NANOBODY-BASED FLUORESCENT CONTRAST AGENTS FOR RAPID AND SPECIFIC INTRA-OPERATIVE TUMOR VISUALIZATION

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Fluorescence molecular imaging aims to support decision-making during surgical oncology by enabling the real-time visualization of malignant tissue through the administration of a fluorescent targeted contrast agent. An important class of targeted molecules are the antigen-binding fragments derived from camelid heavy chain-only antibodies, also called Nanobodies®. Nanobodies are renowned for their rapid and highly specific tumor uptake, and combined with their fast blood clearance, high contrast imaging as soon as 1h post-injection is feasible. Hence, they are already widely investigated as radiotracers for nuclear imaging, with several clinical studies ongoing. We focus on their exploitation as contrast agents in the context of fluorescence guided surgery. Fluorescently-labeled anti-HER2 Nanobodies were demonstrated to clearly visualize soon after injection intraperitoneally implanted cancer lesions, even submillimeter in size. Likewise, cell line- and patient-derived pancreatic and colon cancer lesions implanted orthotopically as well as satellite lesions could be highlighted with fluorescent CEA-specific Nanobodies. More recently, anti-uPAR Nanobodies were developed and validated that can demark various cancer types via uPAR expression on both tumor and tumor-associated stromal cells, including glioblastoma, colon cancer and liver metastasis.

However, we also showed that the choice of fluorescent dye and conjugation chemistry can have a major impact on the pharmacokinetics of Nanobodies. Our latest results indicate that the novel near-infrared fluorescent dyes s775z, FNIR-Tag1.0 and FNIR-Tag766 exhibit the best properties for the labeling of Nanobodies, resulting in very low background signals in vivo as compared to Nanobodies labeled with the more widely used IRDye800CW.

As intermediate step between mice and humans, we are currently investigating the potential of Nanobody-based fluorescent tracers in larger animals. As such, we demonstrated that intravenous injection of 1 mg of fluorescently-labeled anti-mannose receptor Nanobody in pigs enables rapid intraoperative localization of all lymph nodes within an area of interest. This could potentially increase the accuracy of lymph node dissections. Finally, in view of a clinical trial in canine patients that is soon to be initiated, a repeated dose toxicity study in Beagles was performed that indicated that the s775z-labeled anti-EGFR Nanobody was well tolerated.
In conclusion, preclinical studies conducted on mice and larger animals have demonstrated the potential of Nanobodies as a platform technology for the development of targeted fluorescent tracers. These tracers show significant clinical potential in various interventional applications given their ease of use in terms of rapid imaging time frame and high specificity.