



Novel PSMA+ and FAP+ mouse models to support the development of novel molecular radiotherapy agents



Sarah Belderbos, Peggy Provent, Solène Fernandez, Maëva Albanese, Marie Grunenwald, Florent Potvain, Kenny Herry, Didier Grillot, Nicolas Ancellin, Eftychia Koumarianou

Oncodesign Services, Dijon, France

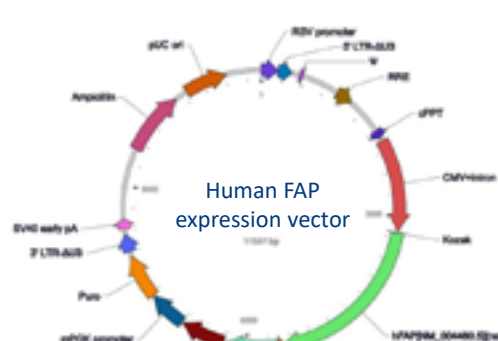
Introduction

- Robust preclinical models are required for the evaluation of novel molecular radiotherapies (MRT):
 - accurately mimicking human tumor microenvironments
 - Expressing optimal levels of target proteins.
- Two biomarkers of interest in the field of MRT and Oncology are:
 - Fibroblast activation protein (FAP):** Minimally expressed in regular tissues, upregulated in cancer-associated fibroblasts within the tumor microenvironment.
 - Prostate-specific membrane antigen (PSMA):** overexpressed in most prostate cancers.
- Oncodesign Services already had **PSMA**-overexpressing models available, but only on Balb/c Nude mice. This strain is radiosensitive due to a mutation and has become unavailable for purchase in the recent months.
- Furthermore, inherently expressing **FAP** models are available, but have minimal FAP-expression
- To close the gap in our catalogue, **Oncodesign Services has recently developed novel FAP and PSMA-overexpressing models in non-radiosensitive mouse strains.**

Methods

Development of two FAP-overexpressing mouse models

- Transduction of two cell lines using a lentiviral vector encoding for FAP and ZsGreen
 - HT-1080: FAP-negative, 0% FAP+ cells
 - U-87-MG: low FAP-positive, 15% FAP+ cells
- Confirmation of *in vitro* FAP expression using immunofluorescence and flow cytometry
- Subcutaneous engraftment of 10×10^6 HT-1080-FAP or U-87-MG-FAP cells in Swiss Nude mice
 - Twice weekly monitoring of body weight and tumor volume
 - Harvesting of tumors at ethical endpoint and *ex vivo* confirmation of FAP expression

