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Bombesin related peptides/receptors and their promising therapeutic roles in cancer imaging, targeting and treatment

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Abstract

Introduction—Despite remarkable advances in tumor treatment, many patients still die from common tumors (breast, prostate, lung, CNS, colon, and pancreas), and thus, new approaches are needed. Many of these tumors synthesize bombesin (Bn)-related peptides and over-express their receptors (BnRs), hence functioning as autocrine-growth-factors. Recent studies support the conclusion that Bn-peptides/BnRs are well-positioned for numerous novel antitumor treatments, including interrupting autocrine-growth via the use of over-expressed receptors for imaging and targeting cytotoxic-compounds, either by direct-coupling or combined with nanoparticle-technology.

Areas covered—The unique ability of common neoplasms to synthesize, secrete, and show a growth/proliferative/differentiating response due to BnR over-expression, is reviewed, both in general and with regard to the most frequently investigated neoplasms (breast, prostate, lung, and CNS). Particular attention is paid to advances in the recent years. Also considered are the possible therapeutic approaches to the growth/differentiation effect of Bn-peptides, as well as the therapeutic implication of the frequent BnR over-expression for tumor-imaging and/or targeted-delivery.

Expert opinion—Given that Bn-related-peptides/BnRs are so frequently ectopically-expressed by common tumors, which are often malignant and become refractory to conventional treatments, therapeutic interventions using novel approaches to Bn-peptides and receptors are being explored. Of particular interest is the potential of reproducing BnRs in common tumors, such as the recent success of utilizing overexpression of somatostatin-receptors by neuroendocrine-tumors to provide the most sensitive imaging methods and targeted delivery of cytotoxic-compounds.

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Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Keywords

Bombesin; cancer; targeted therapy; nanoparticles; gastrin-releasing peptide; prostate cancer; breast cancer; lung cancer; CNS tumor

1. Introduction

Mammalian bombesin(Bn)-related peptides [Gastrin-releasing-peptide(GRP) and neuromedin-B(NMB)] not only occur widely in peripheral-tissues and the central-nervous-system(CNS)[1], they have a wide spectrum of actions in both physiological and increasingly, pathophysiological processes[1,2•,3,4]. Their biological actions are mediated by two G-protein-coupled receptors, the GRPR(BB2) and NMB R(BB1)[1,2•,3]. A third receptor is included in the Bombesin-receptor(BnR) family, bombesin-receptor-subtype 3(BRS-3,BB3) because of its high homology(47–51%) to GRPR/NMBR, however, at present its natural ligand is unknown and is likely to have a unique structure, because BRS-3 has low-affinity for all natural-occurring Bn-peptides[2•,5,6]. GRPR, NMBR and BRS-3 activation stimulate a large number of cellular signaling cascades, which are principally mediated by phospholipase-C activation resulting in stimulation of protein-kinase C and cellular calcium changes[1, 2•,7].

Recently a number of reviews/papers have covered various aspects of BnR's pharmacology, physiology and role in various pathophysiological states. These include reviews of general advances in all these aspects[2•,3]; the role of BRS-3 in obesity and diabetes[5]; the role of BnR's in feeding disorders[2•,8]; pruritis[2•,9]; CNS function/disorders including memory, stress[2•,10]; lung diseases[2•]; inflammatory disorders[2•,11]; the signaling especially in cancer[2•,7] and the development of BnR-ligands as possible imaging modalities[2•, 12,13]. In the present paper the possible role of BnRs in neoplastic processes will be concentrated on with a view to the use of their overexpression/growth effects by many human-cancers as a potential novel target for treatment. Particular attention will be paid to papers within the last five years and to recently described novel approaches using BnR tumoral overexpression to either image these tumors or to delivery cytotoxic agents(radioactive compounds, chemotherapeutic agents including using nanoparticles, immunological agents or other cytotoxic-compounds).

2. General: Bn-related peptides-structures, receptors, pharmacology

This family of peptides are named bombesin-related peptides because the original member of this family, bombesin, a 14 amino-acid peptide was isolated from the skin of the frog, *Bombina orientalis*, and subsequently a large number of related peptides were isolated from invertebrates[1]. Subsequently two mammalian members, GRP, a 27-amino-acid peptide that has a COOH terminus of Gly-His-Leu-Met-NH₂ and thus resembles Bn, and a decapeptide, NMB, having a COOH-terminus ending in Gly-His-Phe-Met-NH₂, resembling the invertebrate peptides litorin-ranatensin[1]. The GRP-gene is located on human-chromosome-18 and has two introns/three exons, whereas the NMB gene is located on human-chromosome 15-q11 and has three exons. NMB is encoded in preproNMB, a 76 amino-acid precursor, which is processed to NMB32 and the decapeptide, NMB. GRP is

processed from a 148 amino-acid peptide preproGRP. The GRPR-gene is localized to chromosome-Xp22 similar to the BRS-3 gene, which is located on chromosome-XA71.7.2, whereas the NMBR-gene is located on chromosome-6p21[1].

hGRPR has high-affinity for the GRP and 650-fold selectivity for GRP over NMB, whereas the hNMBR has the reverse pattern, with a high-affinity for NMB and 640-fold selectivity for NMB over GRP[2•,6]. Numerous studies show there is considerable species variation in not only the affinities of various Bn-related-peptides for GRPR/NMBR, but also variation in whether they function as full agonists/antagonists, so that it is important to examine the pharmacology of possible therapeutic-agents for use in human-studies in cells containing human-BnRs[6,14,15]. In addition to GRP/NMB, there are a number of selective-natural/synthetic Bn-related-analogues, that function as selective-agonists for these receptors, especially the GRPR, and in many cases they are much more metabolically-stable than GRP/NMB, which are rapidly degraded[1,6,12, 16•]. In contrast, with BRS-3, even though the natural ligand is unknown, there are a number of nonpeptide agonists (MK-5046, compound 9D, G, etc.) which functional as selective-agonists[5]. With the GRPR there are no potent selective nonpeptide antagonists, although there are numerous classes of different peptide antagonists[2•,16•,17–20]. With the NMBR the most potent antagonist is a peptoid-analogue, PD168368[17], and with BRS-3 there is one potent peptide antagonist, Bantag-1[21]. A synthetic Bn-related-peptide with the unique pharmacology([DPhe⁶,βAla¹¹,Phe¹³,Nle¹⁴]Bn(6–14)), of having high-affinity for all three human-BNRs, as well as GRPR/NMBR of other species(not m,rBRS-3), has received considerable attention as a possible pan-BnR-targeting-agent for tumor-imaging or targeted-delivery[2•,16•,22].

