ECELLFRANCE Montpellier platform: therapeutic use of mesenchymal stem cells in murine models of osteoarticular and autoimmune diseases

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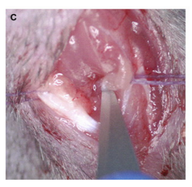
Introduction

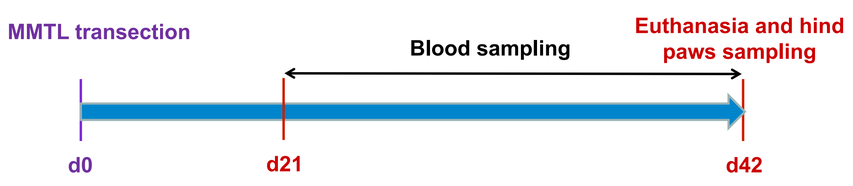
- The ECELLFRANCE Montpellier platform is dedicated to the evaluation of the immunomodulatory and regenerative properties o mesenchymal stem cells in osteoarticular and autoimmune diseases.
- The expertise relies on the use of relevant pre-clinical murine models of these diseases and the follow-up of clinical, biological, immunological and structural parameters.

Osteoarthritis murine models

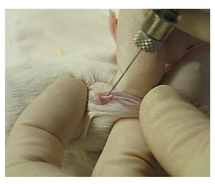
DMM model: Destabilization of the Median Meniscotibial ligament

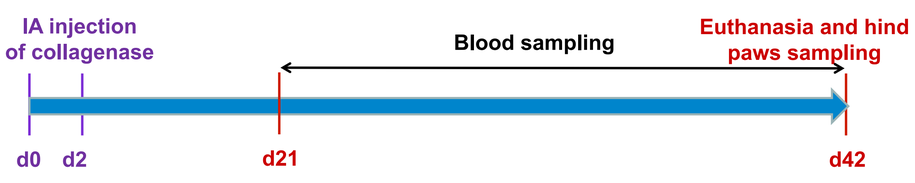


S. S. Glasson et al., *Osteoarthritis and Cartilage*, 2007



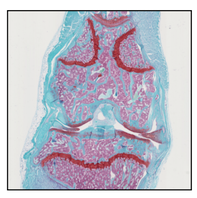
CIOA murine model: Collagenase-Induced OsteoArthritis



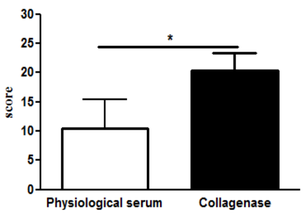


➢ Osteoarthritis can be induced on immunocompetent C57BL/6 mice or immunodeficient mice.

Histological analysis

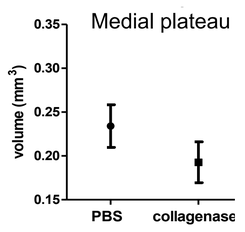


Safranin O-Fast Green staining

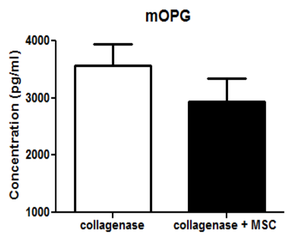


Histological scoring

CLSM analysis



OPG dosage in blood

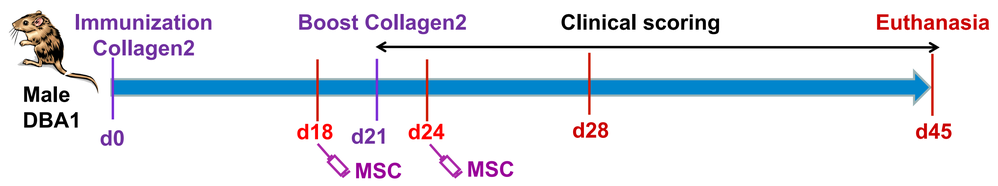


➢ Cartilage degradation is evaluated after safranin 0 staining on histological slides.

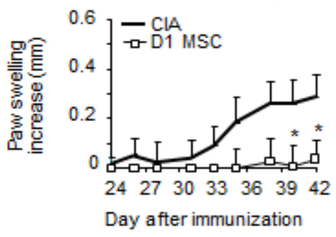
➢ Complementary analyses: CLSM and dosage of biomarkers in serum (OPG, CTX-II, IL-6,…) can be performed

Collagen-Induced Arthritis model

➢ The pre-clinical model of Collagen-Induced Arthritis (CIA) is one of the most relevant model of Rheumatoid Arthritis.



