Chapter 1:

General Introduction
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GENERAL INTRODUCTION

Prostate cancer (PCa) is a heterogeneous disease affected by genetic and environmental factors such as increasing age, ethnic origin and heredity. It is concluded that that exogenous factors drive the risk of progression from a so-called “occult PCa” to a clinical PCa (1). Food consumption, sexual behavior, alcohol consumption, ultraviolet radiation exposure, inflammation are all considered as etiologically important exogenous factors. Nowadays, exogenous factors and hereditary factors are important in determining the risk of developing clinical PCa.

PCa mortality ranges differently from country to country in the developed world. The incidence of prostate cancer has increased markedly over the past century. Prostate cancer is the second most diagnosed cancer (second only to non-melanoma skin cancers) and the second leading cause of cancer death after lung cancer. In the USA in 2008 the estimation was 186,320 new cases and 28,660 deaths (1). In 2009, the incidence of new cases and deaths from this disease were 192,280 cases and 27,360 cases showing a proportional growing epidemiology over the years (2). In Europe, PCa has an incidence rate of 214 cases per 1000 men. This makes PCa the most common solid neoplasm, outnumbering lung and colorectal cancer. The early diagnosis of PCa is an important goal for cancer researchers because it has been estimated that when diagnosed early, the 5-year survival rate of PCa is almost 100% (3). This is proved by the decline of death rates from prostate cancer, and the 5-year survival rate has seen a large increase (now 99% when combined for all stages), thought to be due primarily to screening, early detection, and changes in lifestyle. This trend also results from improvement in successful treatment of prostate cancer. Anyhow this high disease specific survival rate is also influenced by lead time bias and length bias due to screening effects (“European Randomized Study of Screening for Prostate Cancer (ERSPC)”). Nonetheless, more needs to be done to understand and manage this disease.

GENETICS OF PROSTATE CANCER

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PCa is curable by surgical intervention and/or radiation therapy when diagnosed in its initial stages. In the initial stages, PCa is confined to the borders of the prostatic capsule and can be surgically removed. If not diagnosed at the earliest stages, prostate carcinoma can advance to stages characterized by local invasion of the seminal vesicles, followed by metastasis primarily to the bone, usually resulting in lethality. This transition to metastatic disease is regulated by molecular pathways, often activated by genetic and epigenetic dysregulation of tumor protein expression (Fig. 1). A number of mechanisms underlying prostate cancer initiation has been investigated focusing on the analysis of chromosomal alterations that are commonly observed in prostate cancer. It was proven that losses of heterozygosity at chromosomes 8p, 10q, 13q, and 17p are frequent events of chromosome abnormalities that can trigger the prostatic cancer progression. Losses of 6q, 7q, 16q, and 18q have also been reported, even if are not as well characterized (5–10). In addition, gains at 8q and 7 can be relevant although gains of function are usually less common (11–13). So far, several candidate genes (e.g., RB, p53, PTEN, NKKX3.1) have been evaluated in terms of localization to regions of allelic loss and functional properties, but none of these candidate tumor suppressors have been clearly shown to be mutated in a statistically high percentage of prostate cancer specimens. These tumor suppressor genes in the regions of allelic loss have often not been identified yet and eventual mutations are difficult to be detected because these mutations may be masked by the inability to obtain relatively pure tissue samples or homogeneous specimens for analysis. Candidate genes may also be inactivated by several molecular alterations, such as mutations in the promoter and in tumor oncogenes, methylation or mutations within regulatory sequences that may affect transcription, translation, or mRNA stability. Lastly, and most importantly, a final possibility is that the loss of a single allele (haploinsufficiency) may play a pivotal role in prostate carcinogenesis. The mechanisms in the tumor progression can be distinguished according to the progression of the disease. In the initial phase, PIN (prostatic intraepithelial neoplasia) is a precursor of carcinoma genesis, followed by loss of chromosome 8p and NKKX3. In the second phase loss of chromosome 10q and PTEN and loss of chromosome 13q and Rb can lead to altered cell-cycle regulatory genes, aging and telomerase dysfunction. In the last stages, advanced carcinoma and metastatic disease
are the consequence of androgen receptor signaling alteration and cancer progression associated to loss of 17p and p53 and altered apoptotic regulatory genes (6,11–13).

