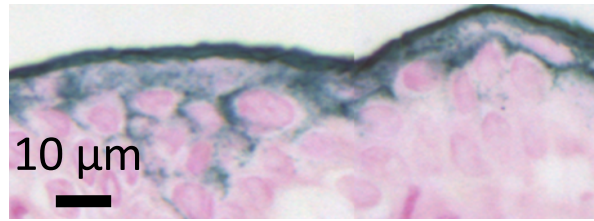


X5

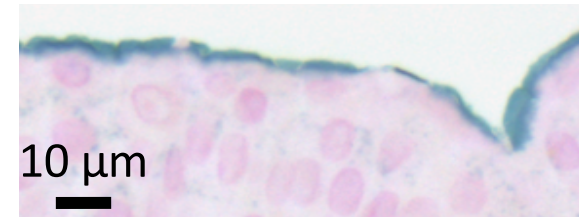
3 rats tested by cystoscopy on 10 rats for this condition

- **MSC treatment decreases Vascular lesions**

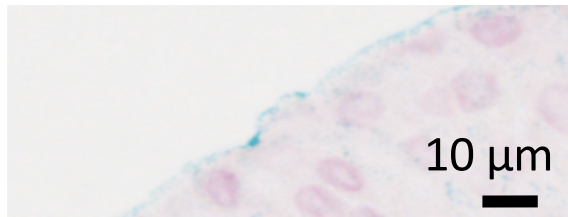
Urothelium integrity alteration related to decrease of uroplakine 3 expression



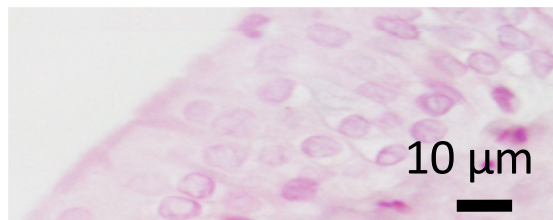
Grade 0: High expression of uroplakine 3



Grade 1: Basic expression of uroplakine 3



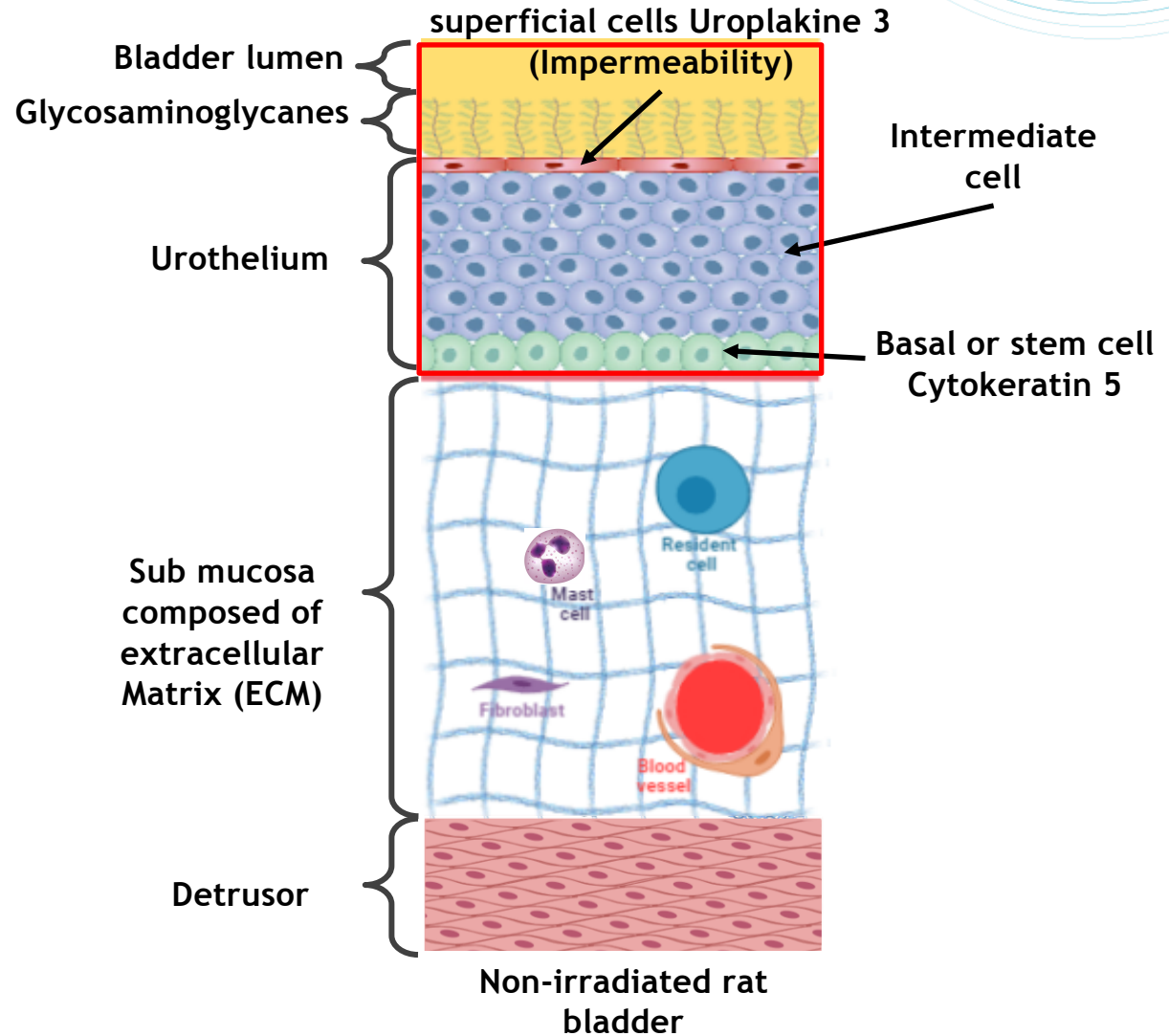
Grade 2: Low expression of uroplakine 3



Grade 3: Absence of uroplakine 3 expression

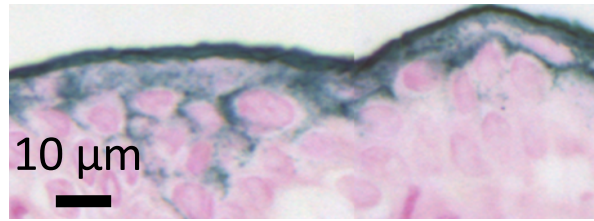
Normal expression of uroplakine 3

Low expression of uroplakine 3

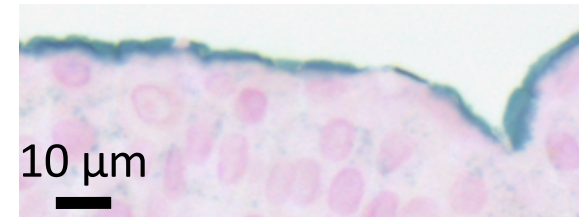


Urothelium integrity alteration related to decrease of uroplakine 3 expression

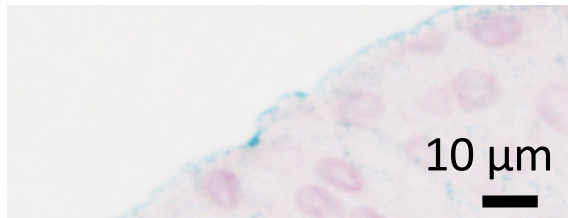
Low uroplakine 3 expression ⇔ decrease in impermeability



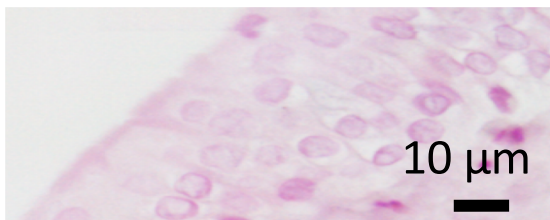
Grade 0: High expression of uroplakine 3