3. General: GRP-NMB and BnR's in tumors

GRP/NMB-immunoreactivity and their mRNAs are found in a large number of tumors, and they are secreted from the tumors and in many cases function as autocrine-growth-factors, interacting with their own receptors on the tumor[1]. GRPR, is overexpressed by a large number of tumors including tumors of the prostate, breast, colon, CNS(gliomas, meningiomas), lung including non-small-cell-lung-cancer(NSCLC), small-cell-lung-cancer(SCLC)], head/neck-squamous-cell-tumors, pancreatic-cancer, and neuroblastomas[1,23, 24•,–25]. NMBR is also frequently overexpressed in neoplasms including by tumors of the lung (NSCLC, SCLC), pancreas, colon and carcinoids (bronchial, intestinal)[1,23]. BRS-3 is found in neuroendocrine tumors, tumors of the lung, pancreas, pituitary, ovary and prostate[23,26]. GRP and NMB stimulate the growth and/or differentiation of a wide range of cancers, and studies suggest GRP functions as an autocrine-growth-factor in a number of tumors, whereas in other tumors, such as colon-cancer, it has weak growth effects, and functions primarily as a morphogen[2•,24•,25,27]. Recent studies demonstrate in a number of different tumors(neuroendocrine, lung, prostate, head/neck-squamous-tumors) that activation of GRPR, NMBR, BRS-3 resulting in growth responses are frequently mediated by transactivation of tumor EGFR or HER2[28•,29–31]. This signaling pathway frequently requires SRC-activation, action of PKCs, stimulation of reactive oxygen species, and stimulation of matrix metalloproteinases with generation of EGFR ligands[28•,29]. In a number of tumors this interaction has been used to define a

novel potential therapeutic approach, because the combination of a BnR-antagonist and an EGFR-inhibitor have potentiating effects on tumor growth such that together they were more potent than either alone[28•,29,31].

4. General: Use of BnR-antagonists for anti-growth effects on tumors

Numerous studies show the possible therapeutic promise of BnR-antagonists or other agents that block the growth-stimulatory action of BnR agonists on tumors[1,32], since the original observation that Bn-related peptides are synthesized, secreted and have autocrine-growth effects on human-small-cell-lung-cancers, and that monoclonal Bn antibodies inhibit growth of these tumors both *in vitro/in vivo* xenografts[33]. The use of BnR-antagonists(GRPR,NMBR,BRS-3)in specific tumors is discussed in more detail in each of the tumor sections below.

In general the use of BnR-antagonists has been reported to effect the growth of a wide range of tumors including cancers of the colon, ovary, lung, breast, kidney, CNS(glioblastoma,medulloblastomas), pancreas, liver, prostate, head/neck, and neuroblastomas [34–36, 37•,38–42]. Furthermore,GRPR-antagonists suppress development of experimental benign-prostatic hypertrophy(BPH),which is due to a pathologic proliferation of prostatic glandular and stromal tissues. In this model of BPH,the GRPR-antagonists reduce the volume of human-prostatic cells, lower prostate-weight and induce significant changes in >90-genes related to growth, inflammatory processes, and signal transduction, which are thought to be important in the pathogenesis of BPH[43••]. In the various BnR-antagonist studies cited above, inhibition of each of the three-BnRs(GRPR,NMBR> BRS-3) decrease growth of various tumors, however, the GRPR has been the most extensively-studied, followed by the NMBR and lastly the BRS-3. In the case of BRS-3 and to some degree with the NMBR, the lower number of studies is related to the lack of availability of specific antagonists until recently.

In the various studies of the tumoral growth-inhibitory effects of BnR-antagonists cited above, the inhibition is reported in various tumors with either the Bn-antagonist alone, as well as in combination with other agents, which in some cases results in increased cytotoxicity. As discussed above, in head/neck-squamous-cancer-cells, medulloblastoma-cells and lung-cancer-cells, the combination of a BnR-antagonist and an EGFR-antagonist are reported to have potentiating growth inhibitory effects, mediated in part by their inhibitory effects on transactivation of the EGFR by the Bn-related peptide[28•,29,31]. Effective Bn-antagonist combinations used include: in rat-glioma-cells the combination of a GRPR antagonist and temozolomide was more potent at inhibiting growth than either single treatment alone[38]. Furthermore, the combination of the GRPR antagonist, RC-3095 and gemcitabine in nude-mice with xenografts of the human-pancreatic-cancer-cell, CFPAC-1, was more potent than either alone[40]. GRPR antagonists potentiate the inhibitory effects of histone-deacetylase inhibitors on lung-cancer-cells[44]; and the combination of a GRPR-antagonist and various cytotoxic-agents(5-FU,irinotecan) produced greater inhibition of growth of colon-cancer-xenografts(HT-29,HCT-116,HCT-115) that either alone[37•]. In contrast, in NMBR-bearing medulloblastoma-cells, whereas a NMBR-antagonist potentiates the inhibitory effects of EGFR blockade on cell-growth[45], but it did not potentiate the

inhibitory effects of histone deacetylase inhibitors, demonstrating the selectiveness of NBMR-antagonist inhibition synergy to the type of combination cytotoxic-agent used.

Silencing of BnR-signaling in cancers has also been recently reported using various siRNA-constructs. Silencing of GRPR/GRP by siRNA-delivery has been shown to decrease the signaling cascades leading to proliferation and the growth of neuroblastomas[39], ovarian-cancers[35] and lung-cancers[46]. Another novel approach reported to be effective[47] in silencing the autocrine-growth effect of Bn-related peptides is to immunize animals containing melanomas which possess GRPR and produce GRP, with a DNA-vaccine contain GRP-fragments coupled to tetanus-toxoid and helper-T cell epitopes. Administration intramuscularly of this vaccine decreased B16-F10 melanoma lung invasion and tumor associated angiogenesis[47].

A phase 1-trial of the GRPR-antagonist, RC-3095, was reported in 25 patients with various solid tumors[48]. No side-effects occurred and no patients demonstrated an objective tumor response. However, one patient with a GRPR-positive, medullary thyroid cancer demonstrated a minor response[48]. The success of various combination therapies of BnR-antagonist with another cytotoxic-agent in studies described above, suggest that this might be a novel approach in patients with BnR-tumors[38]. An attempt in patients with small-cell-lung-cancer to block the autocrine effect of Bn-like peptides on the tumor was reported in 13 patients[49]. Infusion of the monoclonal antibody-2A11 which binds to the biologically active COOH-terminus of Bn/GRP amidated-peptides was performed, and one patient demonstrated an objective tumor response, four had stable disease, and further evaluation was recommended[49].