Figure 1: Schematic presentation of PC progression. Different chromosomal and gene changes are highlighted with other factors according to the PC development timeline.

DETECTION, DIAGNOSIS AND THERAPY

Accurate localization and determination of PCa tumor is critically important for selecting the most effective treatment. The choice of the right treatment is crucial for improving cancer control and reducing the risk of intervention-related complications. Detection of PCa is based on clinical examination, such as digital rectal examination (DRE), prostate specific antigen screening (PSA test), histopathological examination of prostate biopsies. All these tests are crucial because the use of imaging tests should be guided according to the patient’s risk category. This risk category is obtained by examination of several factors like the patient’s age, PSA level, clinical TNM (Tumor, Node, Metastasis) (Tab. 1), Gleason score, and number of positive biopsy cores. PSA is a protein produced by cells of the prostate gland which is not cancer specific. The screening must be interpreted carefully accordingly to the age of the patient, the size of the gland, and the presence of infection.
Thereby, high PSA blood levels may also result from prostatic hyperplasia or prostatitis. However, PSA should be considered a continuous parameter: the existence of PCa is more likely with high values. Absolute PSA serum levels evaluation have limitations but high levels are a strong indicator of stage and prognosis. PSA is also helpful in monitoring response to therapy. An abnormal digital rectal examination (DRE) result or elevated serum PSA measurement could indicate PCa. The Gleason grading score is the sum of the two most common patterns of tumor growth which are observed on a scale from 1 to 5.

For clinical grading the patterns 1 and 2 are not of importance as they are not found in tumor bearing areas. The clinical Gleason score in tumor biopsies and prostatectomy specimens ranges between 6 and 10, with 6 being the least aggressive and 10 the most aggressive.

<table>
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<tr>
<th>T</th>
<th>Primary Tumor</th>
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<tbody>
<tr>
<td>TX</td>
<td>Primary tumor cannot be assessed</td>
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<tr>
<td>T0</td>
<td>No evidence of primary tumor</td>
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<td>T1</td>
<td>Clinically inapparent tumor not palpable or visible by imaging</td>
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<tr>
<td>T1a</td>
<td>Tumor incidental histological finding in 5% or less of tissue resected</td>
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<tr>
<td>T1b</td>
<td>Tumor incidental histological finding in more than 5% of tissue resected</td>
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<tr>
<td>T1c</td>
<td>Tumor identified by needle biopsy (e.g. because of elevated prostate-specific antigen (PSA) level)</td>
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<tr>
<td>T2</td>
<td>Tumor identified by needle biopsy (e.g. because of elevated prostate-specific antigen (PSA) level)</td>
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<tr>
<td>T2a</td>
<td>Tumor confined within the prostate</td>
</tr>
<tr>
<td>T2b</td>
<td>Tumor involves one half of one lobe or less</td>
</tr>
<tr>
<td>T2c</td>
<td>Tumor involves more than half of one lobe, but not both lobes</td>
</tr>
<tr>
<td>T3</td>
<td>Tumor involves both lobes</td>
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<tr>
<td>T3a</td>
<td>Tumor extends through the prostatic capsule</td>
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<tr>
<td>T3b</td>
<td>Extracapsular extension (unilateral or bilateral) including microscopic bladder neck involvement</td>
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<tr>
<td>T4</td>
<td>Tumor invades seminal vesicle(s)</td>
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<td></td>
<td>Tumor is fixed or invades adjacent structures other than seminal vesicles: external sphincter, rectum, levator muscles, and/or pelvic wall</td>
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When diagnosed, the treatment may occur via several strategies; Active surveillance is the first choice in men with organ confined, low volume and low grade (Gleason sum 6) disease which is recognized as insignificant cancer. In cases of higher volume or Gleason sum 7-10 cancer active treatment is recommended (EAU Guidelines) by radical prostatectomy (RP), external beam radiotherapy or brachytherapy. RP consists in the removal of the entire prostate gland between the urethra and bladder, and resection of both seminal vesicles. In cases of non-organ confined (T3) disease external beam radiotherapy in combination with adjuvant androgen deprivation therapy is the standard. In cases of metastatic PCa androgen deprivation therapy is the first line palliative treatment. Although all these treatments do usually permit to control the progression of prostate cancer, eventually PCa relapse may occur despite androgen ablation therapy causing castration resistant PCa (CRPC), which has high lethality. In metastatic castration-resistant prostate cancer (CRPC), chemotherapy with docetaxel improves the life expectancy. In addition, palliative surgery, radiopharmaceuticals and medical treatments for pain and symptoms can be used ("European Randomized Study of Screening for Prostate Cancer (ERSPC)).
NUCLEAR MEDICINE FOR PROSTATE CANCER IMAGING