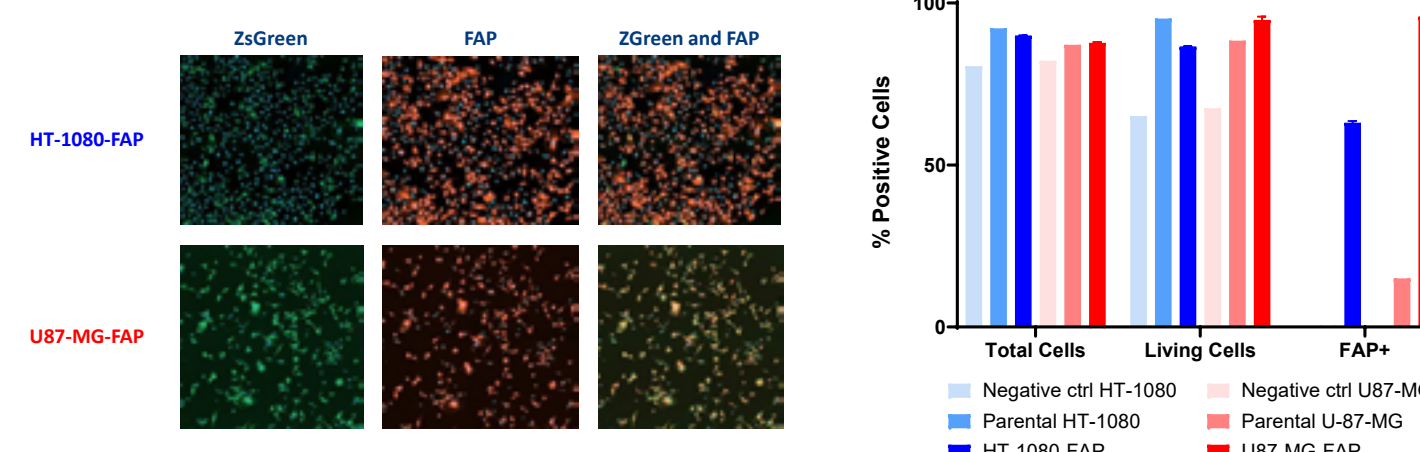


Development of two PSMA-overexpressing mouse models

- Subcutaneous engraftment of 10×10^6 22RV1-Luc-mCherry or Ln-Cap C4.2 cells (with matrigel) in male BRGSF mice
- Before the engraftment of 22RV1-Luc-mCherry, one group of mice was castrated
- Use of enriched diet (JL Mouse) to prevent known cachexia
- Twice weekly monitoring of body weight and tumor volume

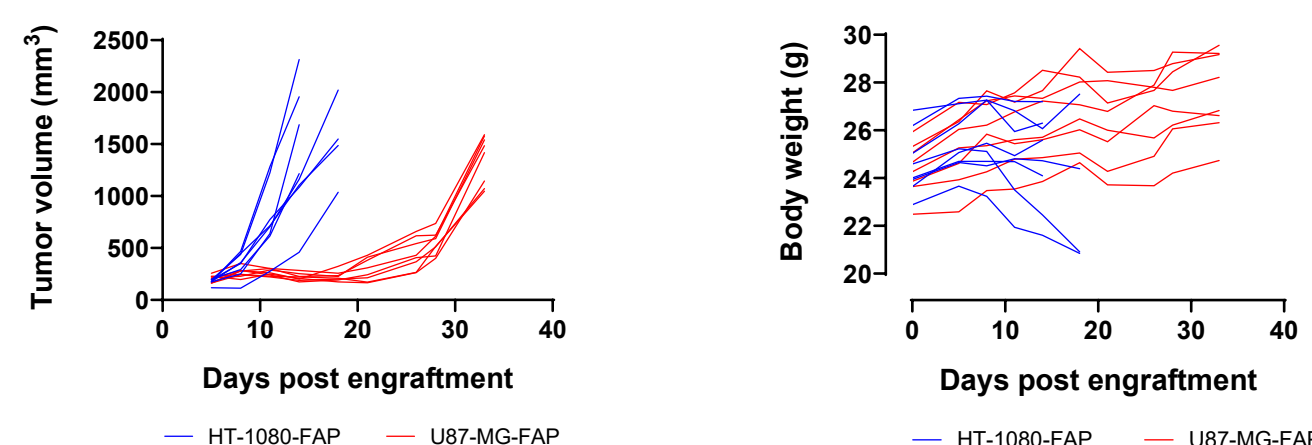
Development of two FAP-overexpressing cell lines

- HT-1080 and U-87-MG cells were successfully transduced with a lentiviral vector encoding for ZsGreen and FAP.
- Expression of both ZsGreen and FAP was confirmed by immunofluorescence and flow cytometry.
 - HT-1080-FAP: 63.00 ± 0.57 % FAP-positive cells
 - U-87-MG-FAP: 95.80 ± 0.28 % FAP-positive cells