Grade 1: Basic expression of uroplakine 3



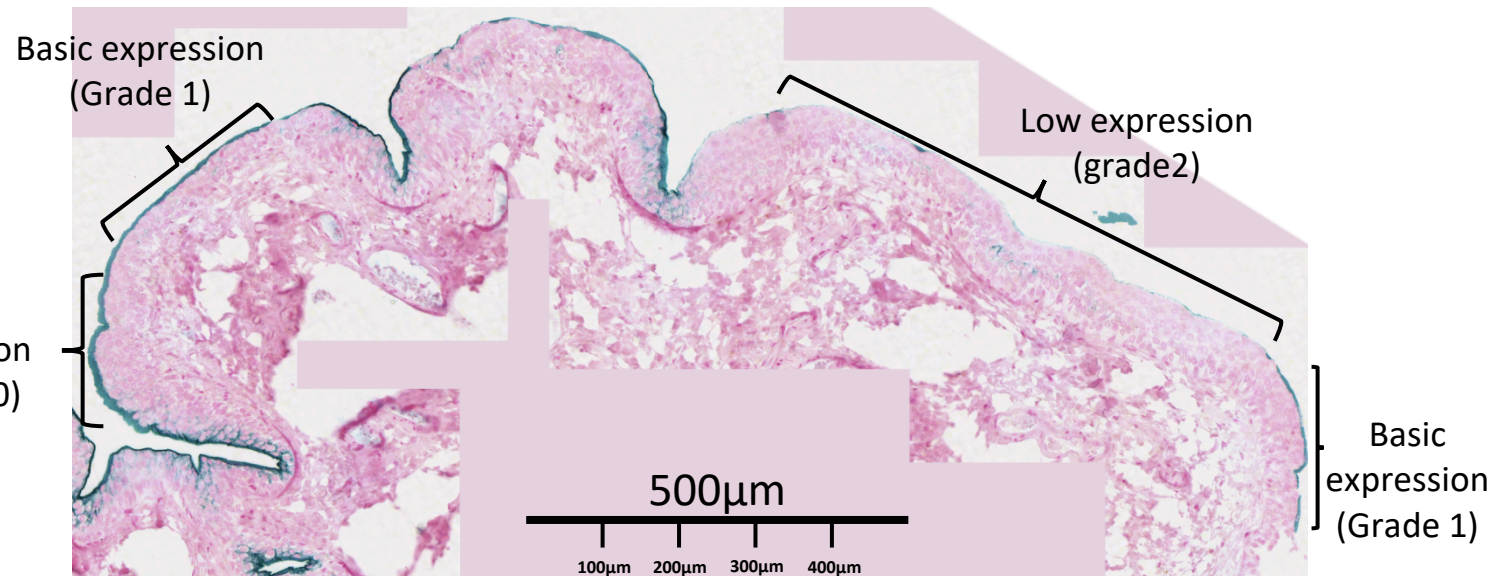
Grade 2: Low expression of uroplakine 3



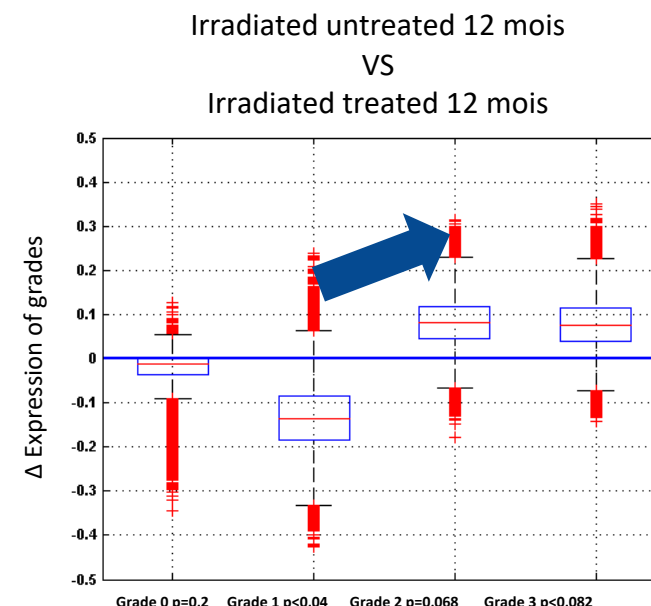
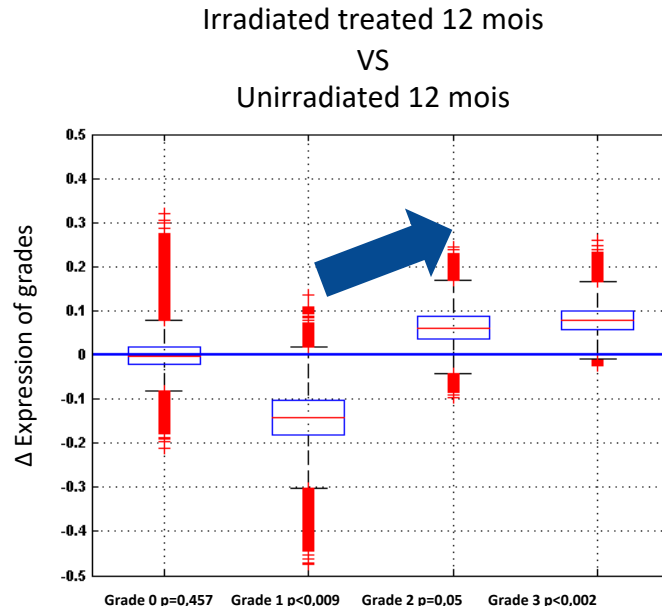
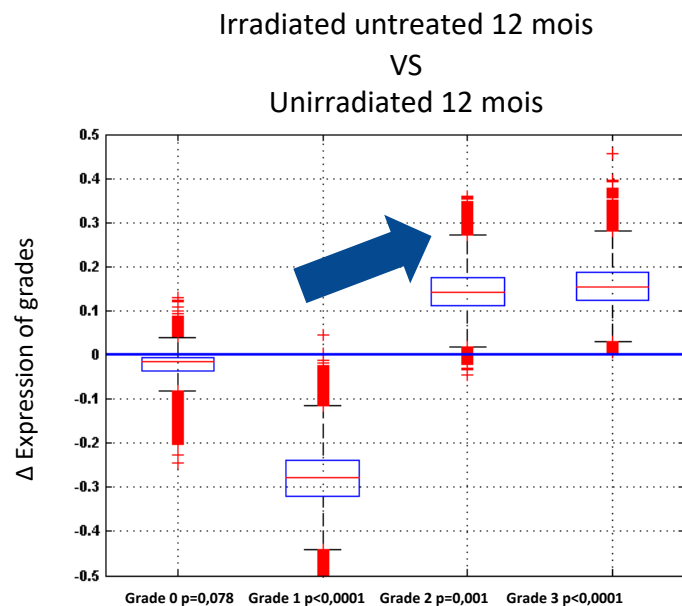
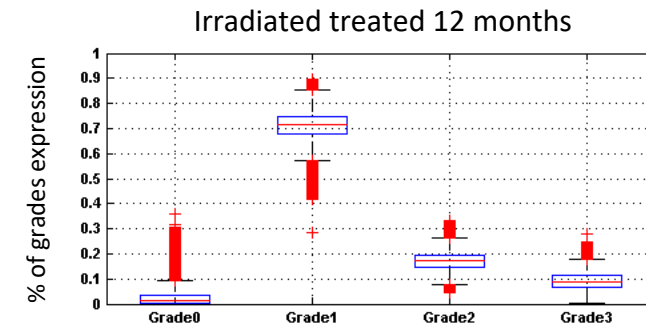
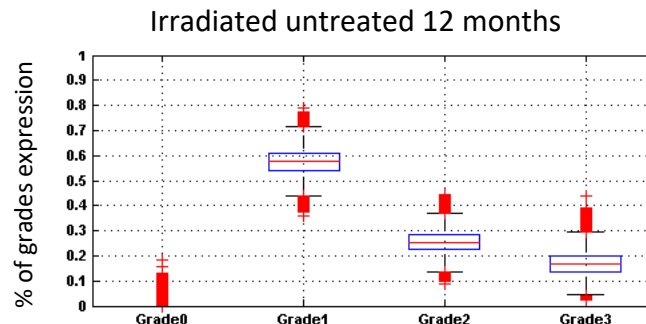
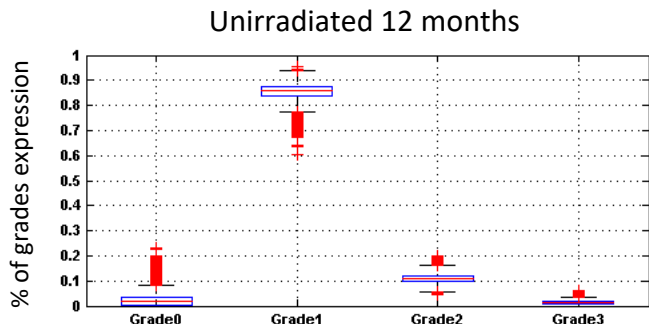
Grade 3: Absence of uroplakine 3 expression

Normal
expression of
uroplakine 3

Low expression
of uroplakine 3



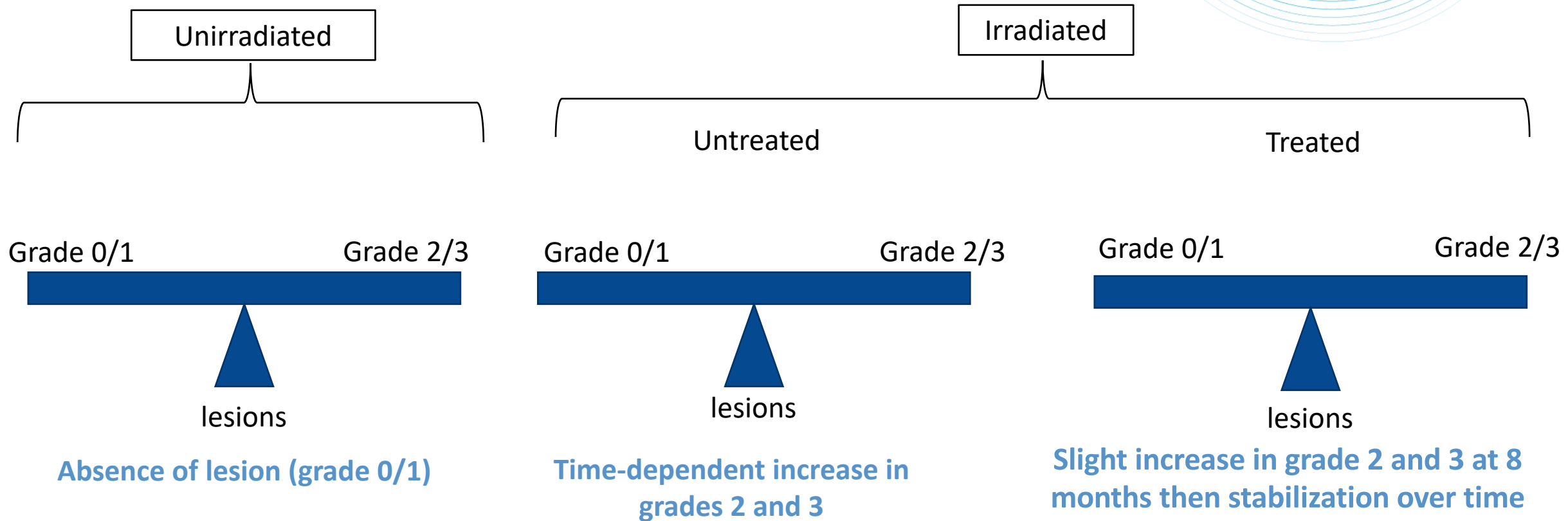
Urothelium integrity alteration related to decrease of uroplakine 3 expression



- After irradiation increase of alteration of urothelium
- MSC treatment reduces severity of alteration compared to irradiated untreated group

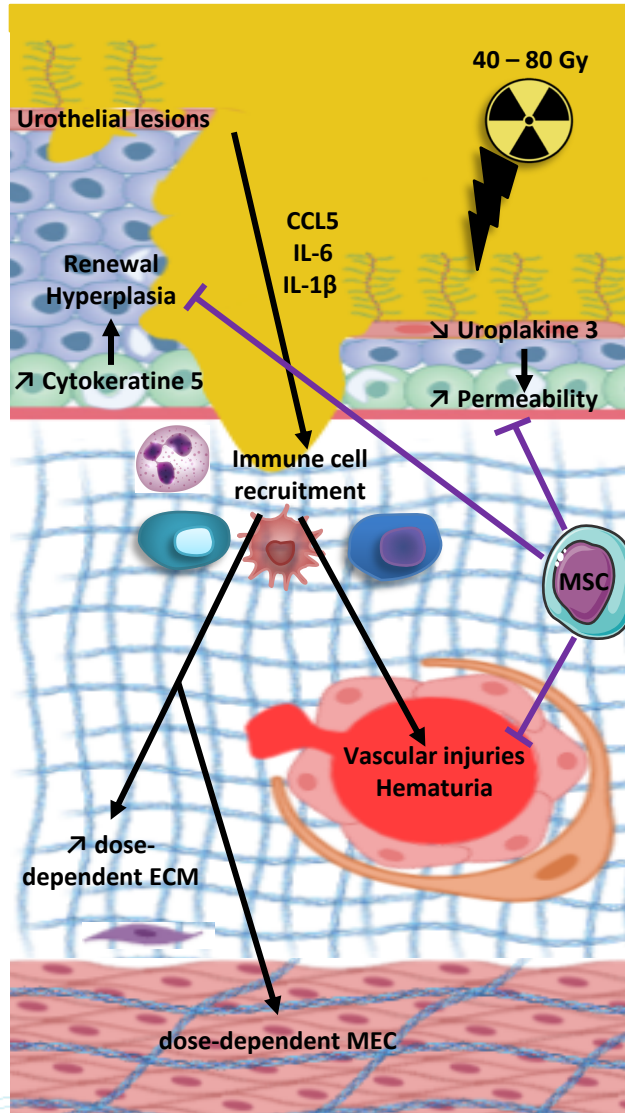


Summary of results of urothelium integrity



- **Decrease in uroplakin 3 expression over time after irradiation → In favor of a decrease of the impermeability of the urothelium**
- **MSC treatment has a protective effect on the maintenance of impermeability of the urothelium**

Chronic radiation cystitis in rats at 6-12 months post-irradiation



Irradiation

Urothelial injuries

Inflammation

Vascular injuries

Fibrosis

Single dose 40 Gy, whole bladder

Decreased uroplakin 3 expression over time in irradiated untreated group

→ Protective effect of MSC treatment

Onset of inflammation at 6 months post irradiation (data not shown)

Protective effects of MSC treatment with delayed onset of hematuria and decreased of vascular injury

Initiation of fibrosis from 12 months post irradiation (data not shown)