What anatomic, functional, and molecular imaging techniques might add to more accurate characterization of disease at initial biopsy? How better staging or evaluation of early response to therapy would allow better patient management, improving effectiveness of therapies or avoidance of unnecessary treatments? How and which imaging tools can be used to inform prostate cancer clinical trial designs and accelerate the evaluation of potential novel breakthrough therapies? These are all questions which need to be addressed to implement PET and SPECT as techniques in the management and diagnosis of PCa disease. Current standard imaging techniques, such as ultrasound, MRI, CT, and nuclear medicine cannot detect early disease, and they provide limited information for disease staging. Current conventional diagnostic imaging techniques methods such as computed tomography (CT) (14,15), ultrasound (16–19), and magnetic resonance imaging (MRI) (20), lack sensitivity and specificity and have limited role in the diagnosis, staging, and monitoring of PCa patients. The reason is that PCa can be indistinguishable from the surrounding normal prostate tissue (21). Only the addition of endorectal coil to paramagnetic super oxide particles for MRI allows optimal MR imaging of prostate cancer for detection and local staging (22,23). Molecular imaging techniques such as PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography) are used as most sensitive tools and applied in the diagnosis and staging of cancer. PET uses positron-emitting radioisotopes and is a non-invasive tool which allows quantitative images of the tracer biodistribution in intact living subjects such animals and humans (24). SPECT is based on the detection of single photon emitting radioisotopes (25–27).