5. Imaging and targeted-delivery of cytotoxic-agents to neoplasms using tumoral overexpression of BnR's to target the tumor: General comments

There is increased interest in the approach to image tumors, as well as in using BnR-overexpression to target cytotoxic-agents to tumors. This increased interest is due in large part to the successful application of this approach to tumors overexpression somostatin receptors (sst1-5). A number of tumors(CNS, endocrine, neuroendocrine, breast, lymphomas) overexpress somatostatin-receptors and are imaged using various radiolabeled-synthetic somatostatin-analogues[50••]. At present the use of ⁶⁸Ga-labeled-somatostatin-analogues with positron emission-tomographic scanning(⁶⁸Ga-labeled-somatostatin-analogues PET-scanning) is the most sensitive method to image neuroendocrine tumors(carcinoid, pancreatic neuroendocrine- tumors). In Europe,⁶⁸Ga-labeled-somatostatin-analogues PET-scanning has become the recommended method to assess neuroendocrine-tumor location and extent, because its use changes management in 20–50% of patients compared to conventional imaging modalities[cross-sectional-imaging with computed-tomography(CT-scanning), magnetic resonance imaging(MRI), ultrasound]. The results of these imaging studies have established that this methodology can target the radiolabeled-probe with very high specificity/sensitivity to neuroendocrine tumors overexpressing somatostatin-receptors[50••]. This finding has lead to the development of somatostatin-analogues that are coupled to cytotoxic-radiolabels

including ^{177}Lu lutetium/ ^{90}Y yttrium, which can be used therapeutically in these patients[50]. A recent prospective, double-blind study (NETTER-1) reported in preliminary form[51], in 230 patients (randomized 1:1) with advanced, inoperable, progressive, small intestinal, metastatic ileal carcinoid tumors, demonstrates for the first time, that using a ^{177}Lu lutetium-labeled-somatostatin-analogue, results in a highly significant extension of progressive-free survival compared to patients not treated with the ^{177}Lu lutetium-labeled-somatostatin analogue ($p < 0.0001$) and likely extension of survival (23 vs 67 deaths), with acceptable toxicity. Unfortunately, most common neoplasms do not overexpress somatostatin-receptors, however, many overexpress BnR's[1,23], which has led to markedly increased interest in using a similar approach using Bn-analogues to both image primary tumor location and extent of these tumors, as well as to target them with cytotoxic-agents[12,13,16•,52].

Because of the high frequency of overexpression of BnRs (particularly-GRPR) by many common tumors, reports of using these receptors to image/target these tumors have markedly increased in number and today there are more than 400 papers dealing specifically with this subject[12,13,16•,52]. A large number of synthetic Bn-analogues have been described with the majority being coupled by various linkers (primarily DOTA, DTPA, NOTA, HYNIC, DPR, DTMA) to various radioisotopes ($^{99\text{m}}\text{Tc}$, ^{111}In , ^{125}I , $^{185/187}\text{Re}$, ^{18}F , ^{64}Cu , ^{68}Ga , ^{90}Y , ^{177}Lu)[12,13,16•,52]. Initially only BnR-agonists were used because of the assumption that for optimum imaging/targeting that ligand-internalization was needed. Numerous previous studies have reported that each of the BnRs internalize agonist ligands, whereas antagonists show no or only minimal internalization, and also agonists stimulate rapid internalization of the BnRs[1,53–57]. The recent finding that radiolabeled-somatostatin antagonists gave superior imaging results to radiolabeled-agonists[58], led to similar studies in BnRs, and recent studies with GRPR report a similar finding with radiolabeled-GRPR-antagonists[16•,56,57,59–65]. In general tumor-localization *in vivo* in animal studies and a few human-studies (Table 1) using radiolabeled-ligands has proven to be a sensitive method to image BnR-positive tumors and these results will be discussed under the specific tumor types in the following sections.

In addition to coupling to radioisotopes for tumor-localization, BnR-ligands have also been coupled to other compounds for tumor-imaging. These include: coupling to fluorescent-probes to be used for optical-imaging[66–68]; coupling to gold-nanorods coated with PEG which can be used for photoacoustical imaging for use particularly in breast-cancers identification[69]; conjugation to superparamagnetic iron oxide nanoparticles[70,71] or to other contrast agents[Gd-TTDA-NP, a protein based contrast agent, ProCA1] enhances detection by MRI; magnetofluorescent polymeric nanoparticles coupled to BnR-agonists improve the localization of the nanoparticles in PC-3 bearing mice compared to nanoparticles without coupling to a Bn-agonist, suggesting this approach could be useful for prostate-cancer imaging[72]; and the use of radiolabeled-monoclonal antibodies to proGRP to image tumors (gastric, lung) overexpressing Bn-related peptides.

Also bivalent probes for imaging and tumor-localization have been shown to be effective including; the combination of a ^{64}Cu radiolabeled-Bn analogue coupled also to DUPA (a pentadioic-acid derivative which is a prostate specific membrane antigen (PSMA) probe or a BnR-agonist coupled to a PSMA inhibitor for use in imaging/targeted-delivery in prostate-

cancer[73]; using a heterodimeric peptide ligand containing a BnR-agonist (with radiolabel) combined with motifs recognizing integrins $\alpha(v)$ $\beta(3)$ demonstrated enhanced uptake in GRPR containing cells/tumors[63,65,74,75, 76•,77–79] and a ^{99m}Tc -radiolabeled-BnR-agonist conjugated to a folate receptor ligand to enhance localization, because many tumors overexpress folate receptors[80]. In addition, radiolabeled-BnR-agonists/antagonists have been co-administered with neutral endopeptidase inhibitors, which are one of the major proteolytic enzymes for Bn related peptides[81], which has resulted in enhanced uptake in tumor xenografts.

In addition to developing BnR-ligands for imaging, BnR-ligands have been coupled to a wide range of potentially cytotoxic-agents to allow peptide-receptor-mediated-targeting (PRRT) by utilizing the over-expression of the BnR by tumors[12,16•,32,82]. These include coupling various BnR-ligands (primarily agonists) to cytotoxic-radioisotopes (^{90}Y , ^{177}Lu)[16•, 82,83]; the coupling of BnR-ligands to phthalocyanine or to porphyrin-photosensitizers[84] to allow photodynamic therapy[85]; the coupling to various siRNA which can affect tumor proliferation/growth/viability[35,86]; coupling to various cytotoxic-chemotherapeutic agents including paclitaxel[87], camptothecin[88,89], and doxorubicin[32,90], as well as to various cytotoxic-marine toxins [hemiasterlin, dolastatin][91]; coupling BnR-agonists to the antimicrobial peptide, magainin 11[92] markedly increase the cytotoxicity of magainin 11 both *in vitro* in a number of GRPR-containing tumor cells and *in vivo* in MCF-7 breast-cancer-cells; and coupling BnR-agonists coupled to antimicrobial cytotoxic-peptides showed enhanced cytotoxicity for breast-cancer-cells[93]. A doxorubicin-containing Bn-conjugated-analogue, AN-215, has been studied in a number of different cancers and shown to have antitumor activity in cancers of the pancreas, lung, prostate, colon, ovary, endometrium, breast, stomach, and CNS (glioblastomas)[16•,32]. Bn-analogues have also been coupled to a number of other cytotoxic-agents including diphtheria-toxin[94], mitochondrial-disruptive-peptides, and to various agents, which activate the immunological system resulting in tumor cell-death[16•,95,96].