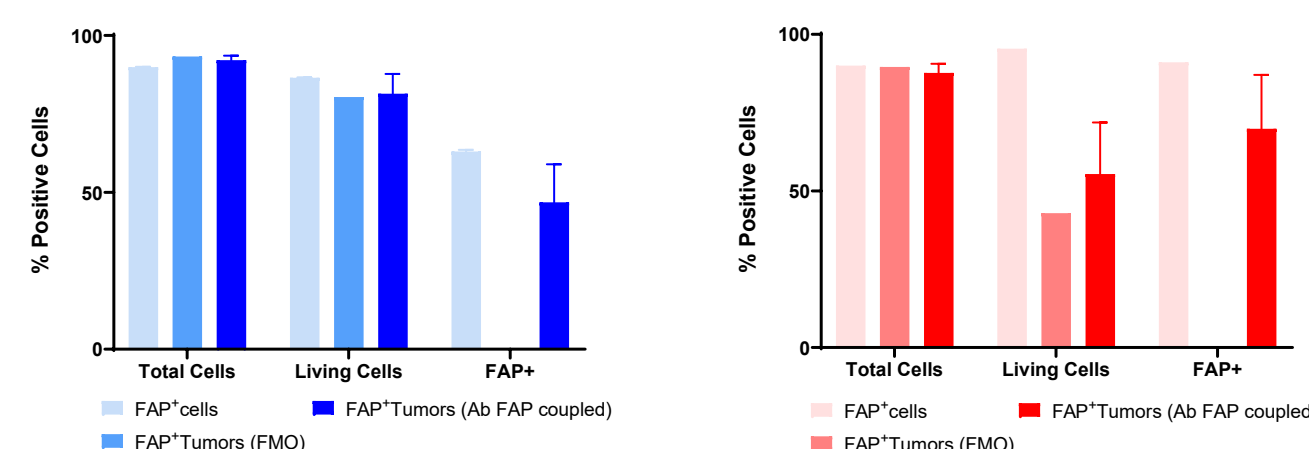
Development of FAP-overexpressing mouse models

- Swiss Nude mice subcutaneously engrafted with HT-1080-FAP cells presented with rapid tumor growth, which was associated with important body weight loss
- Swiss Nude mice subcutaneously engrafted with U-87-MG-FAP cells presented with a very slow, but homogenous tumor growth.



Ex vivo confirmation of FAP expression using flow cytometry

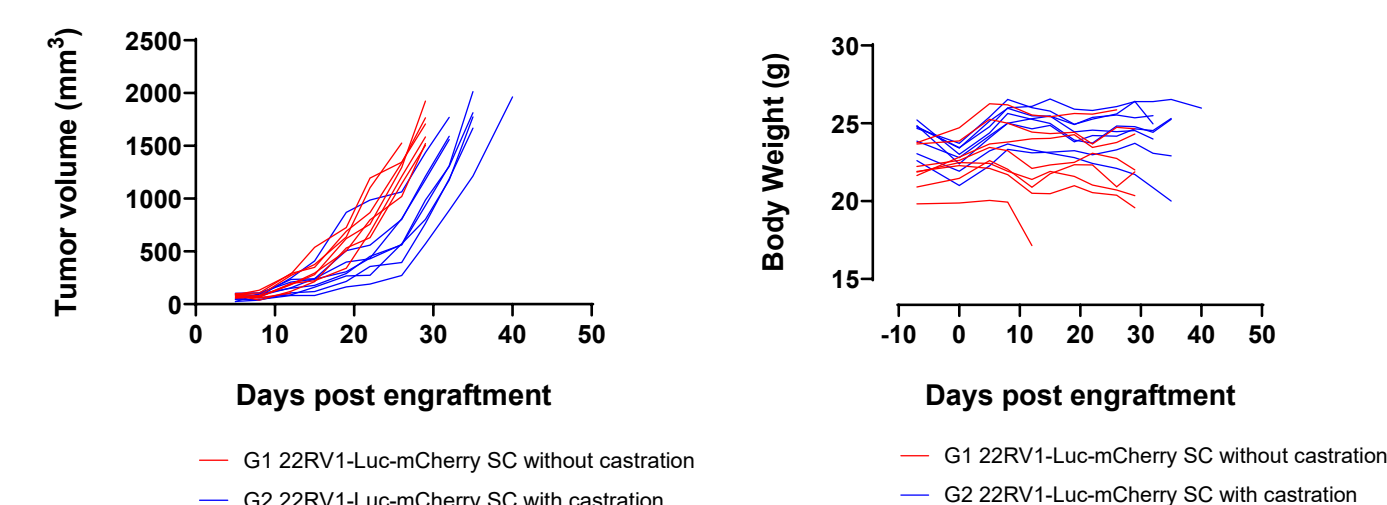
- Ex vivo* flow cytometry confirms FAP expression in both tumor models:
 - HT-1080-FAP tumors: 46.85 ± 12.11 % FAP-positive cells
 - U87-MG-FAP tumors: 69.88 ± 17.20 % FAP-positive cells
- Ex vivo* FAP expression results correspond to the *in vitro* FACS results.



