Chapter 7:

Conclusions and future perspectives
CONCLUSION

New molecular imaging probes are of crucial interest and the growing know-how with respect to prostate cancer metabolism and its cellular biological characteristics need to be implemented in the clinical management so that nuclear imaging could definitively be employed for PCa targeted imaging. We could provide evidence for GRPR targeted imaging as we synthesized different bombesin tracers with high tumor uptake. Unfortunately, preclinical studies in prostate cancer animal models showed some drawbacks in preclinical settings with regard to aspects as stability and proteases degradation, accumulation in non-targeted organs and slow wash out in some cases. GRPR is an ideal candidate for targeting because it is massively over-expressed in several human tumors including PCa, but also shows a low expression in benign prostate tissues. Our BN analogues showed their high potential for PCa imaging and are candidate for clinical translation. However, none of them was totally fulfilling all the aspects necessary to become a possible benchmark, so following research is further necessary. Although the probes described in this thesis showed high affinity and specificity to GRPR and were obtained with high radiochemical yields and purities, further studies are necessary to improve pharmacokinetics properties and stability. Future studies should focus on developing high affinity BN-like radiopharmaceuticals that are highly stable in a pre-clinical and more important for the translation into clinical setting. To optimize the in vivo kinetics of bombesin homodimer further improvements are needed. Chelators, labeling methods, linkers (different in length and flexibility), scaffold/backbones insertion and ligand-receptor recognition are already under evaluation, but further studies need to be performed to evaluate the most efficient methods. These modifications could provide higher selectivity and binding affinity. The evaluation of different linkers and chelators can facilitate the stabilization of radionuclides and, at the same time, avoid steric hindrance. The development of new or improved in vitro stability tests or better preclinical in vivo tumor models, as suggested by Ananias et al. (1), may help to identify a better strategy for selecting radiolabeled BN tracers with appropriate stability and affinity for clinical studies. Additionally, instead of using the relatively low-resolution SPECT for imaging, the use of
radiopeptides suitable for PET imaging will aid in improvement of imaging. In this case, the introduction of long half-life PET radionuclides as $^{89}$Zr (~3.3 days) on BN peptides via new stable chelating agents may allow to combine the properties of the homodimer described in this thesis with the properties of long-lived positron emitter radionuclide. Recently, a new chelating agent named NODAGA (1,4,7-triazacyclononane, 1-glutaric acid-4,7 acetic acid) has recently been evaluated after conjugation with RM1 and AMBA bombesin analogues for their GRPR targeting abilities (2). RM1 and AMBA bombesin were radiolabeled with either $^{64}$Cu or $^{18}$F AIF and tested both in vitro and in vivo. $^{64}$Cu– and $^{18}$F–AIF–labeled NODAGA–RM1 demonstrated excellent serum stability and tumor imaging properties in the in vitro stability assays and in vivo imaging studies. $^{18}$F AIF–NODAGA–RM1 exhibited tumor uptake values of 4.6 ± 1.5, 4.0 ± 0.87, and 3.9 ± 0.48 %ID/g at 0.5, 1, and 2 h, respectively $^{64}$Cu NODAGA–RM1 exhibited tumor uptake values of 3.3 ± 0.38, 3.0 ± 0.76, and 3.5 ± 1.0 percentage injected dose per gram of tissue (%ID/g) at 0.5, 1.5, and 4 h after injection, respectively. Moreover, in a recent study, it was directly compared the radiochemical properties of DOTA, NOTA and NODAGA when used for the labelling of a bombesin AMBA analogue. This study demonstrated that NODAGA is superior to NOTA and DOTA in terms of faster reaction kinetics, higher specific activity and better in vitro stability (3). Our results already have demonstrated good characteristics of these stabilized peptides C5 and C6. It would be interesting to evaluate NOGADA or some other chelator in combination to C5 and C6 to evaluate if their stability, tumor uptake and pharmacokinetic could be even more implemented or improved. This might be a crucial step in the establishment of C5 and C6 as agents for clinical application for the PET imaging of prostate cancer.

