Summary

Cancer is characterized by an uncontrolled cell division, which triggers a steep increase in the demand of nutrients such as sugars, amino acids and nucleotides. Supply of sufficient nutrients to the tumorous tissue is anticipated by the recruitment of new blood vessels, a process called angiogenesis. Subsequent influx of the delivered nutrients inside the tumor cells is enhanced by the expression of the nutrient transporters (e.g., GLUT-1, LAT-1, ASCT-2). In a wide variety of cancer types, the expression of these nutrient transporters is increased and additionally, their expression is sometimes correlated to the patient's prognosis. These two characteristics make nutrient transporters an interesting target for molecular imaging techniques such as positron emission tomography (PET).

In this dissemination the alanine serine cysteine transporter-2 (ASCT-2) was the topic of research. The ASCT-2 is a sodium dependent transporter of neutral amino acids (e.g., glutamine, alanine, serine, cysteine), with glutamine being its preferred one. Glutamine is massively consumed by cancerous tissue, with several cancers being highly dependent on glutamine for survival and proliferation. Some tumors even have a glutamine-addiction with a switch to glutaminolysis, possibly explaining the [^{18}F]FDG negative PET scans of some tumors. The role of glutamine in cancer has evoked a search for PET-tracers visualizing the transport of glutamine, more specifically its main transport protein: ASCT-2. Currently, the two best ASCT-2 tracers are [^{18}F]fluciclovine, which is promising in prostate cancer but also has LAT-1 affinity, and (2*S*,4*R*)4-[^{18}F]fluoroglutamine, a probe with applications in brain and breast tumors but suffering from *in vivo* defluorination.

The aim of this PhD dissertation was the development of fluorine-18 radiolabeled amino acids as positron emission tomography probes for visualizing the glutamine transport in oncology. It is hypothesized that the development of a stable molecular imaging probe, that visualizes the glutamine transport selectively, would provide physicians more information on the glutamine status of the patient, possibly helping in determining prognosis and therapy planning.

Chapter 1 of this dissemination contains an extensive literature study, which provides the reader with the necessary background information regarding the field of oncology and molecular imaging, essential for a proper comprehension of the conducted research. The scope and aims of this project are set out in **Chapter 2**.

In **Chapter 3**, the financial feasibility of amino acid PET tracer interventions in oncology was evaluated. More specifically, the cost-effectiveness of [^{18}F]FET PET for assessing the therapy response of patients with glioblastoma to TMZ treatment was investigated. Incremental cost-effectiveness ratios of around 1,350 euro were calculated. The results of the sensitivity analyses indicated that these values were obtained with an acceptable level of certainty, given the robustness of the data. This case example indicates that fluorine-18 labeled amino acid PET tracers can be used as cost-effective tools in an oncological setting.

After evaluating the cost-effectiveness of amino acid PET tracers, a set of ASCT-2 targeting radiotracers was developed. Five lead compounds, ASCT-2 targeting molecules, were selected as backbone compounds for labelling with fluorine-18. In **Chapter 4**, derivatives of these molecules were synthesized as reference compounds, which can be fully characterized and used as precursors for subsequent radiolabeling. The reactions successfully yielded the reference and precursor molecules of the envisaged radiochemical tracers, except for the biphenyl serine ester analogues that could not be synthesized.

Fluorine-18 derivatives of the precursor compounds were developed in **chapter 5**. A labelling strategy using a ruthenium-enhanced aromatic fluorination was used to synthesize the *L*-γ-glutamyl-*p*-nitroanilide analogues [^{18}F]FPG and [^{18}F]FBPG. The fluorination of the 1,2,3-dithiazole moiety was more challenging, and required a disconnection approach to avoid interreference of the heterocyclic group. In a first step, hydroxyaniline was fluorinated using the ruthenium-enhanced aromatic fluorination and in a second step [^{18}F]fluoroaniline was coupled to the 1,2,3-dithiazole to yield [^{18}F]ClFPDi. The radiochemical fluorination of the V-9302 analogue was successful using an aliphatic nucleophilic fluorination resulting in the synthesis of [^{18}F]FABABA, albeit with low radiochemical yields. Finally, an analogue of biphenyl-proline

could be fluorinated, synthesizing [^{18}F]FBPP with the ruthenium-enhanced aromatic fluorination method.

To obtain a preliminary idea on the tracer potential of the developed radiolabeled probes, the compounds were characterized *in vitro.* Therefore, in **Chapter 6**, the radiotracers were tested for their stability in phosphate buffered saline during an incubation of 3 hours, a log$D_{7.4}$ assessment and an evaluation of their affinity towards ASCT-2, determined in a [^{3}H]glutamine concentration depended uptake study. [^{18}F]ClFPDi displayed degradation in PBS formulation and was not further tested for *in vitro* affinity towards the ASCT-2. Reference products of [^{18}F]FPG, [^{18}F]FBPG, [^{18}F]FABABA and [^{18}F]FBPP were subjected to [^{3}H]glutamine displacement assays for K_i determination. [^{18}F]FPG, [^{18}F]FBPG and [^{18}F]FABABA inhibited the [^{3}H]glutamine uptake and were retained for *in vivo* evaluation. Observed K_i values of [^{18}F]FBPP revealed that the tracer had an almost non-existing affinity towards ASCT-2, thereby excluding the radiotracer from further experiments.

In **Chapter 7**, the tumor uptake of the radiotracers was evaluated in both PC-3 and F98 tumor models using dynamic PET acquisitions and biodistribution experiments**.** [^{18}F]FABABA, a fluorine-18 labeled derivative of compound V-9302, displayed very limited uptake in tumor tissue despite having good *in vitro* K_i values. Rapid uptake in liver and intestines suggest fast metabolization for this radiotracer. Similar to [^{18}F]FABABA, [^{18}F]FBPG also displays a limited uptake in tumor tissue. The affinity of the tracer appears to be insufficient for proper delineation of the tumors. In contrast to [^{18}F]FABABA and [^{18}F]FBPG, [^{18}F]FPG is capable of visualizing the tumors of the PC-3 xenograft. This compound is a promising lead molecule, where further optimization of the arene substituents can result in a high-quality glutamine-based PET radiotracer.

In **Chapter 8**, the general conclusions of the experimental sections were presented. The future perspectives and broader international context of this research project are elaborated on in **Chapter 9**.