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

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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.

- Tow biomarkers of interest in the field of MRT and Oncology are:
  1. **Fibroblast activation protein (FAP):** Minimally expressed in regular tissues, upregulated in cancer-associated fibroblasts within the tumor microenvironment.
  2. **Prostate-specific membrane antigen (PSMA)**: overexpressed in most prostate cancers.

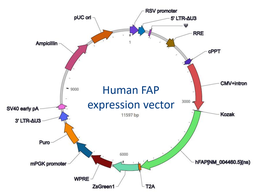
- Oncodesign Services already had **PSMA**-overexpressing models available, but only on Balb/c Nude mice. This strain is radiosensitve due to a mutation and has become unavailable for purchase in the recent months.
- Furthermore, inherently expressing **FAP** models are available, but have minimal FAP-expression

- To close the gap in our catalogue, **Oncodesign Services has recently developed novel FAP and PSMA-overexpressing models in non-radiosensitve 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
- Subcutanous 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



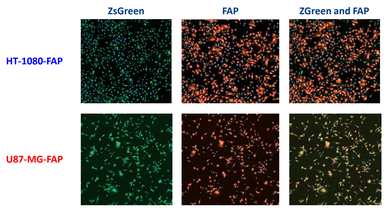
Development of two PSMA-overexpressing mouse models

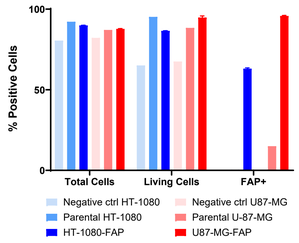
- Subcutanous engraftment of $10*10^6$ 22RV1-Luc-mCherry or Ln-Cap C4.2 cells (with matrigel) in male BRGSF mice
- Before the engraftment of 22RV1-Luc-mCherry, one group of mice was castrated
- Use of enriched diet (JL Mouse) to prevent known cachexia
- Twice weekly monitoring of body weight and tumor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

Results

Development of two FAP-overexpressing cell lines

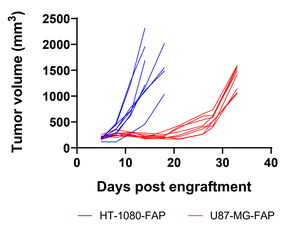
- HT-1080 and U-87-MG cells were successfully transduced with a lentiviral vector encoding for ZsGreen and FAP.
- Expression of both ZsGreen and FAP was confirmed by immunofluorescence and flow cytometry.
  - HT-1080-FAP: 63.00 ± 0.57 % FAP-positive cells
  - U-87-MG-FAP: 95.80 ± 0.28 % FAP-positive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

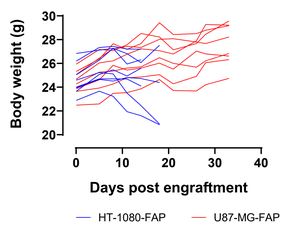




Development of FAP-overexpressing mouse models

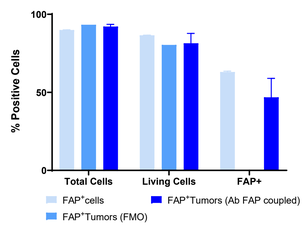
- Swiss Nude mice subcutanously engrafted with HT-1080-FAP cells presented with rapid tumor growth, which was associated with important body weight loss
- Swiss Nude mice subcutanously engrafted with U-87-MG-FAP cells presented with a very slow, but homogenous tumor growth.

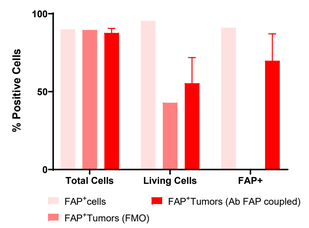




Ex vivo confirmation of FAP expression using flow cytometry

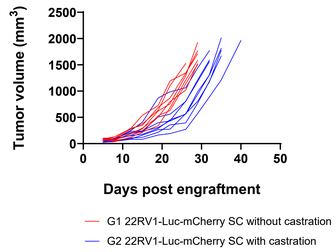
- *Ex vivo* flow cytometry confirms FAP expression in both tumor models:
  - HT-1080-FAP tumors: 46.85 ± 12.11% FAP-positive cells
  - U87-MG-FAP tumors: 69.88 ± 17.20% FAP-positive cells
- *Ex vivo* FAP expression results correspond to the *in vitro* FACS results.

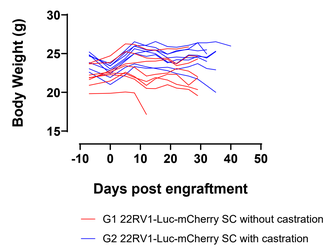




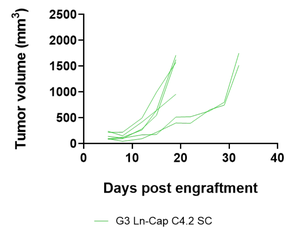
Development of PSMA-overexpressing mouse models

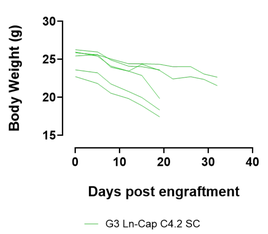
1. *22RV1-Luc-mCherry*
   - Non-castrated BRGSF mice subcutanously engrafted 22RV1 cells present with rapid and homogenous tumor growth.
   - Tumor growth in castrated BRGSF mice subcutanously engrafted with 22RV1 cells was slightly slower and more heterogenous.





2. *Ln-Cap C4.2*
   - BRGSF mice subcutanously 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 succesfully developped 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-radiosensitve, 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.

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