One approach receiving increasing attention as an effective novel method to deliver cytotoxic-agents/image cancer-cells, is to use the over-expression of BnRs on the tumor-cells to target nanoparticles, liposomes or siRNAs to the cancer-cells. BnR-agonists coupled to liposomes and ^{99m}Tc showed high specificity/selectivity for imaging breast-cancer-cells in tumor-bearing nude-mice[97]. BnR-peptide-agonist ligands have been coupled to liposomes, which can, when loaded with doxorubicin or other cytotoxic-agents, demonstrate specific cytotoxicity for cancer-cells[98–102]. In a number of studies a liposome-doxorubicin-complex conjugated to a BnR-agonist showed greater tumoral cytotoxicity than free liposome-doxorubicin-complex[100,102]. Furthermore, an ^{188}Re -liposomal-doxorubicin-complex conjugated to a BnR-agonist caused greater prolongation of survival in nude-mice with pancreatic-cancer-xenografts than animals treated with this complex not coupled to Bn-agonist[103]. Conjugation of Bn-analogues to doxorubicin-loaded-nanoparticles demonstrate excellent cytotoxicity against MCF-7/breast-cancer-cells *in vitro* and in a breast-cancer animal-model[90]. Furthermore, the use of this approach reversed the resistance of the breast-cancer-cells to doxorubicin[90]. Conjugation of a GRPR-agonist-ligand to nanoparticles loaded with docetaxol were 12-times more cytotoxic-for breast-cancer-cells than free-docetaxol[104].

Gold-nanoparticles are now being widely investigated for their use in antitumor-treatments because they can be adjusted to different sizes producing biological responses of interest, and can be used to carry cytotoxic cargo to the tumor-cells[105]. Bn-ligands coupled to gold-nanoparticles retain high-affinity for GRPR[106] and have enhanced selectivity and antitumor activity[105]. Au-nanoparticles conjugated to the GRPR-agonist,[Lys³]Bn and radiolabeled-with ^{99m}Tc, are internalized into nuclei of prostate-cancer-cells and thus could be suitable for delivery of photosensitizing agent or other cytotoxic-agents for prostate-cancer therapy[107]. Bn-conjugated to gold-nanoparticles are internalized in PC-3 prostate-cancer-cells by clathrin-mediated endocytosis[108] via clathrin-coated-pits and intracellularly the gold-nanoparticles are released in the lysosomes. ¹⁷⁷Lu-coupled-nanoparticles have been proposed as a new class of theranostic radiopharmaceuticals and these have been recently conjugated to BnR-agonists, and show preclinical efficacy in prostate-cancer[107].

siRNA's are receiving increasing attention because of their specificity and therapeutic potential, especially for treatment of cancer, however, one of the main problems is to increase their specific-uptake and target them to the desired site. Receptor-mediated-endocytosis as well as their incorporation into nanoparticles/liposomes are commonly used carriers to deliver siRNA. The conjugation of siRNA to Bn/BnR-ligands increases apoptosis and decreases invasiveness of ovarian-cancer-cells[35], as well as suppresses tumorigenesis and metastatic potential of neuroblastomas[109,110]. In the prostate-cancer-cell-line, PC-3, siRNA-constructs coupled to a Bn-agonist-analogue, undergo receptor-mediated endocytosis with a Km-value similar to that seen for pharmacological responses of the cell, utilizing a clathrin-, actin-and dynamin-mediated pathway. Intracellularly the GRP-antisense construct localizes to endomembrane-vesicles associated with Rab7/Rab9 and is transported to late-endosomes/the trans-Golgi-network, demonstrating the deep intracellular transport of the siRNA. An anti-hypoxic-inducible-factor alpha(anti-HIF α) siRNA in nanoparticles coupled to a Bn-agonist-peptide, is rapidly internalized in tumor-cells by receptor-mediated-endocytosis, and the nanoparticles facilitate endosomal escape of the siRNA. Both in vitro and in vivo when given by systemic delivery this siRNA-construct inhibits human-Glioma, U87 growth and in vivo demonstrates greater inhibition of xenograft's growth in nude-mice than seen with nontargeted-delivery systems.

6. Bn-peptides-BnR: Breast-Cancer

In breast-cancers, 38–96% possess GRPR and 0–50% NMBR,BRS-3[23,26, 111•,112,113]]. A number of studies report GRPR-activation stimulates breast-cancer cell-lines, the migration of tumor in vitro cells[114]; their growth: and that GRPR-overexpression enhances cell-invasiveness[113]. In nude-mice with breast-cancer xenografts, induction of high titers of anti-GRP antibodies by a novel GRP-vaccine(HSP65) results in both protective and tumor-immunity[115]. Furthermore, knockdown of GRP in MCF-7 breast-cancer-cells markedly reduces tumor-invasion[113].

GRPR-receptor-antagonists inhibit metalloproteinase-9 activity, secretion of bFGF,IGF-1 And VEGF-A by breast-tumor-cells[116], as well as decrease vessel-density[116] and growth, both in vitro and in vivo breast-cancer xenografts in nude-mice[116,117]. Similarly,

a NMBR-antagonist inhibited growth of MDA-MB-231-breast-cancer-cells both *in vitro* and *in vivo* in xenografts in nude-mice. A GRP-monoclonal antibody inhibited proliferation of breast-cancer-cells[115] suggesting that Bn-like peptides have an autocrine role in breast-cancer-cells.

In breast-cancer patients, higher expression levels of GRP-IR in lymph node metastases is associated with decreased survival and higher primary tumor-expression of GRP correlates with the presence of lymph node metastases[113]. In breast-cancers the expression of GRPR correlates positively($p=0.026$) with estrogen-receptor-expression[111•].

