Characterization of fluorescent probes for imaging-guided surgery

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Cancer is a leading cause of death and disability worldwide and surgical removal still remains the most important curative option for the majority of solid tumors. Currently, intra-operative tumor assessment is based on visual inspection and palpation by the surgeons. This makes it challenging to distinguish cancer tissue from the adjacent tissue, potentially resulting in subtotal tumor resection. The presence of residual tumor tissue at the resection border, defined as positive margin, has been associated with increased rate of local recurrence [1]. Obtaining cancer-free margins is a main medical need in surgical oncology, which is crucial for patient outcome.

Fluorescence guided surgery recently emerged as a technology that allows to identify, in real-time, cancerous tissue and to delineate tumor margins. In particular, probes that specifically accumulate in pathological tissue can support tumor resection through visualization. Fluorescent probes in the Near Infrared Region (NIR, 700-900 nm) have been proposed, due to the relatively high tissue penetration and low background absorbance by water and biomolecules at these wavelengths [2].

Integrins are an attractive biomarker for targeted imaging-guided surgery. One prominent member of this protein family is $\alpha_v\beta_3$ integrin, a receptor for extracellular matrix (ECM) proteins containing arginine-glycine-aspartic acid (RGD) motifs. Integrin $\alpha_v\beta_3$ is expressed at low or undetectable levels in most adult epithelia, but it is highly up-regulated in tumors and correlates positively with disease progression [3]. Noninvasive visualization of $\alpha_v\beta_3$ integrin expression *in vivo* could provide a way for the specific detection of tumors during surgery.

The PhD project is focused on the biological characterization of a new NIR contrast agent, named B26100, designed to target $\alpha_v\beta_3$ integrin, intended for intra-operative use during tumor resection. B26100, developed and patented by Bracco Imaging, is composed of a cyclic RGD (cRGD) peptidomimetic conjugated to a NIR fluorophore (Cyanine 5.5).

In vitro characterization of B26100 included the evaluation of the affinity for $\alpha_v\beta_3$ integrin on both the isolated protein and tumor cells. Moreover, the capability of the probe to enter tumor cells in culture, allowing their visualization by fluorescence was also tested. *In vivo* optical imaging experiments were conducted to assess the performance of B26100, using different xenograft tumor models, with high or low integrin expression. The probe accumulated in tumor masses, while showing lower distribution in healthy tissues. Furthermore, a preclinical model of prostate cancer was adopted to simulate tumor resection under B26100 fluorescence (Figure 1). Given the positive results obtained in preclinical models of cancer, B26100 was characterized in a more complex scenario closer to clinical settings. Oncologic canine patients with spontaneous tumors were enrolled in a veterinary study to assess the feasibility of tumor identification during surgery, using this new probe. The study is still ongoing, in collaboration with Ghent University (Belgium). Some preliminary results are here reported.

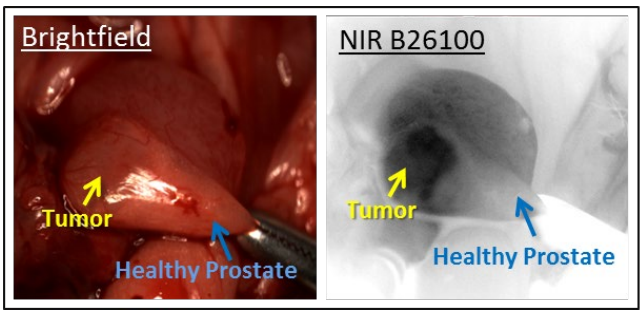

Figure 1. B26100 fluorescence-guided surgery in a preclinical model of prostate cancer. In brightfield, visual tumor inspection during surgery. In NIR fluorescence, tumor mass highlighted by B26100

[1] Zhang, Nat. Rev. Clin. Oncol., 2017; [2] Keereweer, Clin. Canc. Res., 2013; [3] Desgrosellier, Nature Reviews, 2010.