Table 2 describes the main differences and characteristics of these imaging techniques. PET is more sensitive, allows quantitative measurements and has better spatial resolution than SPECT. However, SPECT is still widely used because it offers the possibility to widen the observational time window by using longer half-life of single photon emitters and allows to observe biological processes in vivo from several hours to few days after administration of the labeled compound.
The most widely used PET tracer for cancer diagnosis is $^{18}$F-FDG (fluoro-2-deoxy-2-D-glucose) (29-30) which is able to detect cancer cells because they are more metabolically active than normal cells. Thereby, $^{18}$F-FDG has been employed for PCa imaging because correlates with the prostate-specific antigen (PSA) level and can be used as a measure of tumor aggressiveness (31) and for monitoring the therapeutic responses of patients with aggressive or hormone refractory diseases (32-33). However, a series of limitations make $^{18}$F-FDG PET useful but not ideal for imaging in PCa. The reasons are that glucose utilization in well-differentiated PCa is often lower than in other tumor types, the intense accumulation of $^{18}$F-FDG in the urinary bladder often overshadows the tumor uptake (34) and does not clearly correlate with tumor grade/ stage in PCa (35). Moreover, the accumulation of $^{18}$F-FDG in prostate cancer may overlap with normal prostate activity and uptake in benign prostatic hyperplasia. Thereby, $^{18}$F-FDG is not suitable for the diagnosis/staging of primary prostate cancer and of local recurrence in general, but is useful in the case of poorly differentiated primary tumors (Gleason scores 8-10) with high serum PSA values (33-35). $^{18}$F-FDG is also used in monitoring the treatment response of
metastatic patients and in castrate-resistant prostate cancer. Other small molecules commonly used in prostate cancer imaging are $^{11}$C- or $^{18}$F-labeled choline analogues (imaging of cell membrane metabolism) (36–39), $^{18}$F- or $^{11}$C-acetate (imaging of fatty acid synthesis) (41,42), and $^{18}$F-3′-deoxy-3′-fluorothymidine ($^{18}$F-FLT, imaging of cell proliferation) (43). Unfortunately, also with these molecules major limitations have been observed. Acetate is incorporated into the lipid pool in cancer tissues with low oxidative metabolism and high lipid synthesis. $^{11}$C-acetate uptake in primary prostate cancer generally is higher than $^{18}$F-FDG, however, Kato et al demonstrated a large overlap for prostate hyperplasia, with a mean SUV of 2.1 (SD, 0.6), and prostate tumors, with a mean SUV of 1.9 (SD, 0.6). Choline enters the cell via choline transporters and is used for biosynthesis of phosphatidylcholine in the tumor cell membrane trough malignancy-induced overexpression of choline kinase. $^{11}$C-choline and $^{11}$C-acetate appear to be equally useful in imaging prostate cancer but, similarly to $^{18}$F-FDG, choline and acetate have insufficient diagnostic accuracy for the staging of the primary tumor. This is due to the minimum detectable tumor size of 5 mm and to the inability to differentiate between tumor and benign prostate disease, hyperplasia, chronic prostatitis and high-grade intraepithelial neoplasia. Other common disadvantages to both choline and acetate are their intestinal uptake and potential not specific accumulation in lymph nodes. Therefore a receptor imaging strategy that targets specifically the malignant cancer cells could overcome the drawbacks described above. The development of PET tracers that can target specific over-expressed molecular markers in PCa is necessary to improve imaging of PCa. A variety of PET tracers have been developed or are being investigated for targeting specific antigens/receptors, such as gastrin releasing peptide receptor (GRPR), prostate-specific membrane antigen (PSMA), prostate stem cell antigen (PSCA), among others. Another promising PET agent is based on testosterone or dihydrotestosterone androgens to visualize androgen receptor status. The normal development and maintenance of the prostate is dependent on androgen acting through the androgen receptor which remains important in the development and progression of PCa (44). The clinical use of $^{18}$F-FDHT ($^{18}$F-fluoro-5α-dihydrotestosterone) (45) is limited but appears to be useful in evaluating clinically progressive metastatic PCa. Its value in castrate resistant
PCa has not been determined yet. Peptides and their derivatives are particularly interesting for PET and SPECT imaging because they have unique favorable characteristics. Rapid tissue penetration, fast clearance from the circulation and low antigenicity are the most important. Optimal synthesis achieved via relatively easy radiolabeling procedures make them attractive for specific receptors/antigens targeting (46). The present thesis is focused on development of novel radiolabeled bombesin peptides for imaging of GRPR which is overexpressed in PCa.