Various radiolabeled (^{111}In , ^{64}Cu , $^{99\text{m}}\text{Tc}$, ^{68}Ga , ^{18}F) BnR-agonists(primarily for GRPR) image[118–122] or allow targeted-delivery of cytotoxic-radioisotopes(^{177}Lu) to bind to breast-cancer-cells both *in vitro*[118,120–122] and *in vivo* to image breast-cancers xenografts in nude-mice[120–123]. In a comparative study *in vivo* of xenografts of breast-cancers in nude-mice, a ^{68}Ga -labeled-BnR-agonist showed greater tumor-uptake than a commonly used PET-imaging agent, ^{18}F FDG. Furthermore, the ^{68}Ga -labeled-BnR-agonist demonstrated tumoral changes with tamoxifen-treatment that were not seen with ^{18}F FDG and thus not only imaged the tumor better than ^{18}F FDG, but also allowed assessment of responses to hormonal-treatment of the breast-cancer-cells, not seen on ^{18}F FDG. A high-affinity Bn-agonist-coupled fluorescent-probe demonstrated both *in vitro* and *in vivo* in breast-cancers xenografts in nude-mice, high-uptake by the tumor of the probe, but the degree of uptake was influenced by the type of linker used. Furthermore, recent studies also report a number of BnR-antagonists(primarily to GRPR) coupled to radioisotopes(^{111}In) are highly effective at binding to breast-cancer-cells *in vitro* and imaging breast-cancers xenografts *in vivo*[111•]. BnR-agonists conjugated to superparamagnetic-iron-oxide nanoparticles, which function as a targeting contrast agent for MRI-imaging[70], retain high-affinity for the GRPR on breast-cancer-cells, and demonstrated good diagnostic ability to localize breast-cancer-xenografts in nude-mice[70]. A hybrid-probe combining a $^{99\text{m}}\text{Tc}$ -labeled-BnR-agonist with a folate-receptor ligand, another receptor, which is frequently overexpressed in breast-cancers[80], was reported to show enhanced uptake in both *in vitro* studies with breast-cancer-cells and in breast-cancer- xenografts.

BnR-agonists conjugated to gold-nanoparticles or to gold-nanorods coated with polyethyleneglycol to produce a potential photoacoustic-imaging-agent[69], retain high-affinity for GRPR-expressing-breast-cancer-cells. $^{99\text{m}}\text{Tc}$ -labeled-liposomes conjugated to a BnR-agonist demonstrate high-uptake and strong scintigraphy images of breast-cancers xenografts in nude-mice[101,123]. Heterodimeric-PET-probes combining the RGD sequence, which binds to $\alpha(v)\beta(3)$ -integrins[119] and a BnR-agonist to various radioisotopes(^{18}F , ^{64}Cu , ^{68}Ga) demonstrates high-uptake and the ability to image breast-cancer-cells in xenografts in nude-mice. The dual nature of this probe allows it to image breast-cancers with high BnR-expression and low $\alpha(v)\beta(3)$ -integrin-expression and those with the reverse distribution[119].

There is considerable interest in the possible treatment of breast-cancers with BnR-ligands conjugated with cytotoxic-radiolabeled-Bn-analogues(^{177}Lu , ^{90}Y) as has been demonstrated so effectively with studies using $^{177}\text{Lu}/^{90}\text{Y}$ -labeled-somatostatin-analogues to treat patients

with malignant, neuroendocrine-tumors[50••]. Studies report such Bn-labeled-ligands bind breast-tumor-cells with high-affinity and can have cytotoxicity for breast-cancer-cells in vitro/in vivo xenografts in nude-mice[111•].

In other studies nonradioactive cytotoxic-agents coupled to BnR-ligands show promise for targeted-cytotoxicity in breast-cancers. Photothermal treatment of breast-cancer-cells incubated with a BnR-agonist coupled to gold-nanorods coated with PEG demonstrate destruction of the breast-tumor-cells[124•]. Docetaxol-loaded-nanoparticles coupled to a BnR-agonist[104] are >12 times more cytotoxic-for breast-cancer-cells than the nonBn-coupled docetaxol-nanoparticles, suggesting this approach could be useful for active targeting of breast-cancer-cells. Doxorubicin-loaded-nanoparticles coupled to Bn[90] demonstrate excellent in vitro cytotoxicity for breast-cancer-cells and in vivo on breast-cancer-xenografts in nude-mice. Targeting GRPR on breast-cancer-cell by the cytotoxic-Bn-doxorubicin-construct, AN-215[125], inhibited growth of 5 breast-cancers cell-lines, while the control doxorubicin-analogue, not conjugated to a BnR-agonist(AN-201), had no effect. Furthermore, low or no expression of multi-drug resistance, protein-1 or multidrug resistance-related protein-1, was seen after treatment of breast-cancer-cells with the AN-215[125].

In 126 female patients with suspicious breast lesions scheduled for biopsy/surgery the ability of ultrasound or ^{99m}Tc -RGD-Bn with SPECT/CT to localize the tumor was compared[76••] (Table 1). ^{99m}Tc -RGD-Bn is a dual receptor-targeting probe combining an integrin $\alpha(v)\beta(3)$ and GRPR-targeting-peptide[76••]. ^{99m}Tc -RGD-Bn with SPECT/CT had a sensitivity of 93.5% which was significantly better than ultrasound(82%), and retained high specificity(79%), equal to ultrasound. ^{99m}Tc -RGD-Bn with SPECT/CT detected significantly more lesions 10mm, and was more sensitive than ultrasound at detecting lymph node metastases[76••]. The specificity/positive-predictive values of ultrasound and ^{99m}Tc RGD-Bn were not different. The authors conclude that, ^{99m}Tc -RGD-BBN SPET/CT shows promise for imaging breast-lesions, however, cannot solely replace ultrasound,but it can be used as an additional imaging approach to eliminate the necessity for surgical biopsy and histopathologic examination, because of its high negative-predictive value. They conclude that the best approach maybe to combine the different imaging modalities.

Various radiolabeled-BnR-agonist probes have been examined for their ability to localize and image the tumor in breast-cancer patients in a few studies (Table 1)[62,76,126–128,128–134]. In 4 women with breast-cancers and seven healthy subjects given the radiolabeled-BnR-agonist, ^{99m}Tc -HYNIC-Lys³-Bn[126], the distribution of the radioligand in normals was examined(kidney>lugs>pancreas> liver>ovaries>bone marrow) and high-uptake in malignant breast-tumors was found. In three women with breast-cancers[127] imaging results with ^{99m}Tc -Bn were compared to that with ^{99m}Tc -alone[127] and in all cases the uptake in the tumor was greater with the ^{99m}Tc -labeled-Bn probe,which also imaged lymph node metastases,suggesting possible utility of this approach(Table 1). In a study of 33 consecutive women with suspicious palpable breast-lesions the sensitivity/specificity of ^{99m}Tc -Bn-scanning for identifying breast-cancers was 100%/66% with a negative-predictive value of 100% [129]. In a study of 13 patients(9-suggestive breast-cancers by

clinical exam, 4-tamoxifen-resistant bone metastases from breast-cancers) underwent imaging studies after receiving a ^{99m}Tc -Bn-labeled-agonist(^{99m}Tc -RP527) (Table 1). [134]. The primary tumor was seen in 8 of the 9 patients with clinical breast-cancers(all had proven breast-cancers with GRPR-IHC positive cells) and in surrounding lymph nodes or distant metastases, but localization was not seen in the tamoxifen-resistant patients with low Bn-IHC[134]. In another study with the same radio-BnR-agonist-ligand(^{99m}Tc -RP527)[135], 4 of 6 patients with breast-tumors showed specific tumor-localization and good tumor-imaging(Table 1).