**FUTURE PERSPECTIVES**

In the future, treatment approaches for metastatic PCa would lead to improve the prognosis of patients but costs and side effects should be balanced against increased life time and quality of life. Several studies are focused on discovering new treatment agents
and approaches that can reduce side-effects and further improve quality of life in patients with prostate cancer on the basis of potent treatment efficacy. It is expected that a personalized approach will be needed given multiple drugs are already available. Selection of patients could be improved by adding knowledge of tumor biology into decision algorithms. Clinical studies on the probes described in this thesis could be performed in small numbers of patients to investigate the feasibility of these radiolabeled peptides. The main challenge for the future is to synthesize high-avidity ligands to be used as biological targets or imaging agents. Overall size, type of linker or backbone, receptor density, receptor geometry and distance between ligands need to be carefully considered. Multimerization can be surely an important strategy because it offers possibility to design high-affinity peptidic probes. The insertion of proper linker and chelator already allowed us to obtain bombesin analogues with favorable kinetics characteristics leading at better imaging quality. Future studies are needed to improve the kinetics properties of bombesin homodimeric tracers. A further optimization of the chemical properties could be done by modification of the spacer. For instance, the introduction of poly(ethylene oxide) group or hippurane-like molecular structure could increase the grade of hydrophilicity. The introduction of these functional groups might enable kidney clearance and fasten the excretion of non-labeled radiopharmaceutical. Moreover, the optimization of $^{111}$In-DOTA-BN homodimer could lead to a higher hydrophilic behaviour and faster wash-out. If we consider the observed prolonged retention and internalization characteristics of $^{111}$In-DOTA-BN dimer, we might speculate that this tracer could be interesting for both, diagnostic and therapeutic purposes. In fact, the Auger electron-emitting $^{111}$In-labeled radiopharmaceuticals used as therapeutic agents, after their binding to the cell surface, are internalized and translocated into the nucleus causing DNA damage and consequently cell death. Because of that, $^{111}$In-DOTA-BN homodimers could be feasible for cancer eradication. To evaluate our hypothesis further in vitro cell studies, in vivo pre-clinical and clinical trials are urgently needed. The vast majority of studies (ours included) evaluates GRPR-targeting by using the GRPR overexpressing, but androgen-unresponsive, AR-negative PC3 cell lines. PC-3 is a stable cell lines well characterised. The cells show
favourable characteristics for our imaging purpose: high GRPR expression and the lack of other receptors (that might interfere with our imaging studies). In future, in vitro and in vivo studies could be performed using GRPR-overexpressing, androgen-responsive and AR-positive human cell lines in order to unravel the effect of hormonal manipulation and AR status on GRPR expression in PC in more detail. One useful cell line could be VCaP, which is derived from a human PC metastasis and has shown to have similar GRPR-specific uptake compared to PC-3 cell line. Some studies on the effect of hormonal manipulation and AR status on GRPR expression have already been reported, but showed that GRPR mRNA expression as well as BN-specific binding of VCaP cells was not affected significantly by androgen manipulations in vitro and in vivo. These observations suggested that GRPR expression was not androgen regulated in VCaP cells and that VCaP, as well as PC3, may represent a more advanced stage of PC, where a constitutively activated GRPR expression is present (4,5). Therefore a different androgen-responsive and AR-positive human cell line should be used. Currently, LNCaP cells (androgen-dependent human prostate cancer) are being used in prostate cancer research because they are stably transfected cells with an expression plasmid. The high GRPR expression in these cells results from an exogenous gene under the control of a constitutively-active promoter. Therefore, LNCaP have been employed in GRPR expression studies with encouraging results for the future (6).

We aimed to improve GRPR targeted imaging by incorporating multiple targeting ligands to enhance binding affinities and we observed that modification on the linkers (e.g. length and flexibility) and different bifunctional chelators combined with metabolically stable BN sequences can improve the performance of the bombesin homodimer and monomers. One other approach that proved to be efficient in improving the targeting abilities of BN in our case was lanthionine stabilization. In our study we tested two stabilized peptides which had optimal imaging characteristics of monomeric peptides such as excellent clearance, accumulation rate and specificity. C5 and C6 bombesins were obtained with the ultimate goal to synthesize stable BN peptides by chemically introducing a thioether linkage. In fact, a large number of BN monomers used for cancer imaging show a strong discrepancy between the optimal behavior in vitro and the low stability in vivo during the
preclinical study. To improve the cell surface concentration of the BN to our target cells, it is needed to decrease the fast proteolytic action from plasma proteases. This may be done by introducing non-natural amino acids in the bombesin peptide sequence without effecting the biological activity of the peptide. For instance, Nock and coworkers synthesized bombesin like peptides by substituting amino acids in positions 6 and 13 with the non-nature amino acids D-Phe and Leu-NHET. Thereby, the Nock’s group further selected a potent bombesin analogue for GRP receptor-targeted tumor imaging which was labelled by $^{99m}$Tc and showed improved stability (7,8). All these approaches could be also combined with new versatile radiolabelling techniques such as click chemistry or can lead to obtain ligands with optimal affinity, pharmacokinetics and metabolic stability which could also be employed in multimodality imaging techniques such as PET/MRI, SPECT/MRI or Fluorescence image-guided surgery (FIGS).
Conclusions and future perspective

References