CIA scoring

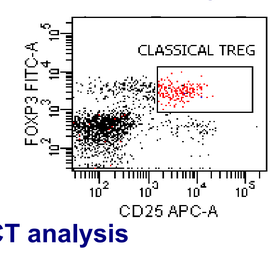


Cytokines dosage

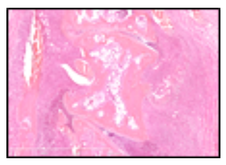
- Pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1β, IL-6,…)
- Anti-inflammatory cytokines (IL-5, IL-10, IL-13 …)

Bouffi et al., *Plos One*, 2010

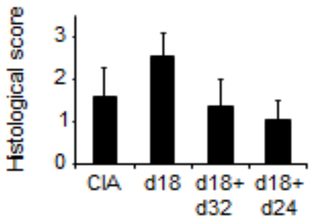
FACS analysis



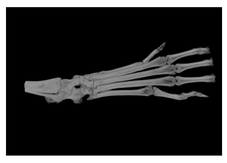
Histological analysis



Hematoxylin Eosin staining



Micro-CT analysis

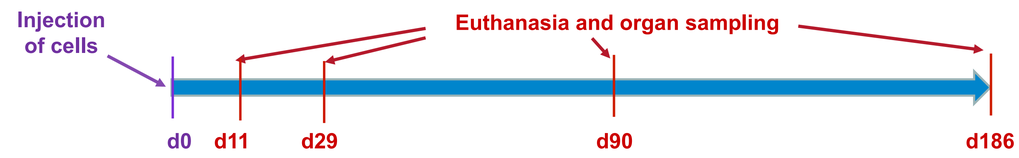


➢ Clinical score is monitored every two days after boost.

➢ Inflammation is evaluated by quantification of immune cell subsets (Th1, Th17, Treg cells) in spleen and draining lymph nodes, cytokine secretion. Cartilage and bone degradation is evaluated by histological and µ-CT analyses.

Biodistribution studies

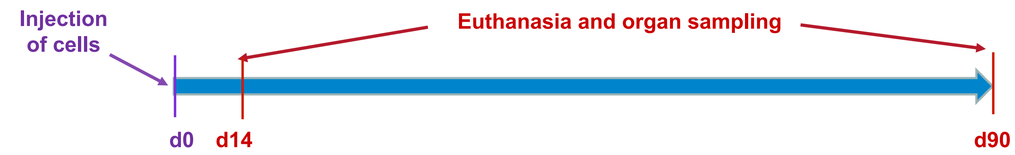
➢ Stem cells are injected in immunocompetent or immunodeficient mice.



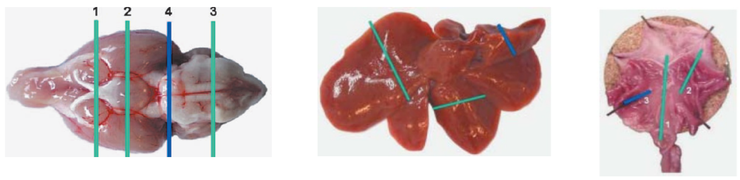
➢ Human cells are detected via quantification of the number of Alu sequences by qPCR or in situ hybridation (FISH) on fixed organs.

Toxicology studies

➢ Stem cells are injected in immunocompetent or immunodeficient mice.



Organs trimmed according to RITA and NACAD procedures



brain liver stomach

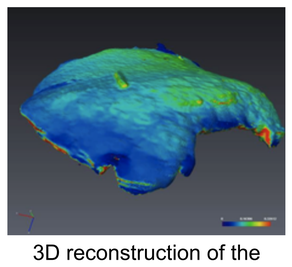
➢ Mice are monitored twice a week (weight, general behaviour).

➢ Organs are processed for routine histology (paraffin inclusion, sections and hematoxylin eosin staining) and analysed by an anatomopathologist.

Cartilage imaging by confocal Laser Scanning Microscopy

Confocal Laser Microscope (Leica, Sp5-II)

Scan of joint cartilage



3D reconstruction of the medial plateau of articular cartilage in the knee joint (Avizo)

Quantification of cartilage degradation

Cartilage thickness, Volume and surface

➢ Degradation of articular cartilage can be evaluated by CLSM after 3D reconstruction of the tissue and the quantification of morphometric parameters: cartilage volume, thickness and surface.

Cartilage and bone imaging by µCT

In vivo micro-CT (Skyscan 1176)

Scan of mice paws

3D reconstruction of murine knee joint

aluation of bone degradation

➢ Bone tissue can be analysed by *in vivo* longitudinal studies. Multiple organs (cartilage, lung, heart, kidney,…) can be analyzed at euthanasia after fixation and process using a contrast agent

➢ After 3D reconstruction, tissue integrity is evaluated by quantification of several parameters (as example for bone: volume, surface, density, trabeculae number,…)