7. Bn-peptides-BnR: Prostate-cancer

In contrast to normal prostate and those showing benign, prostatic hyperplasia[136], in which by IHC, 73% are negative, 23% show weak-moderate staining and only 4% strong staining, prostate-cancers generally show over-expression of BnRs[23,136–139]. In primary prostate-cancers in various studies, 62–100 % possess GRPR with NMBR,BRS-3 being uncommon((0–20%)[23,136–139] and in lymph node metastases and bone metastases from prostate-cancers, 85% and 63% have GRPR. In one study the Gleason-score showed a significant inverse correlation with GRPR-expression($p=0.009$) and in another study a positive correlation was found. In patients with prostate-cancer with high androgen-receptor tumor expression, those with also GRPR-expression had better survival[136]. It has been proposed that GRPR-overexpression in focal non-invasive, prostate glands with low-grade atypia may represent a novel, specific marker of early prostatic neoplastic transformation[137,140]. Prostatic-cancer expression of GRPR in a number of studies is reported to be influenced by androgen status: increasing with administration of androgens, and decreasing with castration[141].

A number of studies report GRPR-activation stimulates growth/invasiveness of prostatic-cancer cell-lines[1,139]. Bn-agonist-analogues stimulate a number of signaling cascades associated with growth/invasiveness including activation of focal-adhesion kinases, COX-2, MAPK(ERK, JUN, p38), and pI3K-kinases,[139,142,143].

The growth inhibitor effects of BnR-antagonists[144], anti-bombesin antibodies[139], and GRP/Bn-vaccines on prostate-cancer cell-lines support the conclusion that Bn/GRP-related peptides may be having an autocrine-growth effect similar to described in an number of other tumors[25]. Furthermore, it has been proposed that prostatic neuroendocrine cells, which secrete Bn-related peptides, may promote the progression and androgen-independence of prostate-cancer[144].

GRPR-antagonists inhibit the growth of prostatic-cancer cell-lines and also decreased the expression of VEGF, bFGF, and binding capacity of EGF receptors as well as their mRNA levels. The growth inhibitory effects of GRPR-antagonists on prostate-cancer-cells are accompanied by inhibition of PKC, MAP kinase and c-Jun. In an experimental model of benign prostatic hyperplasia(BPH)[43••], treatment with potent GRPR-antagonists results in shrinkage of the BPH, which co-incided with a change in the expression >90 genes, including decreases in Ki₆₇-proliferative antigen, NF-kB, COX-2 and androgen-receptor expression.

A number of recent studies using various radiolabeled(^{188}Re , $^{55/57}\text{Co}$, ^{111}In , ^{64}Cu , $^{99\text{m}}\text{Tc}$, ^{68}Ga , ^{18}F) BnR-agonists(primarily-GRPR-agonists) show imaging[16•,73,74,100,121,145,146,147•,148–155] or targeted-tumoral delivery of cytotoxic-radioisotopes(^{177}Lu , ^{188}Re , ^{111}In , ^{64}Cu , ^{18}F)[16,74,77,100,147•,154] to prostate-cancer-cells both *in vitro*[16,73,74,81,121,145–147•,149,152–155] and *in vivo* to image prostate-cancer xenografts in nude-mice[16•,73,74,77,81,100,121,145,146,147•,148,149,151–155]. In two comparative studies *in vivo* of xenografts of the prostate-cancer-cell-line[155,156], PC-3, in nude-mice, a ^{68}Ga -labeled-BnR-agonist or ^{18}F -labeled-Bn-antagonist(BAY 86-4367) showed greater tumor-uptake with lower background than a metabolic probe, which is increasingly used in prostate-cancer patients. Furthermore, recent studies also report a number of BnR-antagonists(primarily-GRPR-antagonists) coupled to radioisotopes(^{111}In , ^{18}F , ^{68}Ga , ^{64}Cu , ^{177}Lu) are highly effective at binding to prostate-cancer-cells *in vitro* and imaging prostate-cancer-xenografts *in vivo*[16•,60,61,65,100,150,154,157–163]. In recent studies with somatostatin-receptors, radiolabeled-receptor-antagonists show better imaging than seen with a comparable radiolabeled-agonist[58]. There are contrasting results in the case of BnR-imaging in different cancer-cells including in prostate-cancer. In two studies[57,164] the BnR-antagonist, demobesin 1 coupled to $^{99\text{m}}\text{Tc}$ or ^{111}In provided superior imaging of prostate-cancer in mouse-xenografts than did the $^{99\text{m}}\text{Tc}/^{111}\text{In}$ -labeled-agonists[57,164]. Similarly, an ^{111}In -labeled-BnR-antagonist demonstrated superior targeting to prostate-cancer-cells,PC-3, than the comparable radiolabeled-agonist. In a study of ^{64}Cu -labeled-BnR-agonist and antagonist[165], the *in vivo* studies demonstrated the radiolabeled-agonist gave the best imaging of prostate-cancer xenografts with the low background and was preferable. At present the basis for differing results in different studies is unclear. However, it could be related to the type of Bn-agonist/antagonist used, because a recent study[56] of three different ^{111}In -labeled-GRPR-antagonists that were from different chemical classes, examining their abilities to image prostate-tumor xenografts, demonstrated they varied in their uptake in different organs and thus, this could affect their backgrounds and ability to provide superior imaging.

BnR-agonists/antagonists conjugated to fluorescent-probes[16•,67,166,167] demonstrate high-affinity/selectivity both *in vitro* and *in vivo* for prostate-cancer-cells. Using MRI-imaging[67] both lymph node and peritoneal metastases were detected in an orthotopic-mouse model of prostate-cancer and had a sensitivity of 89%, specificity-93% and accuracy-90% for detecting prostate-cancer metastases in mice. In another study[166] the photo-acoustic-agent, AA3G-70, which consists of a GRPR-antagonist coupled to the fluorescent dye, ATT0740, had high-affinity for GRPR-overexpressing prostate-cancer-cells and identified even small lesions in xenografts in mice, as well as providing an enhanced photoacoustic signal[166]. There is increased interest in optical-imaging using MRI and this has been studied in prostate-cancer using a BnR-agonist conjugated to $[\text{Gd}(\text{TTDA-BP})\text{H}(2)]_2^-$, a dual-imaging probe acting as a contrast agent for MRI and for optical-imaging. This agent was effective at targeting *in vitro* and *in vivo* in xenografts, prostate-cancer-cells. A BnR-agonist coupled to a MRI contrast agent, ProCA1 demonstrated *in vitro* and *in vivo* using prostate-cancer xenografts, imaging of the tumors, as well as prolonged retention of the probe by the tumor.