**BOMBESIN AND GASTRIN RELEASING PEPTIDE RECEPTOR (GRPR)**

Bombesin (BN) is a 14-amino-acid amphibian neuropeptide (pyroglutamic acid-glutamine-arginine-leucine-glycine-asparagine-glutamine-tryptophan-alanine-valine-glycine-histidine-leucine-methionine-NH$_2$) with a high affinity to the gastrin-releasing peptide receptor (GRPR). Gastrin-Releasing Peptide (GRP) is a 27-amino acid neuropeptide that is the mammalian homologous of the linear tetradecapeptide BN. This homology is in the seven amino acids C-terminal sequence which is the responsible region for receptor binding. Three different BN mammalian receptor subtypes are known: BB1, BB2 and BB3. BB1 is known as Neuromedin B receptor, BB2 is designated as GRPR and BB3 is an orphan receptor. GRPR is a 7-transmembrane domain receptor of the G-protein coupled receptor superfamily expressed in both non-neuroendocrine and neuroendocrine tissues such as breast, pancreas brain, lung, prostate and gastrointestinal tract (47,48). GRP-like-proteins bind GRPR and this binding triggers a complex cascade of intracellular pathways (49). GRPR is highly expressed in a variety of human malignancies, including prostate cancer (50). To prove this overexpression of GRPRs autoradiography was performed (51). These studies also demonstrated the selective overexpression of GRPR in prostate cancer cells in comparison to the hyperplasic prostate tissue (52,53). Kristiansen and coworkers recently confirmed the higher expression of GRPRs in primary carcinomas and metastases. The expression of this receptor was not observed in normal prostate tissue. Inverse correlations of GRPR and Gleason score, PSA value, tumor size was found (53). It has been published by Reubi et al. (49) that the receptor density is about 25 times higher in invasive carcinoma than in
hyperplastic prostate tissues. There is a positive correlation between the overexpression of the receptor and the cancer progression. The more the prostate cancer invasiveness, the higher is the expression of the receptor. Autoradiography studies showed high accumulation of bombesin-like-peptides in malignant tissues but not in the surrounding normal prostate gland indicating the specific expression of GRPR in cancer cells. Taken together, the increased overexpression and the high specificity for the tumor cells make this receptor an optimal target for prostate cancer imaging.

BN is a very attracting peptide for targeted PC imaging because of its high specificity to GRPR. BN peptides are divided in two subcategories based on their structures. There are full length and truncated sequences. The truncated peptides have a shorter amino acid sequence (usually truncated at the C-term) and are generally more stable than the tetradeca-full length peptides (54,55). GRP and bombesin share aminated C-terminus sequence homology (48) in the final 7 amino acids, Trp-Ala-Gly-His-Leu-Met-NH2. Deletion of the N-terminus sequence pGlu1-Gln2-Arg3-Leu4-Gly5- from the BN molecule caused practically no loss of affinity and intrinsic activity, but further shortening of BN gave rise to a gradual reduction of both parameters (56). On the other hand, deletion of only two (Leu13-Met14) or three (His12-Leu13-Met14) amino acids from the C-terminus afforded BN fragments with low affinities BN(1-12) and BN(1-11) and showed for BN(1-11) also a reduced intrinsic activity compared to BN(1-12) (57). The sequence His12-Leu13-Met14-NH2 seems to be critical to fully activate BN receptors and the sequence BN(7-14) was regarded to be sufficient for the specific binding interaction with the gastrin-releasing peptide receptor (57). At present, only a few BN analogues have entered the clinical phase. Low tumor accumulation and/or peptide instability are still major issues in many preclinically tested BN analogues. Both BN agonists and antagonists are studied to define a sequence which could be successfully introduced in clinical practice. A ligand that triggers a cascade of biochemical downstream transformations by activating receptors to produce second messengers is an agonist; differently, a peptide antagonist binds the active site of membrane receptors and makes the binding of other agonistic peptides impossible. It has been suggested that the mechanism of internalization, that is typical of agonistic peptides, could allow higher tumor accumulation because of its internalization
into the tumor cells. Yang. et al. compared $^{18}$F-labeled BN antagonist and agonist (58) and demonstrated that the tumor uptake of $^{18}$F labeled agonist MAGBBN (Gly-Gly-Gly-Arg-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-MetNH$_2$) was much higher that the tumor uptake observed with the $^{18}$F labeled antagonist MATBBN (Gly-Gly-Arg-Asp-Asn-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NHCH$_2$CH$_3$). Their results indicated that $^{18}$F-labeled BN peptide agonists may be more useful for prostate cancer imaging due to their relatively higher tumor uptake and retention. Schroeder et al. (55, 59) compared BN antagonists and four agonists by evaluating the targeting efficacy observed in PC-3 tumor-bearing mice. They observed that, although the tumor uptake of the antagonist Demobesin-1 was 3.0±0.4 %/ID/g at 1h p.i., it was not significantly different from that of AMBA (2.7±0.5 %/ID/g) and PESIN (2.3±0.5 %/ID/g). AMBA and PESIN were more stable than Demobesin-1 after 24h p.i. and were more indicated for imaging at later time points. A few comparative studies were published in recent years and suggested that the in vivo behavior of receptor antagonists is superior to receptor agonists (58-61). It is still unclear which between agonistic or antagonistic behavior is preferred to design candidate for GRPR-positive cancer imaging with desirable tumor accumulation, appropriate pharmacokinetics and sufficient in vivo stability.