Thank you for your attention

GSEA member: Animal monitoring and assistance in animal experimentation

9 researchers
1 post-doctoral
4 technical
6 PhD students

C. Demarquay
MSC culture

C. Linard
Immunolabelling and
inflammation

F. Milliat
Head laboratory

N. Mathieu
Cystoscopy

V. Buard
Microscopie and
Immunolabelling

Tarlet
PCR

Brossard



A. Benadjaoud :
Biostatistique



M. Dos Santos : rats
irradiation (SARRP)

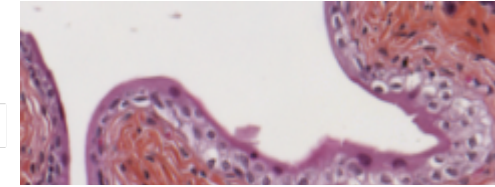
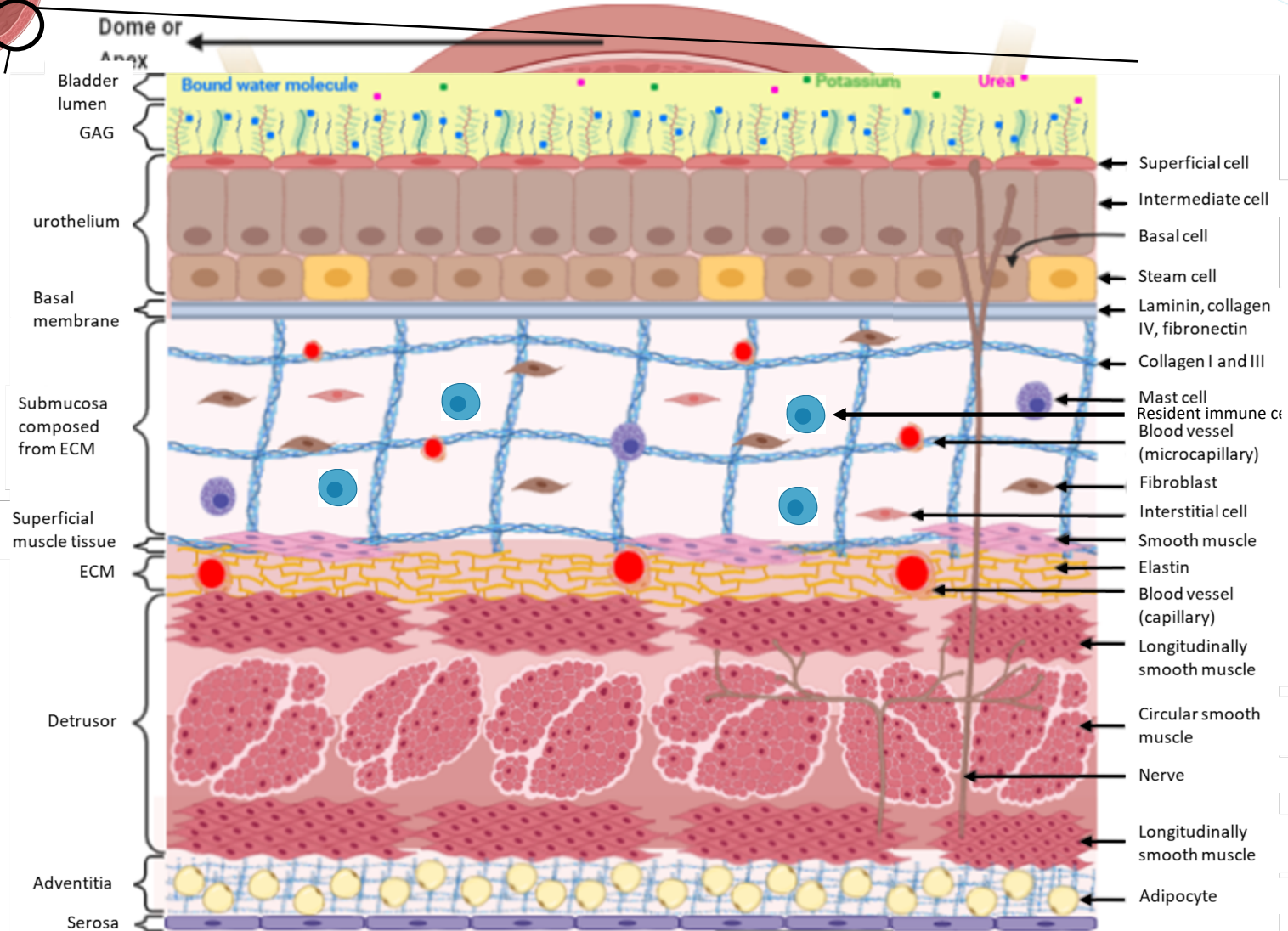
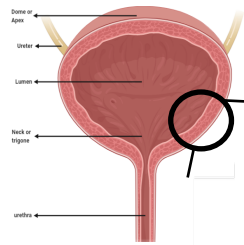


Alain Chapel
Director thesis

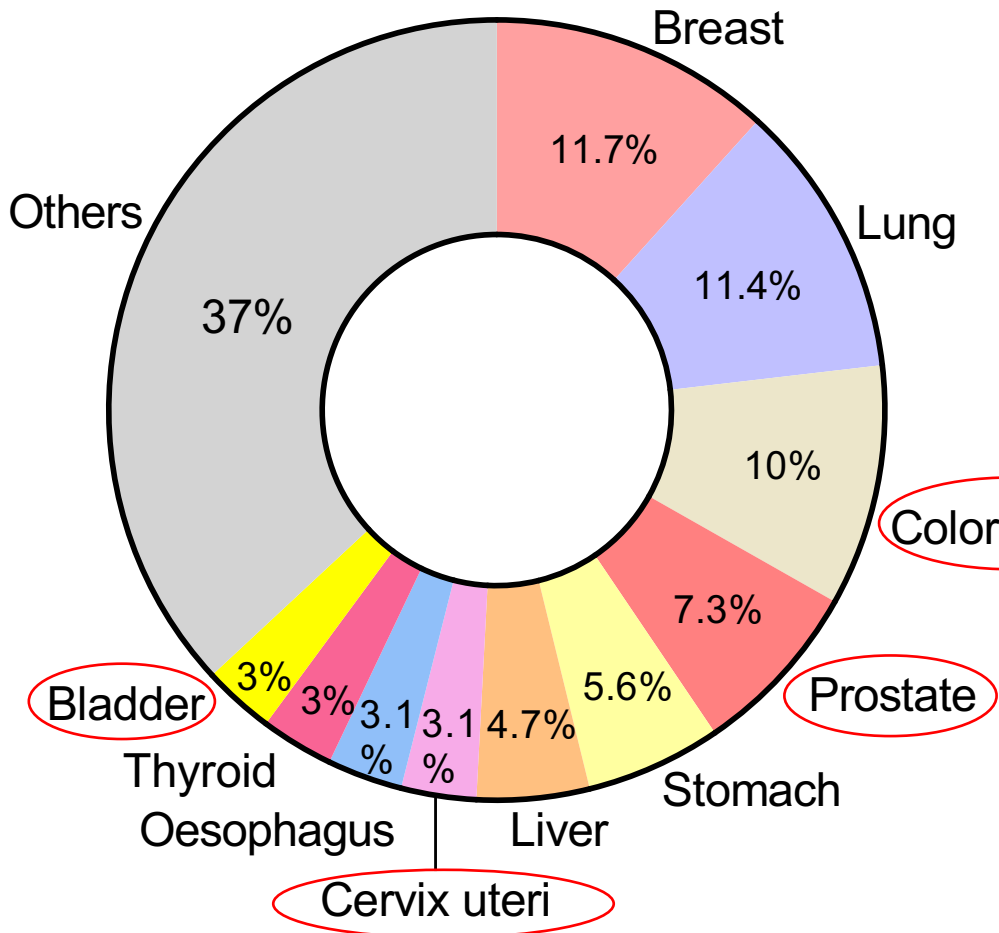


Anne-charlotte Lefranc:
Master 2 in CRC project

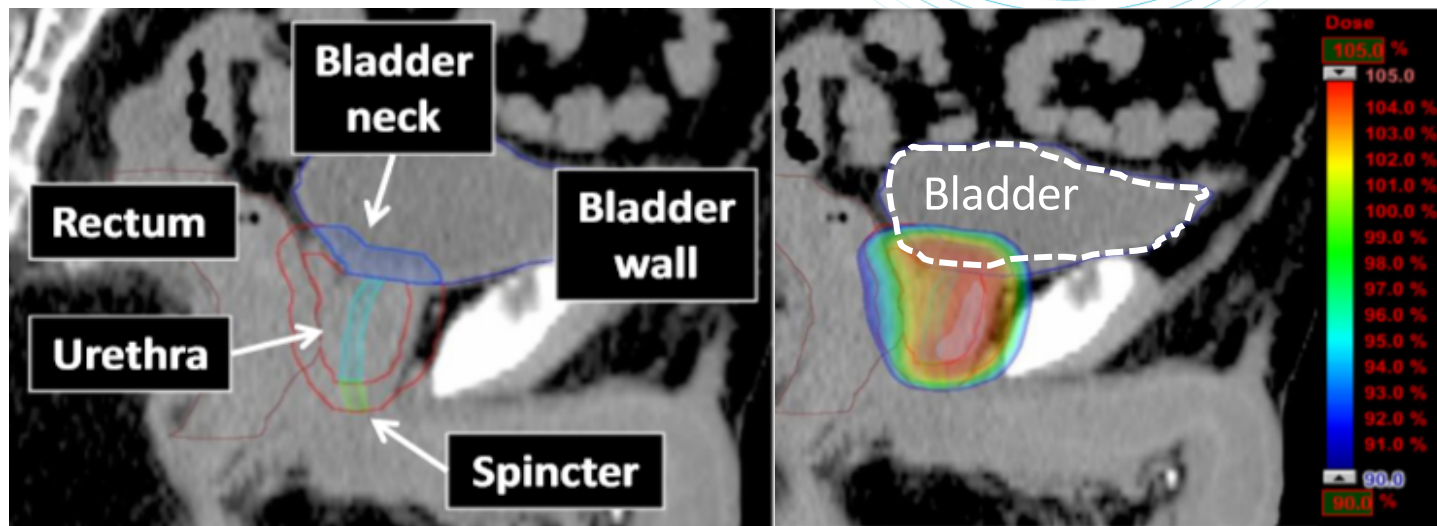
Laboratoire de Radiobiologie des expositions médicales (LRMED)



Cancers and radiotherapy



Total : 19 millions of people



Inokuchiet al.; 2017

60% treated by RT



Radiotherapy :

- Anal canal
- Cervix
- Endometrium
- Vulva
- Prostate

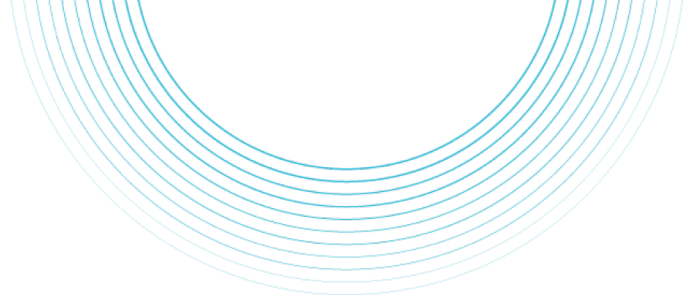
Organ at risk :
Bladder

Grading of CRC

Grade	Droller (11)	Brugieres (12)	Bearman (13)	CTCAE v5.0	Arthur (14)	RTOG/EORTC (15)	SOMA-LENT (16)
I	Microscopic hematuria/ dysuria	Macroscopic hematuria with thrombocytopenia	Macroscopic hematuria after 2 days from the last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Minimal or microscopic bleeding intervention not indicated	More than 50 erythrocytes	Slight epithelial atrophy Minor telangiectasia (microscopic hematuria)	Hematuria occasional and microscopic Dysuria occasional Incontinence weekly episodes
II	Macroscopic hematuria	Macroscopic hematuria with clots	Macroscopic hematuria after 7 days from last chemotherapy dose not caused by infection or hematuria after 2 days with subjective symptoms of cystitis not caused by infection	Gross bleeding, medical intervention or urinary tract irrigation indicated	Macroscopic hematuria	Moderate frequency Generalized telangiectasia Intermittent macroscopic hematuria	Hematuria intermittent, macroscopic and <10% decrease hemoglobin Dysuria intermittent Incontinence daily episodes
III	Macroscopic hematuria with small clots that were passed on micturation	Macroscopic hematuria with clots and urinary obstruction	Hematuria cystitis with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomy or other surgical procedure	Transfusion, interventional radiology, endoscopic or operative intervention indicated, radiation therapy (ie hemostasis of bleeding site)	Clots	Severe frequency and dysuria Severe generalized telangiectasia (often with petechiae) Frequent hematuria Reduction in bladder capacity (<150 cc)	Hematuria persistent, macroscopic and 10-20% decrease hemoglobin Dysuria persistent and intense Incontinence daily episodes
IV	Massive clotting often necessitating instrumentation for clot evacuation			Life-threatening consequences, major urgent intervention indicated	Macroscopic hematuria clots, renal insufficiency	Necrosis/ Contracted bladder (capacity <100 cc) Severe hemorrhagic cystitis	Hematuria persistent, >20% decrease hemoglobin Dysuria refractory and excruciating Incontinence complete refractory

CRC grading based on hematuria





Prostate cancer

1 year after RT = **14,2 % [9-21]** The incidence of hemorrhagic cystitis is approximately 11%.