BnR-agonists conjugated to gold-nanoparticles/nanorods[107,108,126,168] retain high-affinity for GRPR-expressing prostate-cancer-cells and are taken up by the tumor-cells by receptor-mediated-endocytosis[168]. The uptake of the Bn-gold-nanorods by prostate-cancer-cells is clathrin-mediated via clathrin-coated-pits and results in intracellularlysosomal mediated-release of the gold-nanorods[108]. Combining the coupling of BnR-agonists to ^{177}Lu -labeled-gold-nanoparticles and to HIV-Tat(49,50••,51–57)a cell penetrating-peptide that reaches DNA[107], resulted in uptake and internalization to the nucleus in the prostate-cancer-cells. $^{99\text{m}}\text{Tc}$ -labeled-nanoparticles conjugated to a BnR-agonist demonstrate high uptake and strong scintigraphy images of prostate-cancer-xenografts in nude-mice. Homodimers of various BnR-agonists demonstrate increased binding affinity to GRPRs on prostate-cancer-cells[169] and $^{99\text{m}}\text{Tc}$ -homodimeric-analogue demonstrated 3-fold greater uptake in vitro and enhanced uptake in vivo in prostate-cancer-xenografts. Similarly a BnR-agonist-homodimer coupled to ^{111}In [147•] demonstrated good uptake and imaging in vitro and in vivo of prostate-cancer-xenografts.

A number of heterodimeric-probes interact with overexpressed BnR's on prostate-cancer and have increased sensitivity for imaging prostate-cancer[73,75] or for possibly targeted-delivery of cytotoxic-agents. A BnR-agonist conjugated to DUPA(small molecule, PSMA-targeting-probe) radiolabeled-with ^{64}Cu to allow PET imaging, as well as a BnR-agonist conjugated to a PSMA inhibitor, Glu-urea-Lys(Ahx)-HBED-CC, which allowed targeting to both BnR's and PSMA which are frequently overexpressed by prostate cancers, demonstrates excellent imaging and targeting to prostate-cancer-xenografts in nude-mice[73] and enhanced affinity for prostate-cancer-cells both in vitro and for xenografts in vivo. Another approach with prostate-cancer to increase the tumor-uptake of the BnR probe is to co-administer a neutral-endopeptidase inhibitor, because this is one of the main proteolytic enzymes for Bn-related-peptides[81]. This approach with both a radiolabeled-BnR-antagonist/agonist[81], increased the uptake of the radiolabeled-ligand in prostate-cancer-xenografts.

Heterodimeric-PET-probes combining the RGD-sequence which binds to $\alpha(v)\beta(3)$ integrins[16•,63,65,74,75,77,79] and BnR-agonists or BnR-antagonists coupled to various radioisotopes(^{64}Cu , ^{188}Re , ^{177}Lu , ^{111}In , ^{18}F) demonstrate high uptake and the ability to image prostate-cancers-cells in xenografts in nude-mice. The dual nature of this probe provides synergistic-effects and allows it to image prostate-cancers with high BnR-expression and low $\alpha(v)\beta(3)$ -integrin-expression and those with the reverse distribution[63,65,74,75,77,79]. Coupling of $^{99\text{m}}\text{Tc}$ -labeled-BnR-agonists to HIV-Tat(49,50••,51–57)[126], a cell-penetrating-peptide that reaches DNA[107], resulted in uptake/internalization to the nucleus in prostate-cancer-cells such that 59% of the tumor cell-bound ligand occurred in the nucleus. Prostate-cancers are among the most hypoxic of cancers[170] and this was explored to possibly effect retention of radiolabeled-Bn-analogues in prostate-cancer-cells[170]. ^{111}In -labeled-Bn-analogues were conjugated to nitroimidazoles, which function as hypoxic-selective drugs, and their uptake examined in prostate-cancer,PC-3. This combination resulted in a marked increase in retention of the Bn-heterodimer in hypoxic conditions[170] in vitro and clear delineation with increased retention in the prostate-cancer-cells in mice with xenografts in vivo[170].

There is considerable interest in the possible treatment of prostate-cancer with BnR-ligands conjugated with cytotoxic-radiolabeled-Bn-analogues (^{177}Lu , ^{90}Y , ^{213}Bi), as has been demonstrated so effectively using $^{177}\text{Lu}/^{90}\text{Y}$ -labeled-somatostatin-labeled-analogues to treat patients with malignant, neuroendocrine tumors [16, 50, 160]. Various $^{177}\text{Lu}/^{90}\text{Y}/^{213}\text{Bi}$ -coupled Bn-agonists/antagonists bind prostate-cells with high-affinity and can have cytotoxicity for prostate-cancer-cells *in vitro/in vivo* xenografts in nude-mice [77, 157, 160]. In one study the α -emitter, ^{213}Bi coupled to either of two different BnR-agonists, showed greater cytotoxicity to prostate-cancer-xenografts in mice than a β -emitting ^{177}Lu -BnR-agonist, with a good safety profile. The mTor inhibitor, rapamycin, sensitizes various tumor-cells to the effects of radiotherapy and in a recent study [157] treatment with the combination of rapamycin and a ^{177}Lu -tagged-BnR-antagonist, demonstrated greater survival in a mouse-model of prostate-cancer than with the ^{177}Lu -tagged-BnR-antagonist alone [157].

In other studies nonradioactive cytotoxic-agents coupled to various BnR-ligands demonstrate targeted-cytotoxicity for prostate-cancer-cells. Doxorubicin-loaded-liposomes coupled to Bn[100] demonstrated excellent cytotoxicity *in vivo* on prostate-cancer-cells, PC-3 xenografts in nude-mice. The Bn-coupled-liposomes-labeled-with doxorubicin showed greater cytotoxicity than the nonBn-coupled-doxorubicin-loaded-liposomes [100]. BnR-agonists conjugated to nanoparticles containing docetaxol [104] demonstrate greater cytotoxicity for prostate-cancer-xenografts in mice than the nonBnR-targeted-nanoparticles or the docetaxol alone. BnR-overexpression on prostate-cancer-cells has been reported to allow targeted-delivery of antisense-constructs to prostate-cancer-cells which are endocytosed by a clathrin-, actin- and dynamin-dependent mechanism and partially localize to endomembrane-vesicles associated with Rab7/Rab9, and thus are trafficked to deep endomembrane compartments. Targeting GRPR on prostate-cancer-cells by the cytotoxic-Bn-doxorubicin-construct, AN-215 [30], inhibited growth of prostate-cancer-cell-lines in xenografts. Furthermore, AN-215-treatment of prostate-cancer-cells decreased the expression of EGF-receptor family members and the activation of EGFR/HER-2, which are associated with a poor prognosis [30].