Development of PSMA-overexpressing mouse models

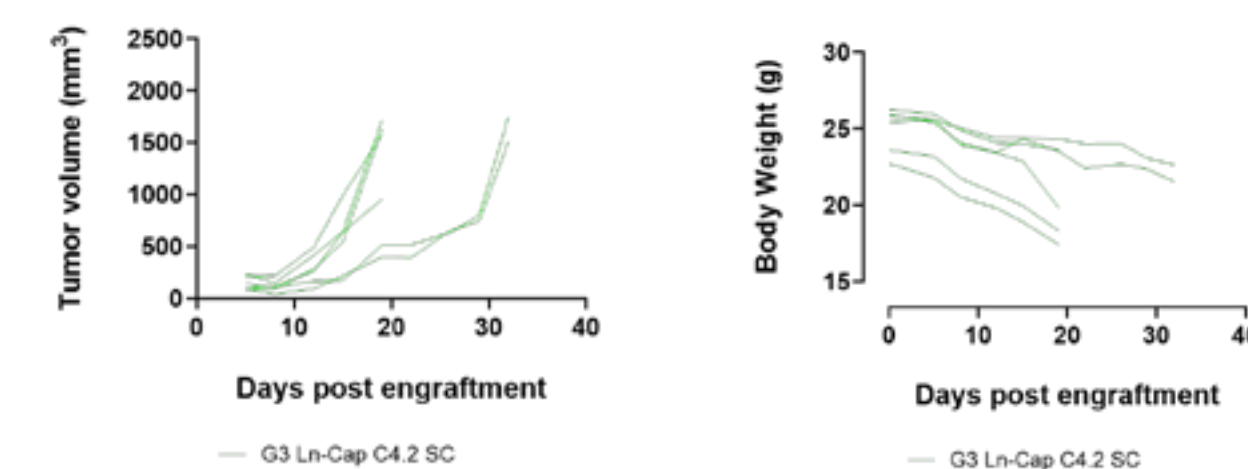
1. 22RV1-Luc-mCherry

- Non-castrated BRGSF mice subcutaneously engrafted 22RV1 cells present with rapid and homogenous tumor growth.
- Tumor growth in castrated BRGSF mice subcutaneously engrafted with 22RV1 cells was slightly slower and more heterogenous.



2. Ln-Cap C4.2

- BRGSF mice subcutaneously engrafted with Ln Cap C4.2 cells presented a very heterogenous tumor growth, which was associated with a slow, but significant body weight loss despite the use of enriched diet



Conclusion

- Four novel preclinical mouse models were successfully developed at Oncodesign Services:
 - Two FAP-overexpressing models with variable expression of FAP, mimicking clinical heterogeneity (negative HT-1080, slightly positive U-87-MG, positive HT-1080-FAP and overexpressing U-87-MG-FAP cells)
 - Two PSMA-overexpressing models, developed in non-radiosensitive, BRGSF mice
 - Further histological validation of the models is on-going.
- These models offer new opportunities to validate novel imaging agents and molecular radiotherapies targeting FAP and PSMA, facilitating their translation to clinical applications.