For 3- to 4-box, small-field therapy (66 Gy), scoring, is as follows:

- Grades 1 and 2: **24-64%**
- Grades 3 and 4: **2-9%**

For conformal beam therapy (70-78 Gy), the frequency is as follows:

- Grades 1 and 2: **65%**
- Grades 3 and 4: **9%**

Cervical cancer

1 year RT = **4,9 % [3-6.7]**

The frequency associated with combination 4-field external beam therapy (70-80 Gy) and cesium implants is as follows:

- Grade 1: **1-3%**
- Grade 2: **1-2%**
- Grade 3: **2-5%**

Bladder cancer

1 year after RT = **17,8 % [2-47]**

The frequency associated with 3- to 6-beam, small-field therapy (32-57.5 Gy) is as follows:

- Grades 1 and 2: **19-49%**
- Grades 3 and 4: **33-48%**

Genitourinary toxicity with intensity-modulated radiotherapy

IMRT is increasingly used for the treatment of prostate cancer; doses of 81 Gy have been delivered.

- Grade 2: IMRT, **17-36%**; 3D conformal radiotherapy, **42-60%**
- Grade 3: IMRT, **0.3-0.5%**; 3D conformal radiotherapy, **1-2%**



Development of a Management Algorithm for Acute and Chronic Radiation Urethritis and Cystitis

Ben G.L. Vanneste^a Evert J. Van Limbergen^a Tom A. Marcelissen^b
Joep G.H. van Roermund^b Ludy C. Lutgens^a Carsten W.K.P. Arnoldussen^c
Philippe Lambin^d Matthias Oelke^e

^aDepartment of Radiation Oncology (MAASTRO clinic), GROW, School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands; ^bDepartment of Urology, Maastricht University Medical Centre, Maastricht, The Netherlands; ^cDepartment of Radiology and Nuclear Medicine, VieCuri Medical Centre, Venlo, The Netherlands; ^dThe D-Lab, GROW, School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands; ^eDepartment of Urology, St. Antonius Hospital, Gronau, Germany

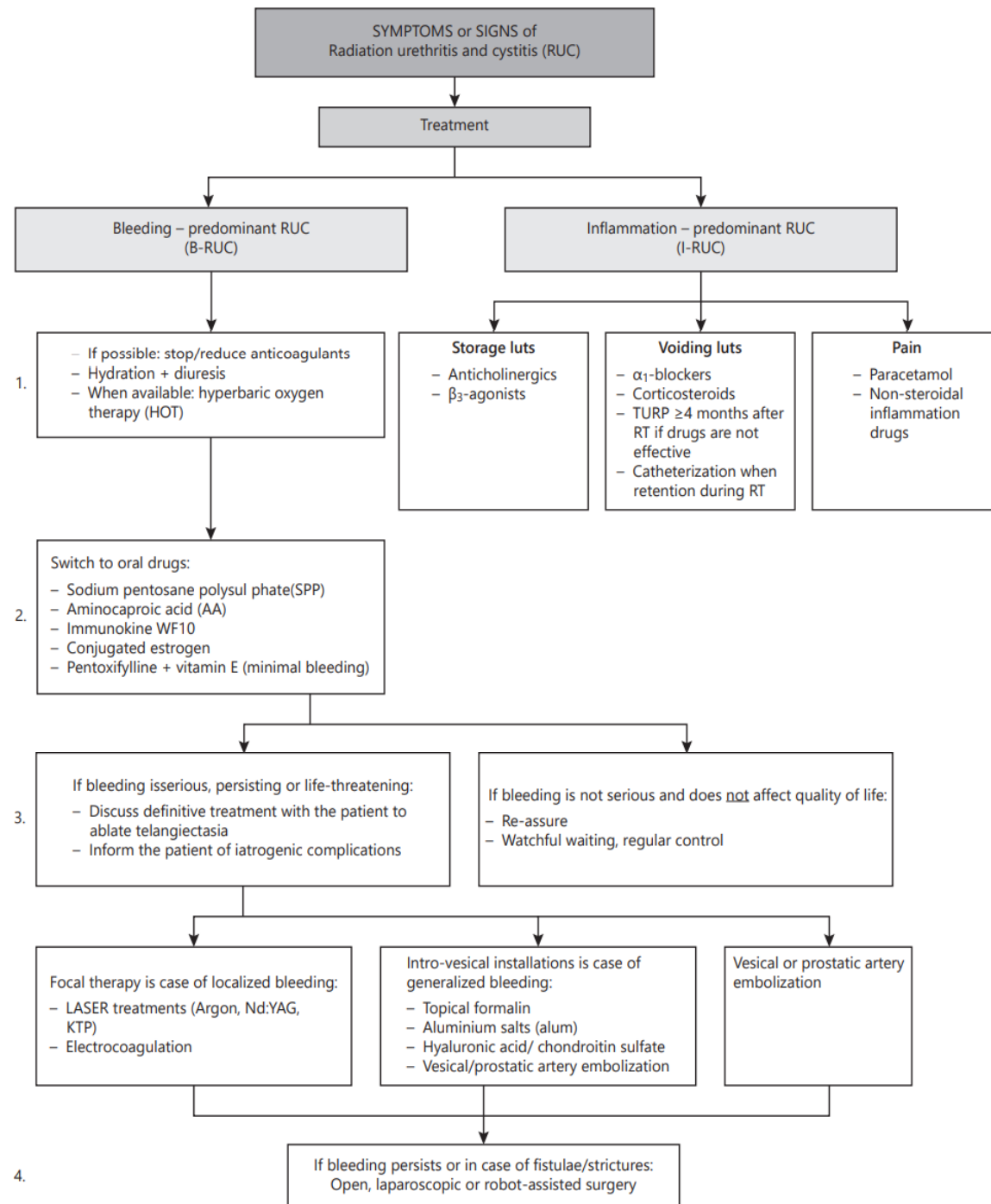
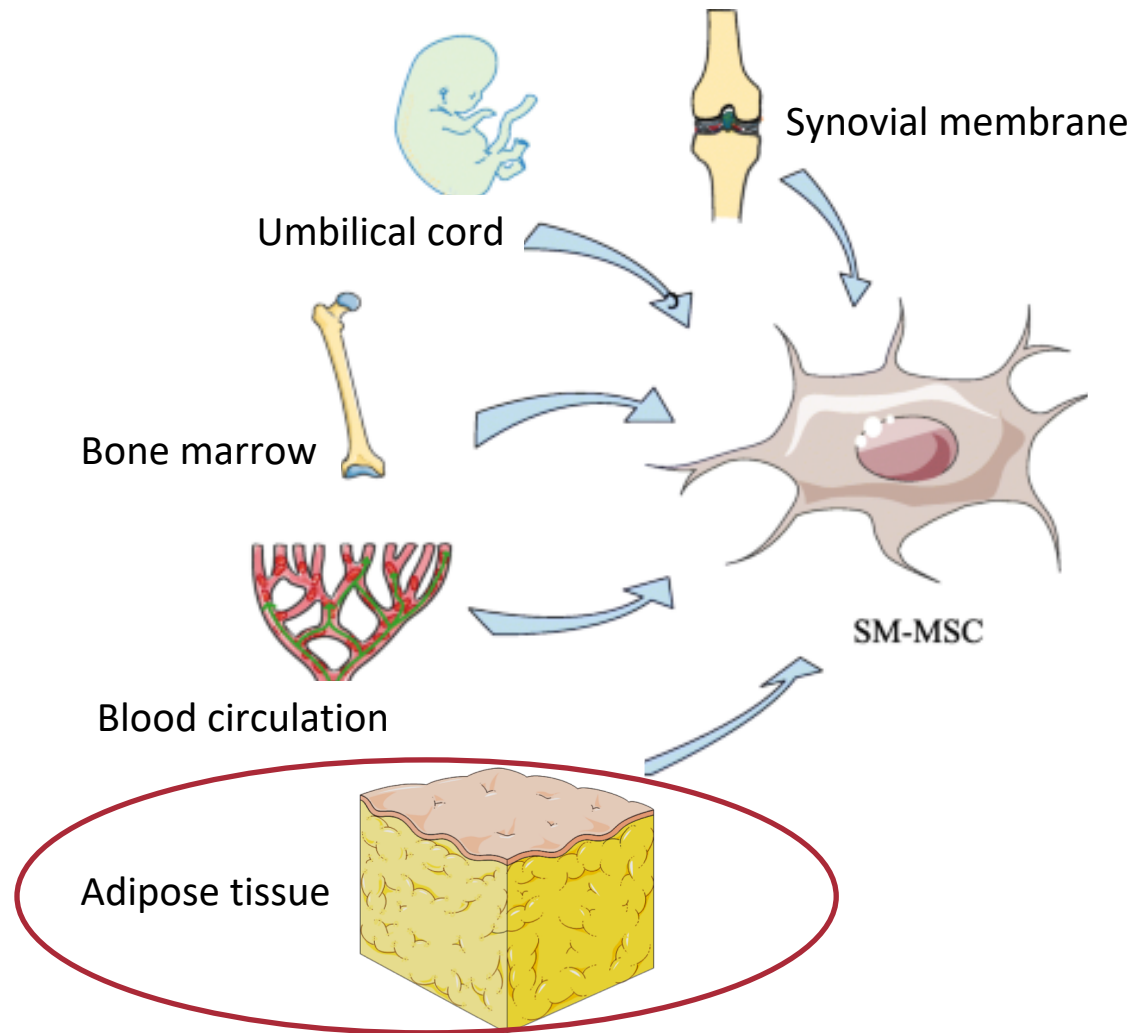


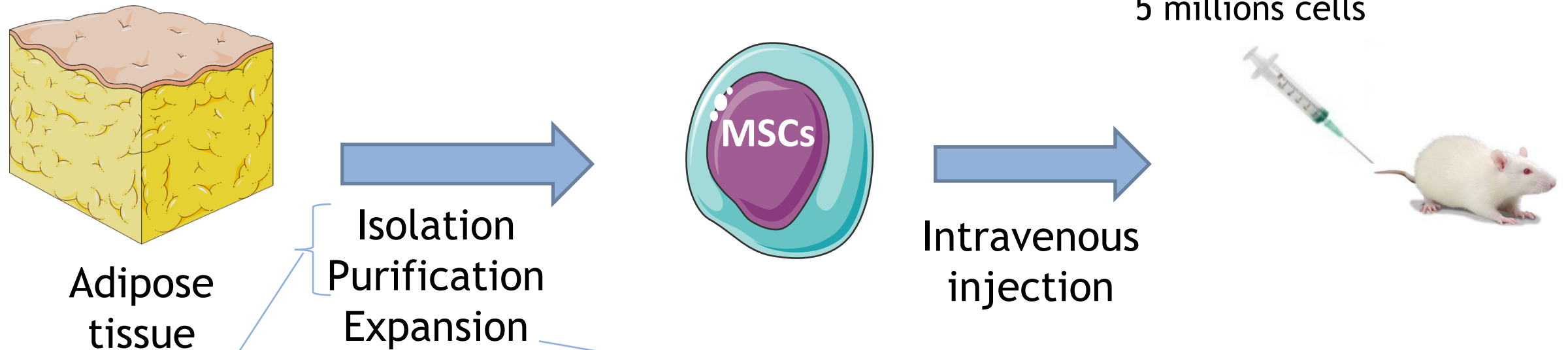
Fig. 3. Algorithm for management of RUC. RUC, radiation urethritis and cystitis.