Linkers are routinely used with BN sequences to modify several parameters as binding affinity, hydrophilicity, and stability of the BN tracers and the retention of these compounds in tumor cells. Linkers play a key role in the performance of radiopharmaceuticals containing radiometals, and have been extensively employed to improve the in vivo kinetics and the targeting abilities of the radiolabeled biomolecules, bombesin included. Garrison et al. showed the importance of the linker in the in vivo clearance but also the in vivo uptake and retention of the radiopharmaceutical in targeted tissues. Different linkers change the hydrophilicity/hydrophobicity behavior of the radiotracer and change the in vivo kinetics (62). Amino acids and PEG are often used as linkers to reduce the abdominal background of the radiolabeled BN. High lipophilicity and positive charge of bombesin tracers lead to hepatobiliary excretion and long retention in kidney, respectively. Thereby, negatively charged or neutral and/or hydrophilic linkers could overcome this issue (63-66). Mu et al. (64) reported that the negatively charge
linkers show better characteristics and in vivo behavior than positively charge linker for the $^{18}$F-labeled non-natural amino acids modified BN. This group demonstrated that the charge of the linker plays a major role in in vivo pharmacokinetics. The comparison of $^{18}$F-7b and $^{18}$F-6b showed that the molecule linked with negatively charged linker, Ala(SO$_3$H)-Ava, exhibited a two-fold higher tumor accumulation (4.67±0.04 %ID/g at 1h p.i. against 2.36±0.47 %ID/g at 1h p.i.) than peptide linked to positive Arg-Ava linker.

Chelators such as DTPA, DOTA, NOTA and their derivatives are commonly available for the labeling of $^{111}$In, $^{68}$Ga, $^{177}$Lu and $^{64}$Cu (67, 68), but are also important to stabilize bombesin or other radio-pharmaceuticals. Prasanphanich et al. reported on $^{64}$Cu labeled NOTA-BN demonstrating a superiority over DOTA-BN in a GRPR expressing prostate cancer animal model. $^{64}$Cu-NOTA-8-Aoc-BBN(7-14)NH$_2$ appears to overcome demetallation and uptake of tracer by hepatobiliary proteins. Moreover the accumulation and retention of conjugate in renal tissue in vivo is significantly lower than the other conjugates that were compared, resulting in better microPET images (69). HYNIC (6-hydazinonicotinic acid) is often used because of its high $^{99m}$Tc-labeling efficiency, the high solution stability of its $^{99m}$Tc complexes, and the easy use of different co-ligands. However HYNIC has been also employed for $^{18}$F labeling of a variety of bioactive peptides, such as Octreotide, IL-2, and of course, BN. Ananias et al. (70) studied the characteristics of $^{99m}$Tc-HYNIC(Tricine/TPPTS)-ε-aminocaproic acid-BN(7-14) ($^{99m}$Tc-HABN) and reported a GRPR-mediate specific tumor uptake (2.24±0.64 %ID/g at 30 min p.i.) which was higher than the tumor uptake observed with $^{18}$F-Aca-BN (0.71 %ID/g at 30 min p.i.). Ananias and co-workers also demonstrated that the radioactivity accumulation in liver was 3-fold lower than that of $^{18}$F-Aca-BN.

