Preclinical models to improve the therapeutic index of radiotherapy

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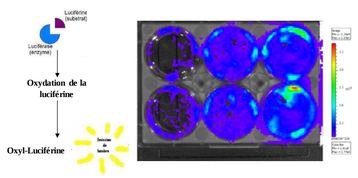
Introduction

Radiotherapy (RT) is a major weapon in cancer therapy, and approximately 50% of all patients with cancer can benefit from RT in the management of their disease. The therapeutic index of the treatment relies on both tumor control and normal tissue tolerance. Efforts to develop new approaches to optimize tumor and normal tissue differential response are crucial to improve the clinical outcomes both by increasing the probability of cancer cure and/or by decreasing normal tissue toxicity.

The research and development of novel strategies to improve the efficacy of radiotherapy or to limit its toxicity require the use of appropriate preclinical models. Our research team since many years develops and optimizes mouse models to study radiotherapy/drug combinations, with a focus on immunomodulators. These models offer key opportunities to study novel therapeutic approaches to improve the therapeutic index of radiotherapy, which can be transferred to the clinic.

Models

1. Bioluminescent orthotopic cancer models

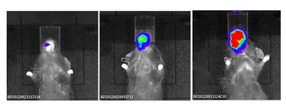


Luciferase-expressing tumor cells injected in syngeneic immunocompetent mice

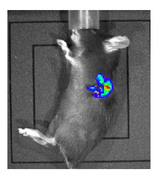
***In vivo* imaging using IVIS systems (Perkin Elmer)**

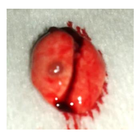
- Head and neck cancer
  - Grafting of HPV-positive TC1-luc cells at a submucosal site of the inner lip in C57BL/6 mice under anesthesia
  - Tumor development monitoring
    Bioluminescence imaging and survival
  - Necropsy
    A large tumor mass onto the snout, body weight loss due to feeding issues

Days after tumor challenge



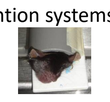
- Lung cancer
  - Transpleural injection of lung carcinoma LL2-luc cells in the parenchyma of the lung performing a small surgery
  - Tumor development monitoring
    Bioluminescence imaging and survival
  - Necropsy
    A localized tumor mass, atrophied lungs, lung nodules, pleural effusion, mediastinal tumor masses, disseminated pleural implants



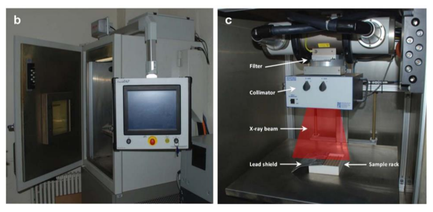


2. Conventional cabinet and image-guided stereotactic irradiations

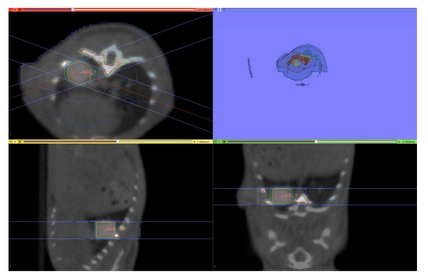
- Localized irradiation using a conventional cabinet
  - Mice are immobilized using contention systems or kept under isoflurane anesthesia



  - A lead shield covering allows localized irradiations



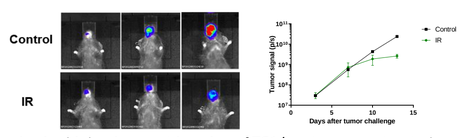
- Stereotactic irradiation using an image-guided irradiation system
  - The SARRP irradiator (Small Animal Radiation Research Platform) uses a X-ray source mounted on an isocentric gantry which revolves at 180°and which produces both the irradiation and the image allowing:
  - to capture high resolution (200 µm) scan images
  - to target a specific target of the animal (accuracy of 0.2 mm) via the Muriplan treatment planning system
  - to deliver the irradiation beam at that point with field sizes ranging from 0.5 mm in diameter (round collimator) to 10 x 10 mm² (square field collimator).



Collaboration with IRSN, F. Milliat-M. Dos Santos

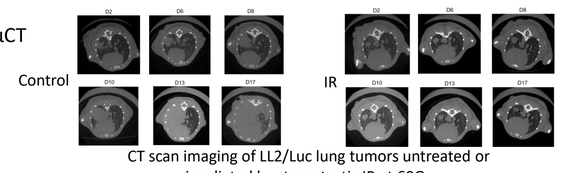
3. Tumor growth and immune environment analyses

- Follow-up of the tumor growth
  - Tumor growth can be followed by *in vivo* imaging and quantification of bioluminescent signals



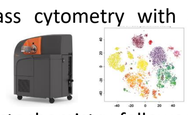
In vivo bioluminescent imaging of TC1/Luc tumors untreated or locally irradiated (IR) at 7.5Gy and signal quantification

  - In collaboration with IRSN we have also access to a µCT scanner



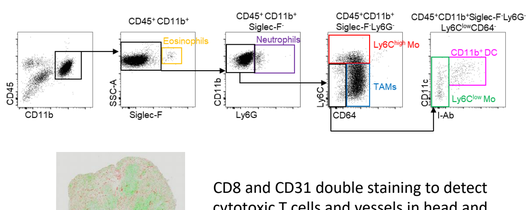
CT scan imaging of LL2/Luc lung tumors untreated or irradiated by stereotactic IR at 60Gy

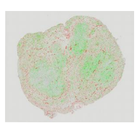
- Analysis of tumor immune infiltrate
  - Multicolor flow cytometry (BD LSRFortessa cell analyzer)
  - High parameter mass cytometry with a panel of 33 immune markers



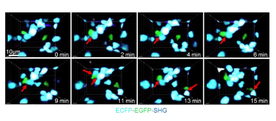
t-sne visualization

  - Multiplex immunohistochemistry followed by automated image detection and quantification
  - Time-lapse intravital biphoton microscopy, in collaboration with A. Boissonnas, Cimi Paris





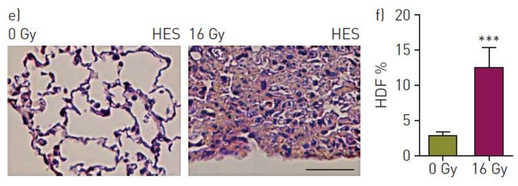
CD8 and CD31 double staining to detect cytotoxic T cells and vessels in head and neck tumors



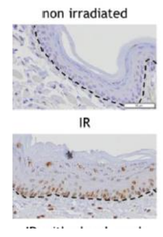
Time-lapse intravital microscopy in irradiated head and neck tumors from transgenic mice bearing fluorescent reporters

4. Radiation-induced toxicity models

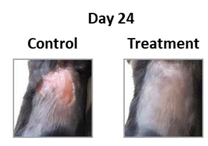
- Radiation-induced lung fibrosis
  - Mice receive a localized irradiation at the thorax at 16Gy
  - Development of lung fibrosis starting 15 weeks post irradiation
  - Detection of fibrosis by CT-scan and histological analysis followed by digital image analysis (performed by Biocellvia, Marseille)



- Radiation-induced oral mucositis
  - Mice receive a localized irradiation to the snouts at 18-20Gy
  - Development of oral mucositis starting 1 week post irradiation
  - Analysis by macroscopic Parkins scoring and by histology



- Radiodermatitis
  - Mice receive a localized irradiation of the dorsal skin at 4 fractions of 12Gy
  - Development of radiodermatitis starting 2 weeks post irradiation
  - Analysis by macroscopic scoring and by histology



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