Origin and differentiation of MSC



- Easy to collect
- Large amount of tissue and MSC

Culture of MSC: Isolation and expansion



5 millions cells

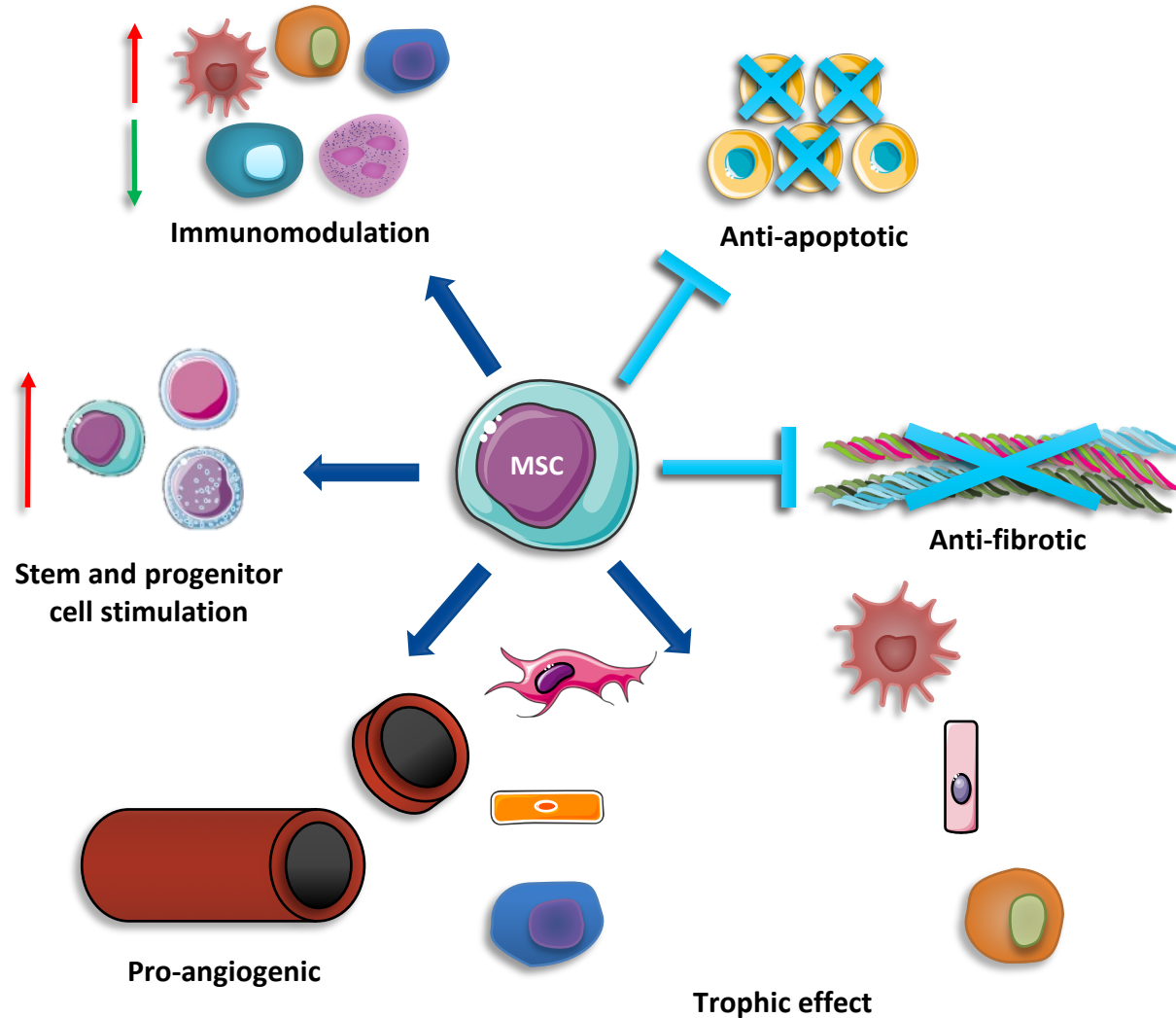


- The fat is cut into small pieces and put into MEMa + collagenase 0.1% (x2 tubes/digestion). Incubation for 30 min at 37°C with agitation.
- The cells are recovered with a 100µm filtration, the non-digested fat is put back in MEMa + collagenase at 0.1%.
- On the filtered cells, medium containing SVF is added to dilute the collagenase.
- Centrifugation is performed to collect all cells, filtration on 40µm and then counting.

- P0 1000ç/cm² or 0.150.106ç/150cm² in MSC medium (MEMa, SVF 20%, PS, Glut)
- Change the media 2 times a week