Efforts are currently made to improve BN tumor targeting effect, imaging quality, and the in vivo stability by using multimerization strategies and lanthionine-stabilized bombesin agonistic peptides. The development of new analogues is mostly aimed at improving the affinity and the specificity of GRPR targeting; several new BN-analogues, both synthetic as well as native sequences, have already been tested and developed. Their potential has been extensively evaluated by both preclinical and, in a few cases, clinical studies. Generally, all the novel designed BN pharmaceuticals have been tested regarding their
peptide characteristics such as stability, biodistribution and toxicity. At this moment, it is not possible to make a real comparison of available analogues for PCa detection because the differences in the preclinical studies performed from different research groups make difficult the standardisation process. As consequence, it is also difficult to establish which analogue shows best characteristics overall the BN peptides making arduous the clinical translation. This also could explain why only few BN-analogues have been studied in PC patients. The published clinical data are those obtained from the clinical studies on $^{99m}$Tc-RP527 (71,72), $^{99m}$Tc-(Leu13)BN (73), $^{177}$Lu-AMBA (74) and $^{68}$Ga-DOTABOM (75), $^{99m}$Tc-HABBN (70,76). The stability issue involves modification of the native sequence to synthesize stabilized peptides.

**OUTLINE OF THE THESIS**

The aim of this thesis was to develop novel bombesin tracers to target Gastrin Releasing Peptide Receptor (GRPR), which is overexpressed in Prostate Cancer (PCa). In chapter 1 we introduce genetics, etiopathology, diagnostics and therapy for PCa stressing the needs and the importance of new radiopharmaceuticals for imaging diagnostic purposes. Bombesin (BN), a promising peptide for GRPR targeting was described in detail, because of its favourable proprieties and selectivity towards GRPR. A general overview of the actual strategies to improve tumor targeting using multimerization is described in chapter 2. The improvement in pharmacokinetics by using multimers, enhancing mechanisms of binding, ligand choice, linkers, scaffolds and synthetic strategies is observed. The properties of a novel $^{99m}$Tc homodimeric BN analogue have been evaluated and described in chapter 3. The aim of this study was to develop a radiopharmaceutical, which could improve the binding characteristics by increasing local ligand concentration and improving dissociation kinetics. A new bombesin homodimer with two identical Aca-BN(7-14) moieties linked with Glutamine acid was developed and labeled with $^{99m}$Tc via the HYNIC-Tricine/TPPTS was developed. The hypothesis is that the increased molecular size of BN dimer, compared to BN monomer, could result in an increased circulation time, a slower clearance and a slower accumulation rate. This hypothesis was tested in vivo in PC-3 xenografted mice. In chapter 4 pre-clinical experiments are described. We aimed to
evaluate the optimal time-point to achieve the highest tumor-to-normal ratios. Because of the results obtained with $^{99m}$Tc labelled BN showing an increase in cellular accumulation over 4 hours, we analysed later time points, using another pharmaceuticals based on the same molecule labelled with an isotope with longer half-life, $^{111}$In. Switching isotope gave us the advantage to analyse the tracer accumulation at longer time points. We did observe a higher tumor accumulation due to the peptide characteristics, but a long kidney retention because of slow excretion. In chapter 5, other radiopharmaceuticals based on BN monomers were developed. In order to stabilize the molecule reducing the peptide degradation in plasma, a lanthionine internal bridge was introduced into the molecule to obtain lanthionine-stabilized bombesins. Two of these newly synthesized bombesin peptides (C5-BN and C6-BN) were selected according to the binding affinity to GRPR for further pre-clinical evaluation. $^{18}$F radiopharmaceuticals were developed via Aluminum Fluoride strategy. $^{18}$F-NOTA-CS and $^{18}$F-NOTA-C6 were compared in vitro in PC3 cell lines and in vivo in human xenografted animal models. Stability and tumor accumulation in comparison to $^{111}$In and $^{99m}$Tc tracers was studied. The In Chapter 6, the final conclusion of this thesis describing the clinical relevance of all the probes analysed and the future perspectives are discussed. An English/Dutch and Italian version of the summary can be found in chapter 7.
Chapter 1

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