

Figures legends

Figure 1: Orthotopic tumor implantation and resection. a) Small tumor fragments were obtained from subcutaneous tumors. b) A small incision was made on the inner aspect of the mouse cheek (arrow), and a tumor fragment was implanted (dashed arrow). c) Internal (arrow) and external aspect of a developed orthotopic tumor. d) Orthotopic tumor resection through an external incision on the cheek. e) Wound closure by absorbable sutures. Complete wound healing was observed after 5-7 days. f) Weight curve of the mice showed normal weight gain after orthotopic tumor implantation. Minimal transient weight loss was observed for approximately 2-3 days following tumor resection.

Figure 2: Microscopic analysis of an orthotopic tumor, in vitro and in vivo expression of $\alpha\beta3$ integrin, and in vitro binding of the probe that targets $\alpha\beta3$ integrin on CAL33 cell line. a) Hematoxylin-eosin (H.E.) staining of orthotopic tumors showed a moderately differentiated squamous cell carcinoma with disorganized architecture, hyperchromatic nuclei, pleomorphism, increased mitotic activity and a greatly altered nuclear-cytoplasmic ratio. Original magnification: 20x objective lenses. b) Flow cytometry analysis of $\alpha\beta3$ integrin expression was performed using the CD51/CD61 R-Phycoerythrin clone 23C6 mouse anti-human antibody (Pharmingen, BD Bioscience). The results showed that the CAL33 cell line shows positive in vitro expression of $\alpha\beta3$ integrin. d) Flow cytometry analysis of binding of the RGD-based peptide RAFT-c(-RGDfK-)₄ which targets $\alpha\beta3$ integrin. The results showed a positive binding of the RAFT-c(-RGDfK-)₄ to CAL33 cells. d) Immunohistochemical staining with an anti-human $\alpha\beta3$ integrin monoclonal antibody of orthotopic tumors developed from the CAL33 cell line. The tumor cells show positive in vivo expression of $\alpha\beta3$ integrin (brown/yellow coloration). Original magnification: 20x objective lenses. e) Immunohistochemical staining with an anti-human $\alpha\beta3$ integrin monoclonal antibody of the control tissue showed no expression of $\alpha\beta3$ integrin. Original magnification: 20x objective lenses.

Figure 3: AngioStamp™ 800 biodistribution and tumor fluorescence kinetic. a) The highest fluorescence intensity is found in the kidney and in the tumor, 24 hours after the injection of AngioStamp™ 800. b) Tumor fluorescence intensity increases gradually to reach a high level between 16 and 24 hours after intravenous injection of AngioStamp™ 800.

Figure 4: Orthotopic tumor resection with the aid of fluorescence imaging. a) Fluorescence of the main tumor. b) NIR optical imaging was able to detect the fluorescence of a cancer tissue rim (10%) left behind (circle) after the macroscopic removal of 90% of the tumour. c) Fluorescent residues (arrow) unintentionally left behind after total macroscopic resection of the tumour. d) No fluorescence was observed in the surgical bed after the excision of all fluorescent tissues. e) *Ex vivo* fluorescence of the tumor and the fluorescent residues compared to the control tissue. f) The FluoStick™ Clinical System device was used for NIR optical imaging.

Figure 5: Microscopic analysis of fluorescent residues. a) Hematoxylin-eosin (H.E.) staining of a fluorescent residue revealed a focus of squamous cell carcinoma. The control tissue shows normal skeletal muscle. Original magnification: 10x objective lenses. b) Positive

correlation between the size of the tumor foci detected microscopically in the fluorescent residues and the intensity of the fluorescent signal ($R^2 = 0.5956$ and P value < 0.0001).

Figure 6: Bioluminescence follow-up of the orthotopic tumors. a) The graph shows that the bioluminescent signal increased gradually till tumor resection. A high rate of tumor recurrence was observed in the group of mice that underwent macroscopic resection compared to the NIR optical imaging-guided resection group. b) Bioluminescence imaging was performed one week after orthotopic tumor implantation, and 100% of the mice presented a unique local bioluminescent signal before the appearance of a macroscopic visible tumor. c) No bioluminescent signal recurrence was observed after complete excision of the orthotopic tumor. d) Macroscopic tumor recurrence confirmed by bioluminescence. e) The graph shows the high tumor recurrence rate in the macroscopic resection groups (two independent groups with 6 mice in each group) compared to the NIR optical imaging-guided surgery groups (two independent groups with 6 mice in each group).