Biological effect of MSC

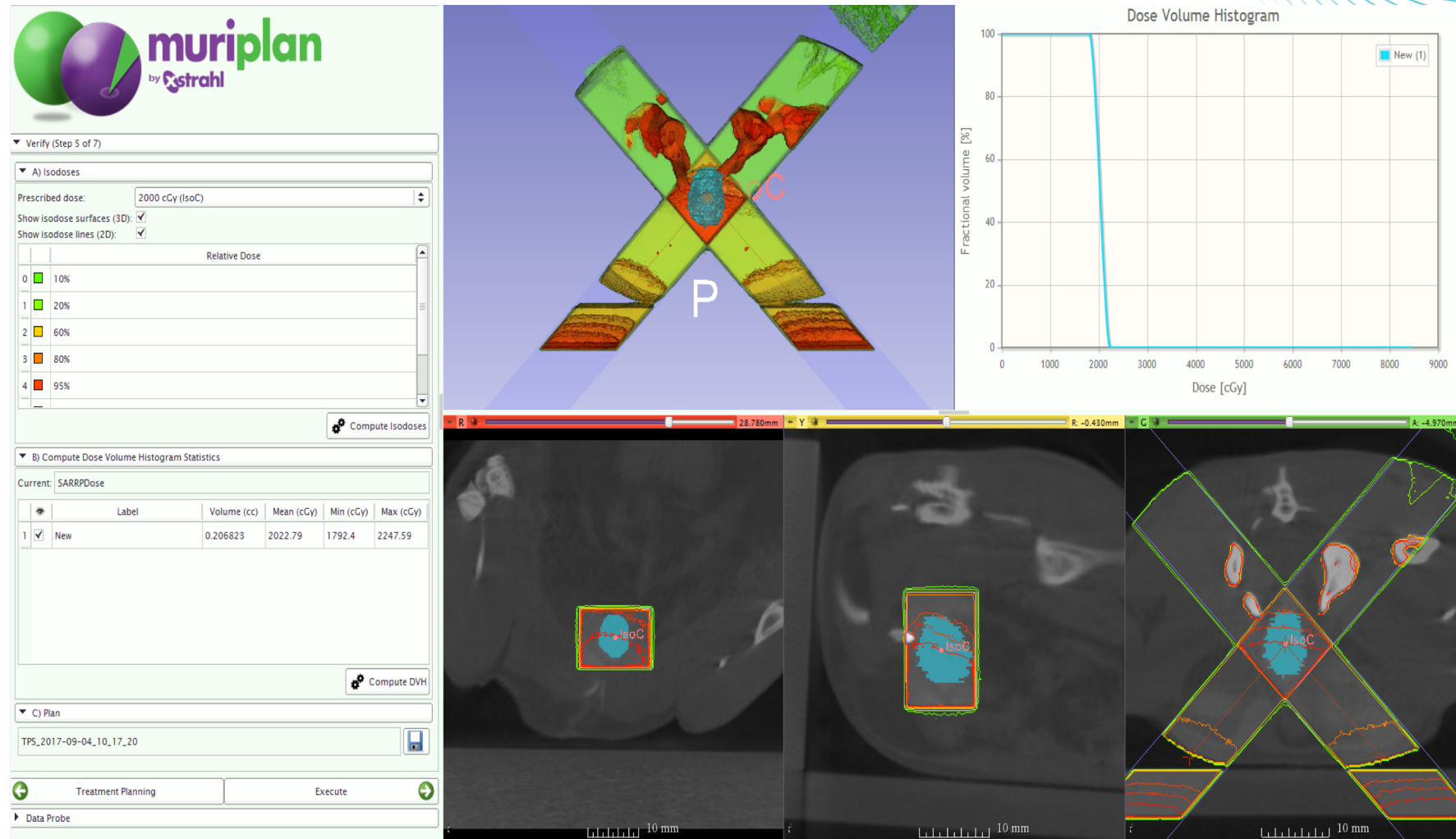


MSC : « injury drugstore »
Allogenic graft



263 clinical trials in the world

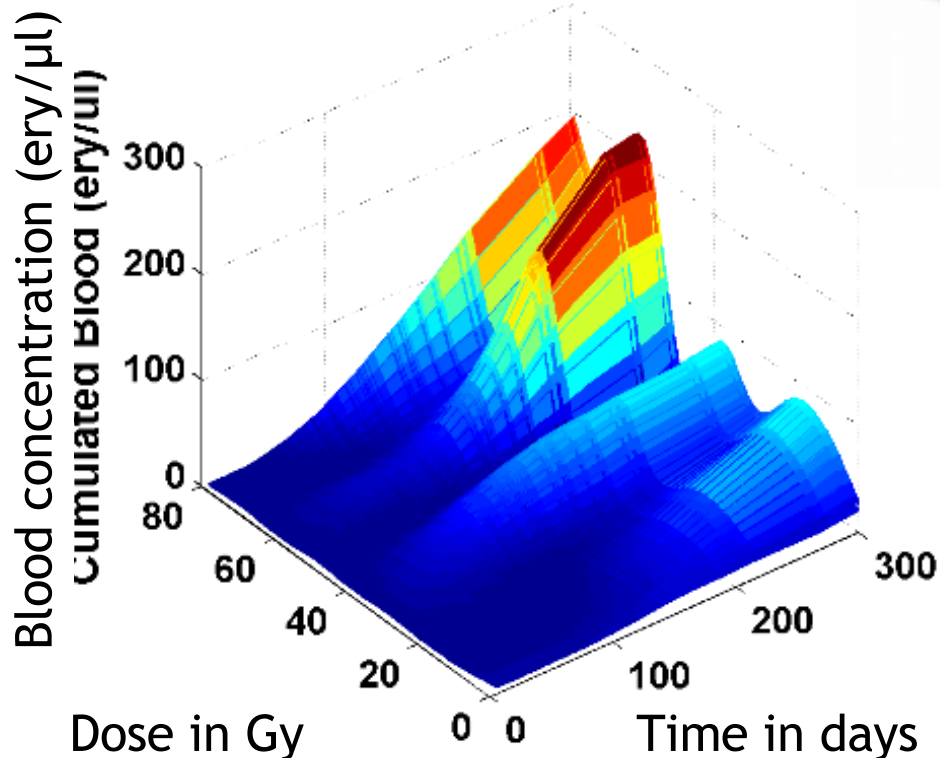




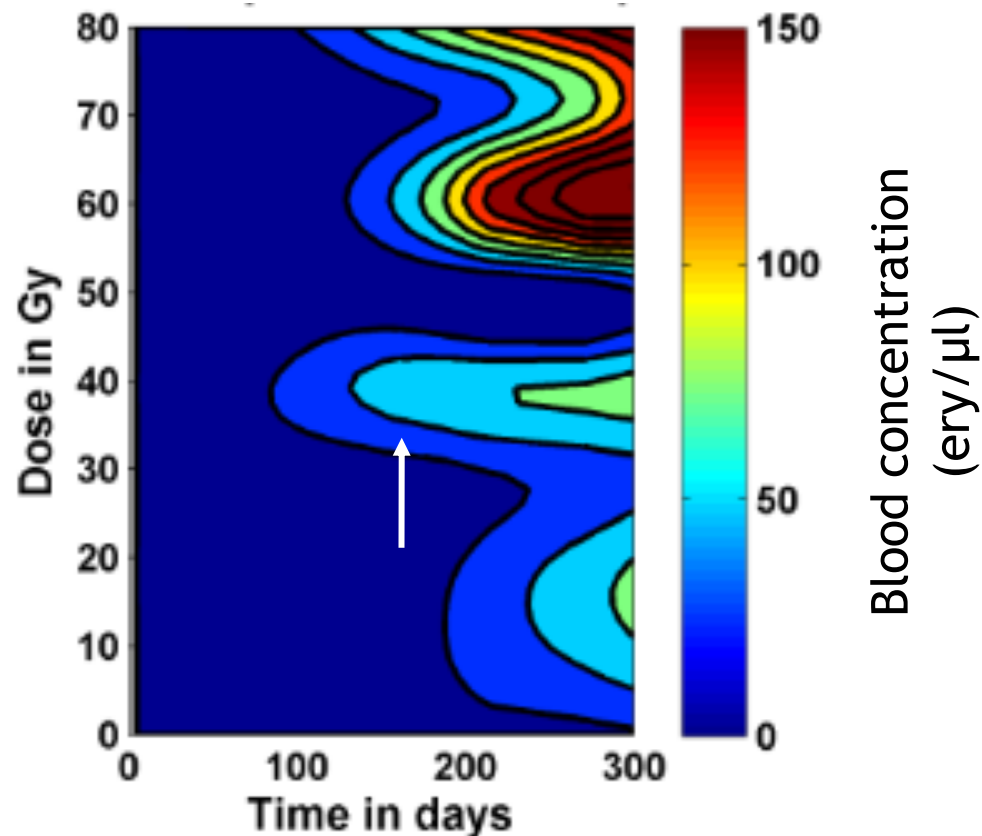
- 2 irradiation beams
- Maximized the dose on the bladder and minimized the dose on surrounding tissues (skin, fat)
- 0 Side effects

Hematuria (urinary strips exacto pro biosynex)

Hematuria
3D kinetics dose-time



Hematuria
2D kinetics dose-time



40 Gy :

- ↗ hematuria, occurring early
- Vascular lesions at 6 months post-irradiation

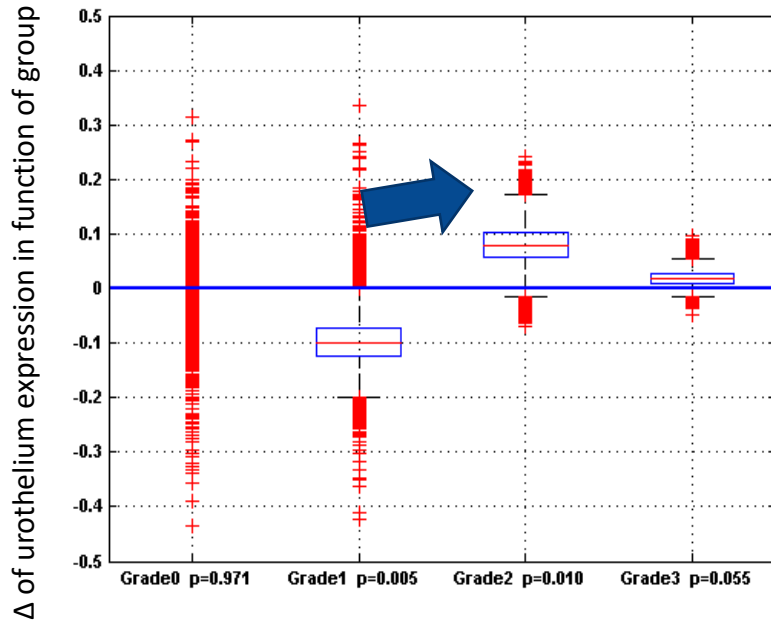


Urothelium integrity alteration: uroplakine 3

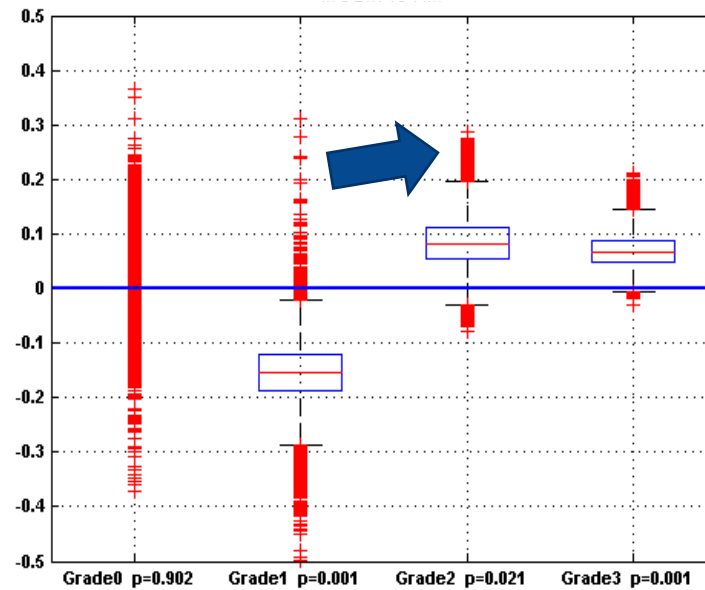
Grading of the whole urothelium for each animal
 Δ expressions of uroplakine 3, **8 months** in function of groups



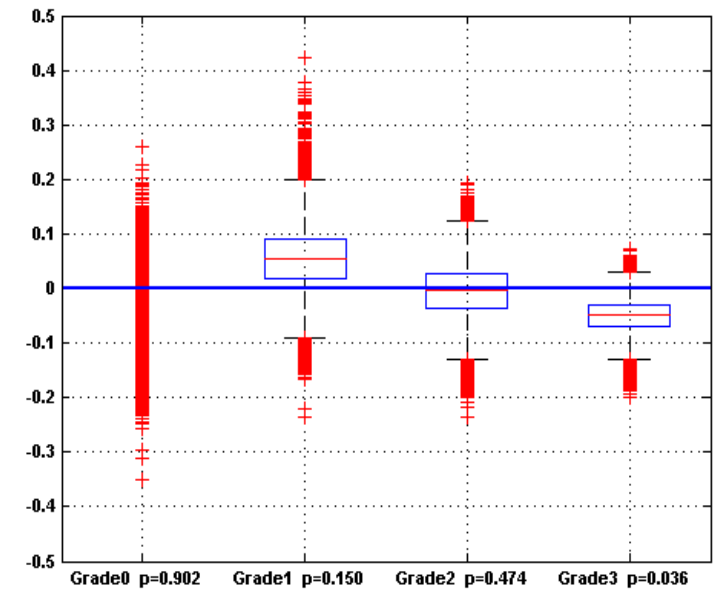
Irradiated untreated
VS
Unirradiated



Irradiated treated
VS
Unirradiated



Irradiated untreated
VS
irradiated treated

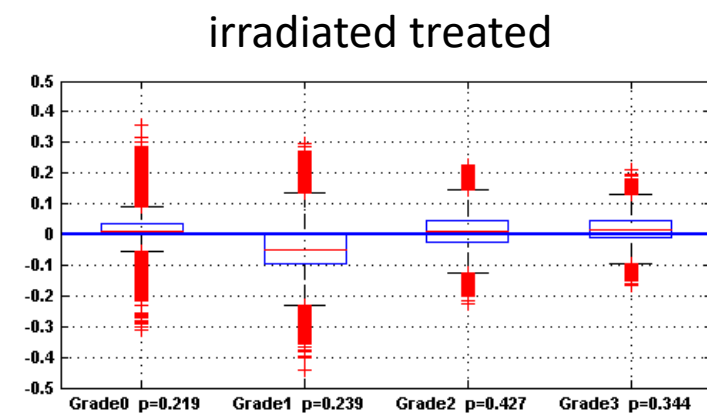
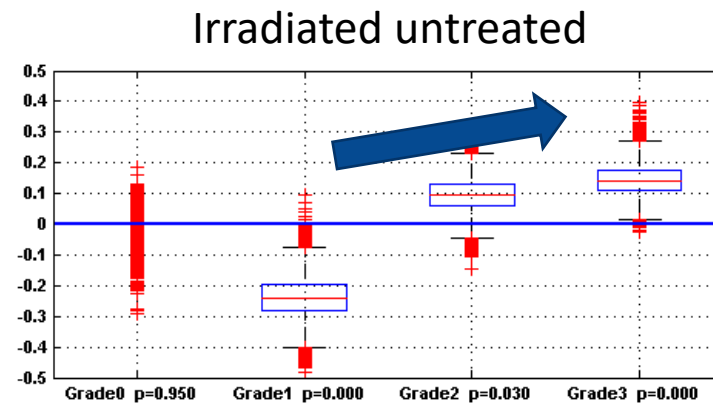
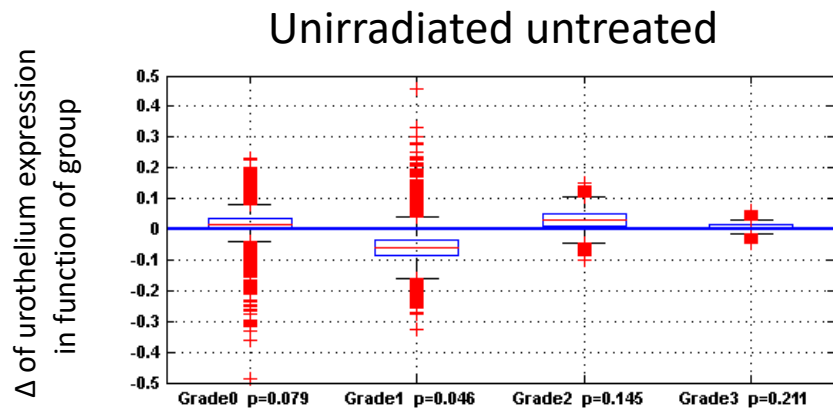
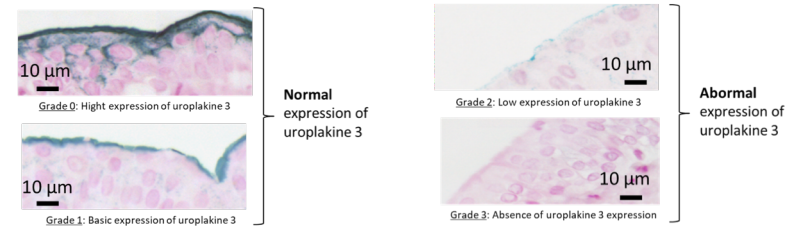


- Increase % of grade 2 and 3 after irradiation with or without MSC treatment
 - No difference after irradiation for treated compared to untreated

