SUMMARY

Glioblastoma (GB) are highly infiltrating, heterogeneous brain tumors characterized by high cellularity, nuclear pleiomorphism, microvascular proliferation and central tumor necrosis. GB patients have a survival rate of only 5% in 5 years, resulting in a median survival of 6 - 12 months, despite the current therapeutic approach known as Stupp's protocol, consisting of maximal surgical resection followed by radiation- and chemotherapy. Even though novel therapies, such as immune therapy, have been evaluated, no major progress has been achieved in the last decade.

At diagnosis, neuroimaging plays an important role in discriminating benign lesions from brain tumors. Computed tomography (CT) and magnetic resonance imaging (MRI) represent the two most common used imaging modalities for obtaining anatomical information. Furthermore, therapy-related changes such as inflammation and radiation necrosis (RN) can hamper the detection of tumor recurrence as they can cause similar signal abnormalities on MRI. In order to provide additional functional information, positron emission tomography (PET) utilizes radioactive tracers that target different specific metabolic and molecular processes.

In general oncology, 2-[^{18}F]Fluoro-2-deoxy-D-glucose ([^{18}F]FDG) has become the most important clinically utilized PET radiopharmaceutical. However, the physiological high [^{18}F]FDG uptake in healthy brain parenchyma and non-specific uptake in inflammatory lesions complicates the delineation of tumor tissue. In contrast, radiolabeled amino acids exhibit more favorable tumor-to-brain contrast due to the lower uptake in normal brain parenchyma. However, the uptake mechanism of the most used amino acid PET tracer, O-(2-[^{18}F]Fluoroethyl)-L-tyrosine ([^{18}F]FET) remains unclear resulting in non-specific uptake in non-neoplastic lesions due to reactive astrocytosis.

The system L transporters (LAT) are a group of sodium-independent transporters with four isoforms: LAT1, LAT2, LAT3, and LAT4. Since LAT1 is overexpressed in various human tumors and is closely correlated with malignant proliferation of glioma, it represents a promising target for both imaging and therapeutics.

In research, small animal models are considered a major keystone prior to clinical experiments. Especially in neuro-oncology, non-invasive *in vivo* imaging of tumors can be valuable for diagnostic and/or therapeutic purposes. In addition to the development of preclinical imaging modalities, small animal radiation research platforms have made the progress in radiation oncology towards personalized medicine possible. In this dissertation, we are interested in the concept of dose painting where a non-uniform dose is given to the GB based on the information obtained by PET images. Dose

painting can be performed using two different techniques: dose painting by contours (DPBC) and dose painting by numbers (DPBN). Depending on the technique used to perform dose painting, a dose is given to a set of embedded subvolumes (DPBC) of at voxel level (DPBN).

In accordance with the title of this dissertation, exploring diagnostic and therapeutic opportunities of LAT1-selective PET imaging in glioblastoma, the following aims are set. Firstly, we are interested to evaluate the feasibility of a preclinical GB rodent model for the application of PET based dose painting and subsequent evaluation of treatment outcome. The second aim is to develop a ^{18}F labeled phenylalanine analogue for PET imaging that has more favorable affinity and specificity for the LAT1 transporter than [^{18}F]FET. Finally, we are interested how the improved *in vitro* characteristics of this new compound are translated *in vivo*.

The first part of this dissertation provides the necessary background on GB and molecular imaging. Chapter 1 gives a general introduction on GB, amino acid transporters, preclinical GB models and molecular imaging modalities commonly used in neuro-oncology (CT, MRI, SPECT & PET). Chapter 2 covers radiopharmaceuticals used for GB imaging. Firstly, special attention is given to some basic aspects of radiopharmaceuticals including ^{18}F radiochemistry. Then an overview is given of SPECT and PET tracers used for GB imaging with a focus on amino acid radiopharmaceuticals. Finally, chapter 3 postulates several research questions that will be investigated in the following experimental chapters.

In chapter 4, an orthotopic allograft F98 GB rat model is described and used in a radiation therapy dose targeting experiment. The hypothesis was that PET-guided radiation therapy targeting the most metabolically active or radioresistant/hypoxic tumor region, using [^{18}F]FET or [^{18}F]Fluoroazomycin arabinoside ([^{18}F]FAZA) respectively, might improve local tumor control and lead to better survival. Therefore, four different treatment groups were defined based on the definition of the target volumes for brain tumor irradiation ([^{18}F]FET, [^{18}F]FAZA, MRI and control group). In the end we were able to perform PET based radiation therapy using the F98 GB rat model. When comparing the dose volume histograms, a significant difference was found exclusively between the D2-values. A significant difference in tumor growth was only found between active therapy and sham therapy respectively, while no significant differences were found when comparing the three treatment groups ([^{18}F]FET, [^{18}F]FAZA and MRI based). In this chapter we showed the feasibility of PET guided subvolume boosting of F98 glioblastoma in rats. No evidence was found for a beneficial effect regarding tumor response. However, improvements for dose targeting in rodents and studies investigating new radiotracers for GB treatment are mandatory.

Chapter 5 discusses the synthesis and characterization of a set of six different *ortho-*, *meta-*, *para-*fluoroethyl substituted phenylalanine derivatives. As there are conflicting reports on the preferred stereochemistry of the phenylalanine analogues for the LAT1 transporter, both enantiomers were provided. To assess the stereospecificity and the influence of the aromatic ring modification, the affinity (K_i) for the LAT1 expressing F98 cell line was determined by means of competitive *in vitro* assays. When considering the K_i values of the L-enantiomers, the preference of the position of the 2-fluoroethyl substituent on the aromatic ring of phenylalanine was as follows: *ortho* > *meta* > *para*. 2FELP has the most promising affinity towards the LAT1 transporter in the F98 GB cell line. Hence, we opted to develop and evaluate 2FELP further in the following chapters.

Chapter 6 covers the radiosynthesis of 2-[^{18}F]FELP by a S_N2 nucleophilic substitution with [^{18}F]fluoride on a tosylated protected precursor, followed by an acidic deprotection step and high performance liquid chromatography (HPLC) purification. Furthermore, the radiosynthesis was successfully implemented on a Synthra RN plus module enabling the production of larger amounts, necessary for larger preclinical and clinical studies. No radioactive metabolites were observed within 360 min after the end of synthesis making this radiopharmaceutical applicable for *in vitro* and *in vivo* experiments. By means of uptake experiments using different inhibitors, we concluded that the LAT1 transporter is more involved in the 2-[^{18}F]FELP than in the [^{18}F]FET uptake. An initial *in vivo* uptake experiment in a orthotopic F98 GB rat model revealed non-inferiority of the novel compound 2-[^{18}F]FELP when compared to [^{18}F]FET.

In chapter 7, two different animal models were set up. Firstly a radiation necrosis/GB rat model was set up to evaluate the capability of 2-[^{18}F]FELP to differentiate this two lesions compared to [^{18}F]FET and [^{18}F]FDG. Secondly, a murine inflammation model was set up to reveal if this would give rise to false positive PET signals using the novel radiopharmaceutical. Semi-quantitative analysis of both experiments showed that 2-[^{18}F]FELP was superior to [^{18}F]FET in discrimination GB from RN and was not taken up in the inflammation lesion. Considering both experiments, the most appropriate tracer for differential diagnosis between tumor recurrence and RN is 2-[^{18}F]FELP.

An answer to the in chapter 3 postulated research questions is given in chapter 8. The broader international context, relevance and future perspectives can be found in chapter 9.