Urothelium integrity alteration: uroplakine 3

Grading of the whole urothelium for each animal

Δ expressions of uroplakine 3, **8 VS 12 months**

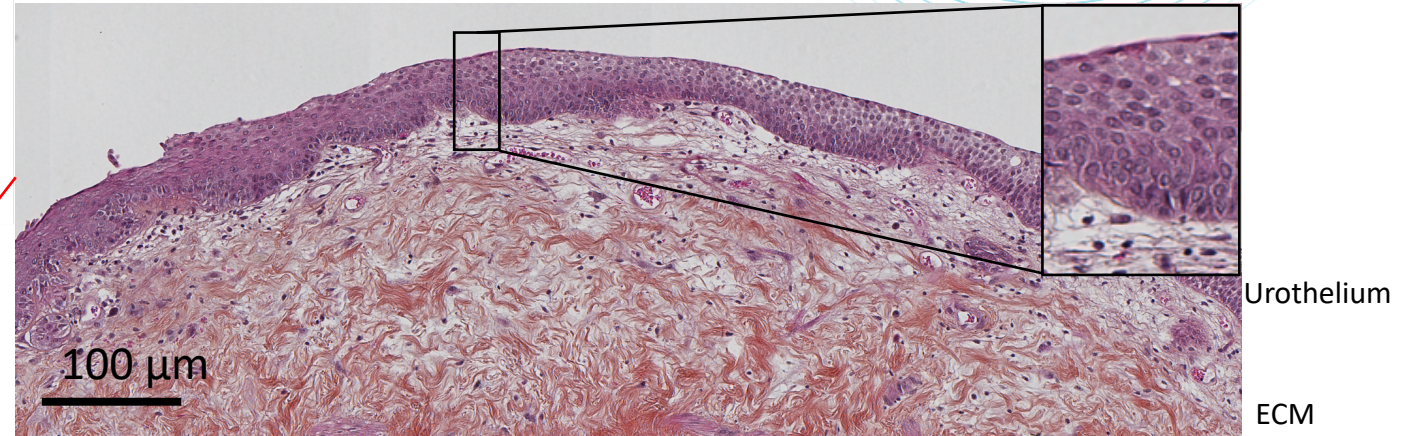
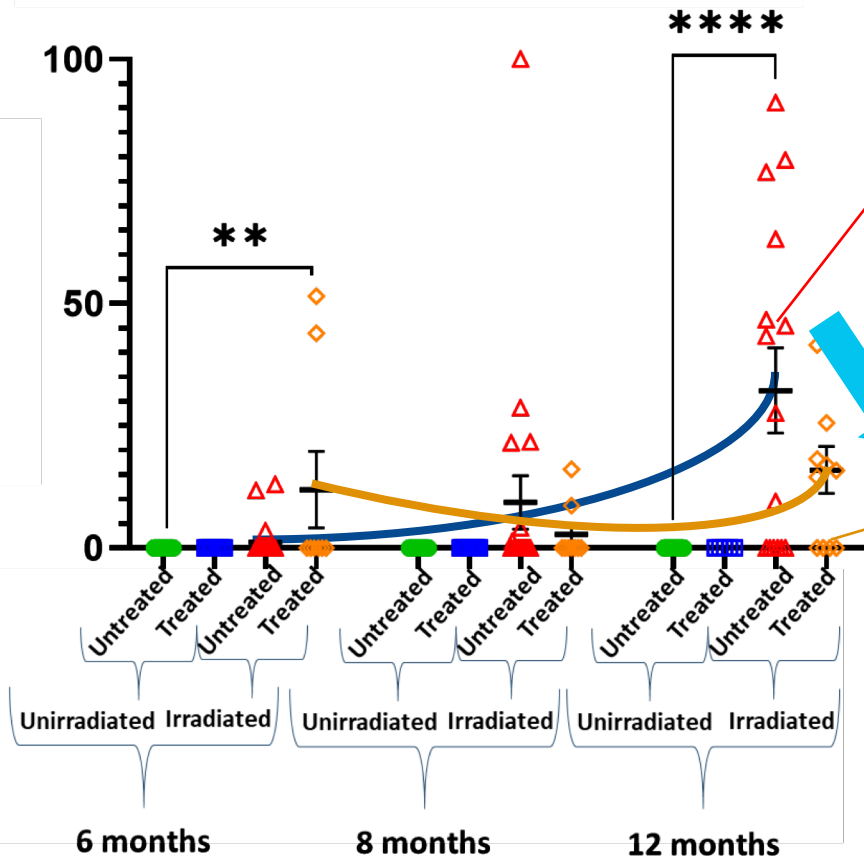


- Increase % of grade 2 and 3 in function of time for Irradiated untreated
 - MSC treatment keeps the grade 2 and 3 lesions low and stable

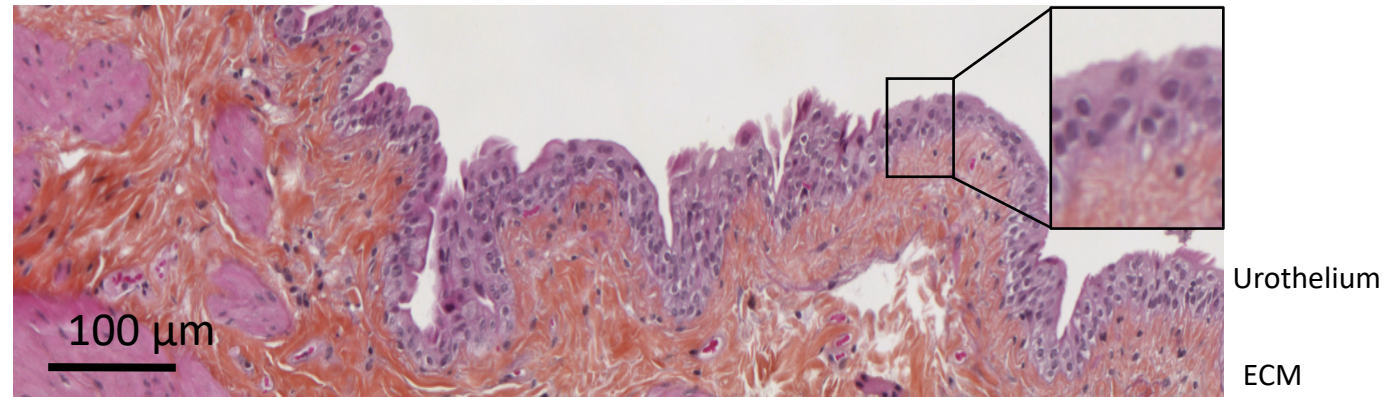


Urothelium integrity alteration: Thickness of urothelium

% of the urothelium concerned by an abnormal increase of thickness in function of time



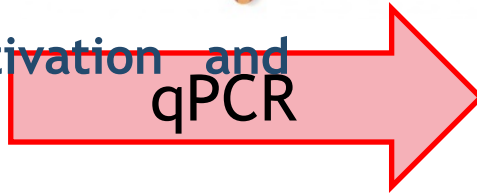
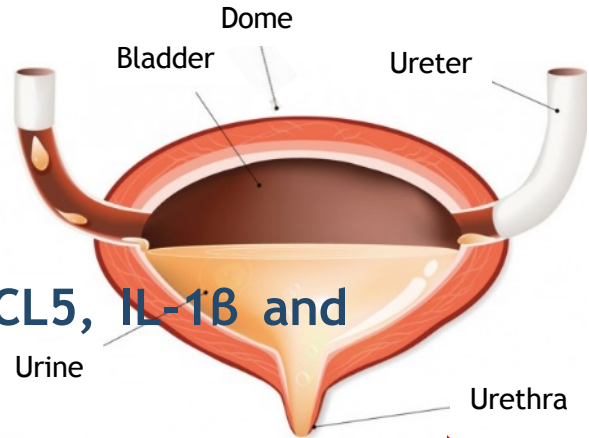
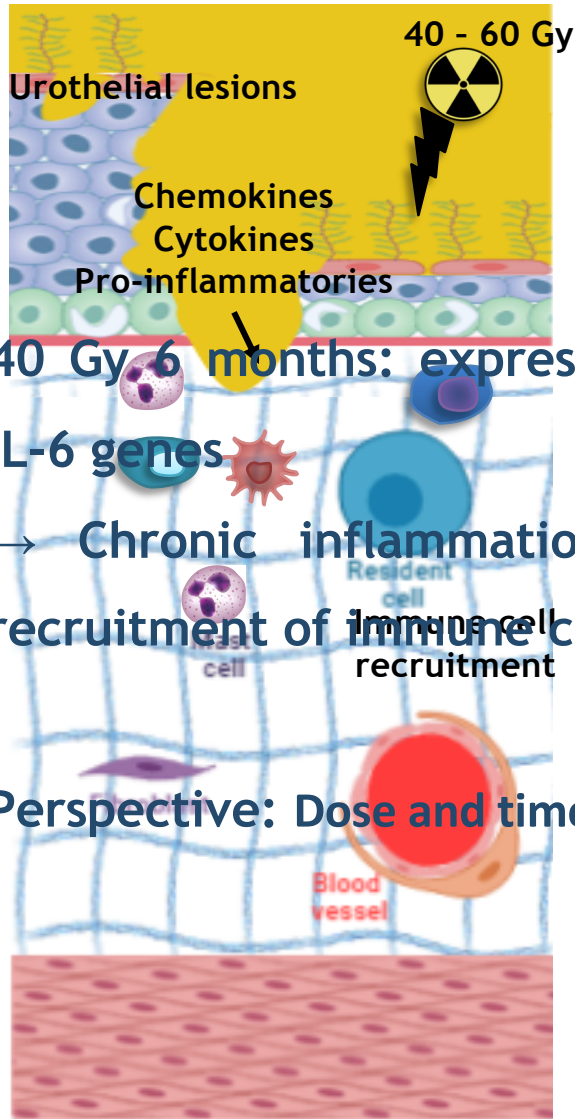
12 months abnormal increase in urothelial thickness



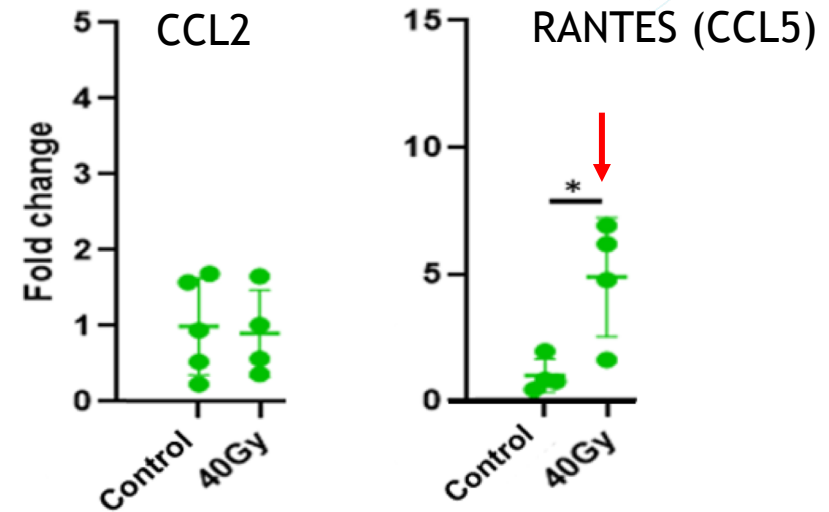
12 months without abnormal increase in urothelial thickness

- Irradiated untreated: Increase of urothelium thickness in function of time for
 - After MSC treatment : Increase in slower of urothelium thickness

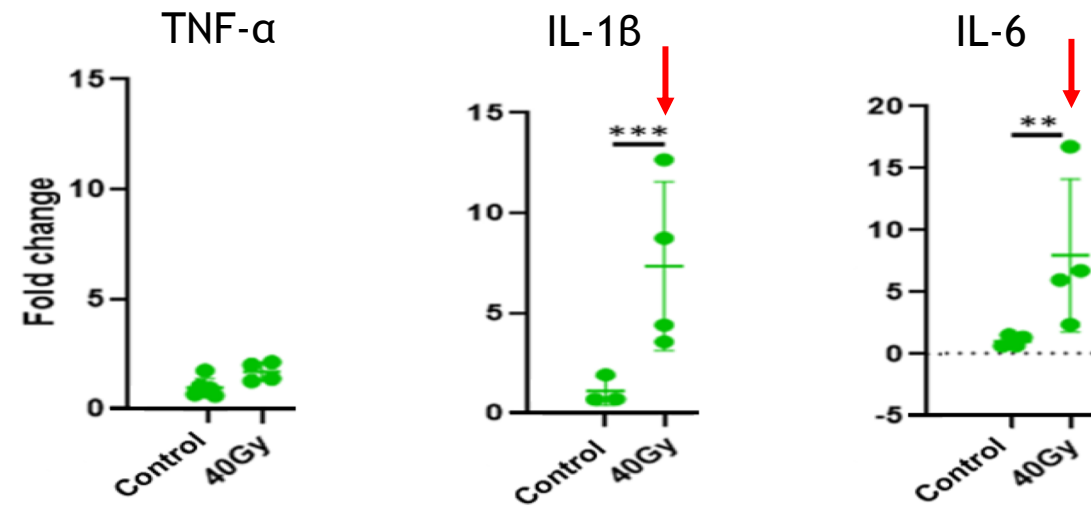
Inflammation at 6 months post-irradiation



Chimiokines

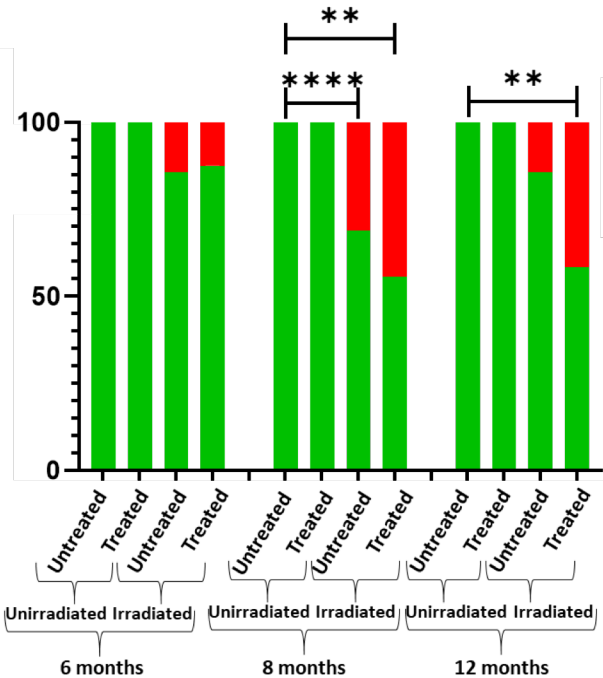


Pro-inflammatory cytokines

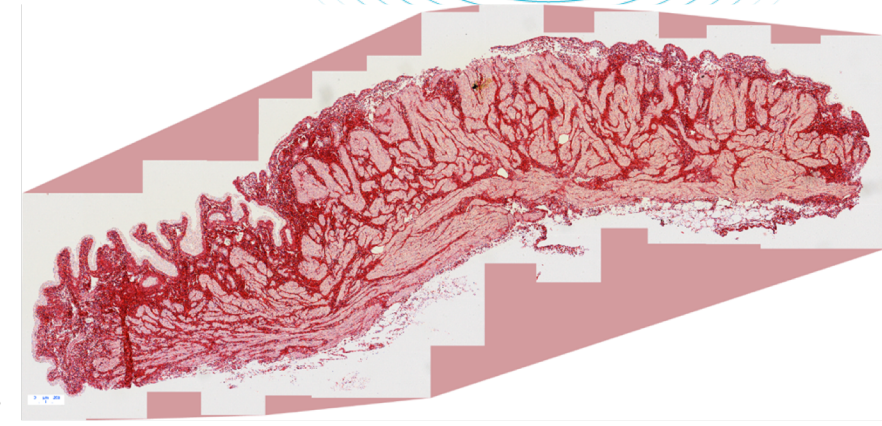
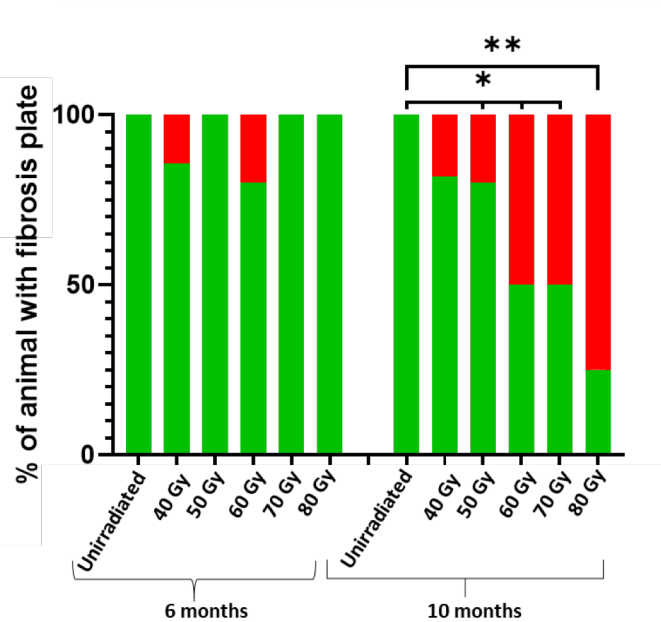


Fibrosis studies

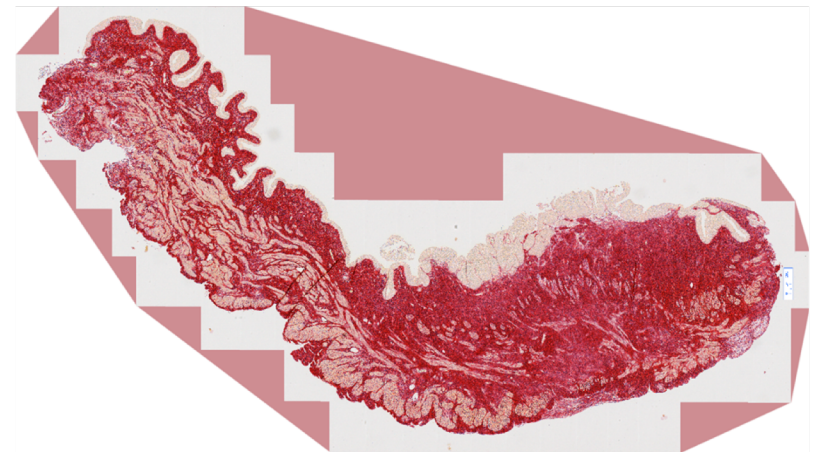
% of animals with fibrosis plaques as a function of time and conditions



% of animals with fibrosis plaques as a function of time and irradiation dose



12 months without fibrosis plaque

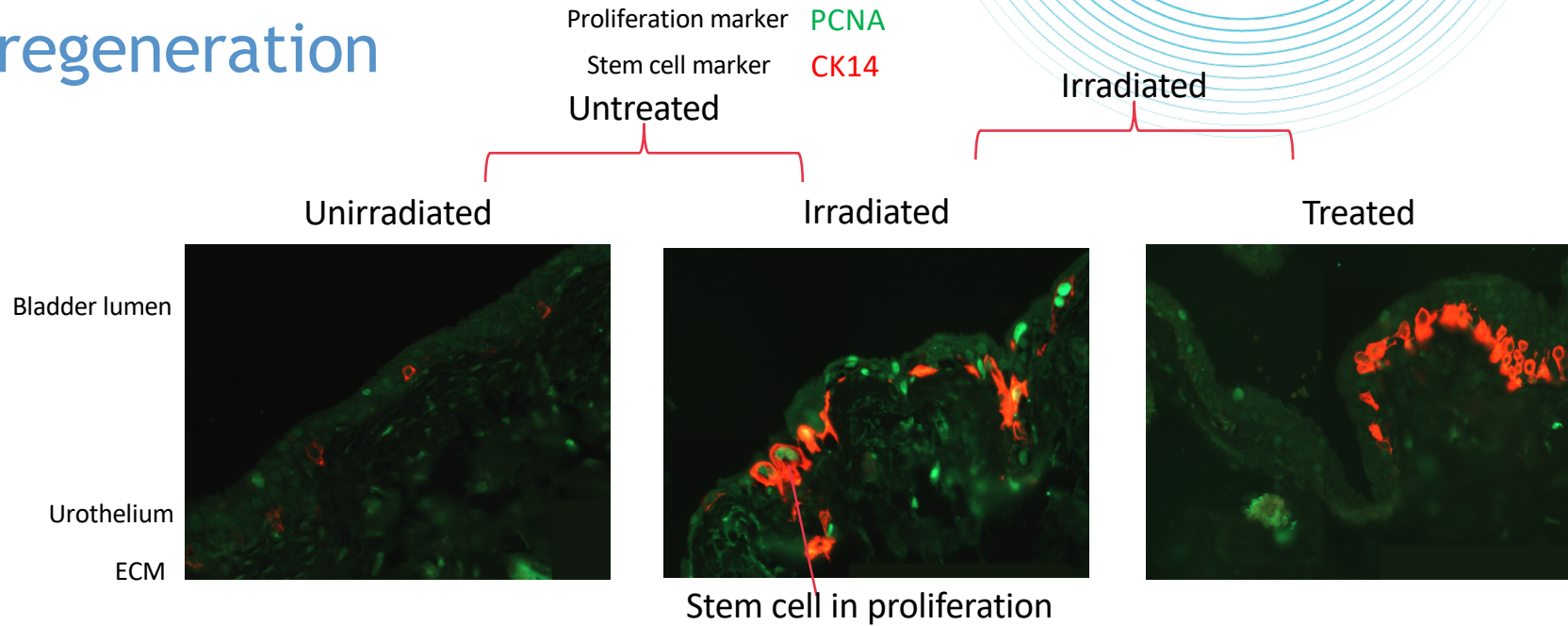
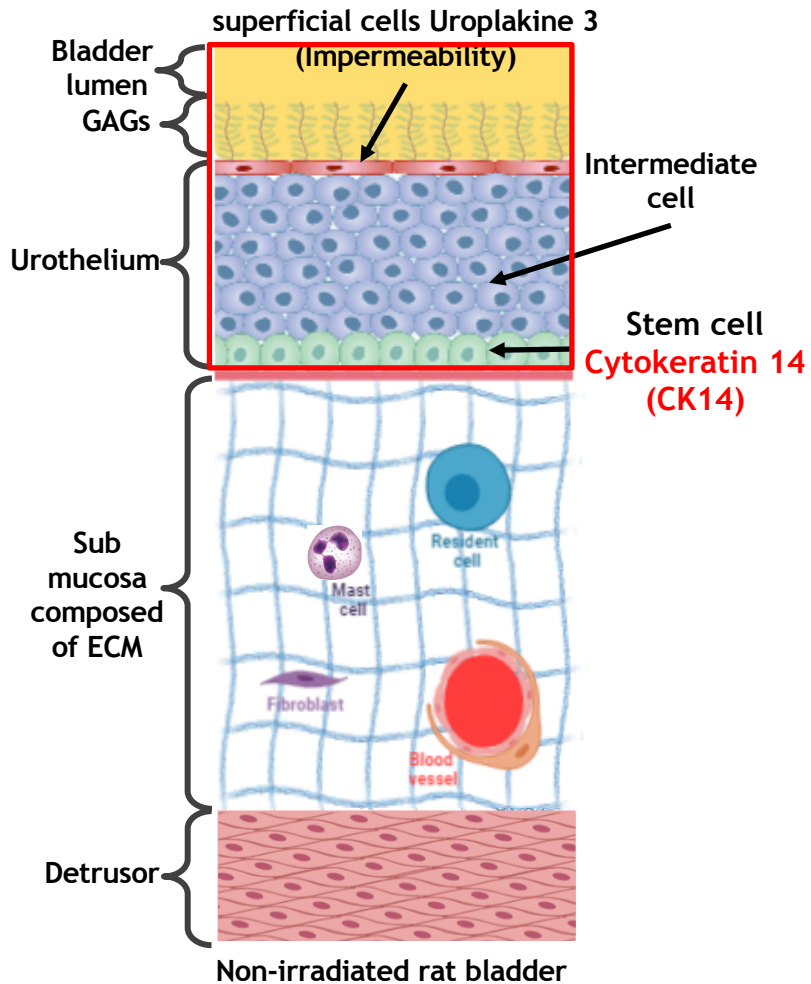


12 months with fibrosis plaque

■ positif
■ negatif

- Irradiation dose increases the number of animals with fibrosis plaque as a function of time
- At 12 months MSC treatment may increase number animal with fibrosis plaque (no significant)

Perspectives: Urothelium regeneration



- **MSC treatment affect the basal stem cell compartment?**
 - Investigation at 6 and 8 months in progress