There are a number of studies investigating the possibility of imaging prostate-cancer in humans with various BnR-probes [130, 133, 171–173, 174, 175–179] (Table 1). In early studies using $^{99\text{m}}\text{Tc}$ -labeled-BnR-agonists [133, 178, 179] involving 4–10 patients with proven/suspected prostate-cancer, specific-uptake by the probe was seen in 25% in one study [133] and in 100% of patients in the other two studies [178, 179], for both the primary tumor and lymph node metastases (Table 1). A recent study [180] investigated the expression of various proteins used for imaging prostate-cancer or reported to be overexpressed and potentially useful for imaging (PSMA, EpCAM, VEGF and GRPR), in recurrent prostate-cancer in patients after surgery or radiotherapy. This study [180] in 17 patients prostate-cancer samples found in the tumor the PSMA; EpCAM; VEGF; GRPR positivity was; 100; 82; 82; 100% and in the surround nontumor, stromal tissue it was 0; 0; 0; 100%. This led the authors to conclude that to evaluate recurrent prostate-cancer after therapy, GRPR should not be a target for bio-imaging and that, PSMA, EpCAM; VEGF should be considered [180]. Two more recent studies report results using $^{99\text{m}}\text{Tc}$ -BnR-agonists [175, 176] to image prostate-cancer lesions (Table 1). One study [176] using $^{99\text{m}}\text{Tc}$ -Demobesin4 examined the ability of to image disease in 8 patients with prostate-cancer (2-localized, 6-met disease). In

the 6 patients[176] with advanced disease 3/6 were found to have bone-metastases,the pelvis was negative in all 6 and no primary lesions were seen, leading to the conclusion that the probe is safe, but hampered by low,metabolic stability in man(Table 1). In the second study[175],^{99m}Tc-HYNIC-ACA Bn(7–14), a radioligand showing excellent stability in vitro in human-serum, was used to image the tumor extent in 8 patients with prostate-cancer(Table 1). In vivo the radioligand was rapidly degraded(only 20% left intact at 30 min) and did not image any of the tumors[175]. It was concluded[175] that there can exist a marked disparity between assessment of the stability of a possible BnR-imaging-probe for prostate-cancer, assessed by in vitro stability studies in serum and found in vivo in humans, and it was recommended either better predictive in vitro assays need to be developed or the potential BnR-imaging-probe be assessed in vivo first for stability.

There have been a number of recent studies[62,171–173,174••] examining the ability of various radiolabeled(¹⁸F,⁶⁸Ga,⁶⁴Cu)GRPR-antagonists to image tumor location and extent in patients with prostate-cancer(Table 1). In 4 of the studies a radiolabeled-statine¹³ Bn-analogue was used[62,172,174••] and in one study a desMet¹⁴-ethylamide-analogue was used[173], which are from two of the most potent GRPR-antagonist classes(Table 1)[14,15]. In general the radiolabeled-antagonists demonstrate better pharmacokinetics than radioagonists, and showed enhanced stability in vivo. The ⁶⁸labeled-Bn-desMet¹⁴ethylamide analogue showed good stability and ability to image prostate-tumors in mice xenografts,and was given to 9 patients with advanced prostate-cancer. No adverse side effects were seen and the radiolabeled-antagonist showed pathological uptake in 5 of the 9 prostate-cancer patients(Table 1)[62]. In the 4 radiolabeled ¹³Statine-Bn-antagonist studies 10[172],7[173], 4,and 14[174••] patients with prostate-cancer were studied. Prostate-cancers were imaged in 50%[172], 14%[173], 75%, and 88%[174••]. In the largest study of 14 patients[174••] imaged prior to prostatectomy or hormonal therapy for recurrence, in addition to having a sensitivity of 88%, the ⁶⁸Ga-labeled-statine¹³-Bn analogue(BAY 86-7548) had a specificity of 81% and accuracy of 83%[174••] for the primary tumor and sensitivity of 70% for identifying metastatic lymph nodes(Table 1). It was concluded in this study[174] that using this Bn-antagonist-radiolabeled-probe with PET/CT-imaging is a promising molecular imaging technique for the detection of extra-prostatic-cancer.

8. Bn-peptides-BnR: Lung-cancer

Lung-cancer has played a very important role in the increasing appreciation of the important roles that Bn-related-peptides and BnR's play in cancer growth, differentiation and now for possible treatment. This occurred because small-cell-lung-cancers(SCLC) have long been known to produce and secrete Bn-like-peptides[24•,25] and in 1985 it was the first human-tumor in which an autocrine-growth effect was shown[33]. In addition to synthesizing Bn-related-peptides, lung-cancers frequently possess BnR's. In SCLC cancers in various studies, 52–100% possess GRPR, 55% NMBR, 25% BRS-3, whereas in nonsmall-cell-lung-cancer(NSCLC) cells, 62–78% possess GRPR, 68% NMBR, 8 %BRS-3 and in bronchial-carcinoids 0–100% possess GRPR, 4–88% NMBR, 35–88% BRS-3[1,23,181].

Activation of each of the three BnR-receptor subtypes on lung-cancer-cell-lines stimulates growth/proliferation[24•,25,29] and activation of BRS-3 stimulates increased adhesion of

tumor-cells. Recent studies demonstrate that transactivation of the EGF-receptor on lung-cancer-cells is a key mechanism for the stimulation of lung-cancer cell-proliferation by activation of each of the three BnR-subtypes[2•,28•,29,31]. Activation of phospholipase-C; stimulation of matrix-metalloproteinases with release of EGF family members; activation of Akt/Src-kinases; and generation of reactive-oxygen-species are all important signaling cascades in mediating the EGFR-transactivation by activation of BnRs in lung-cancer-cells[9,29]. Studies demonstrate that the combined use of an EGFR tyrosine-kinase-inhibitor such as gefitinib, with a BnR-antagonist(GRPR,NMBR-antagonist), causes a synergistic,inhibitory effect on growth[2•,28•,29,31].

Specific BnR-receptor-antagonists for each of the three classes of BnRs(GRPR,NMBR,BRS-3) inhibit the proliferation/growth of lung-cancer-cells and can inhibit growth of lung-cancer-xenografts in nude-mice[24•,25]. The growth-inhibitory effect of GRPR-antagonists in lung-cancer-cells is accompanied by reductions in levels of K-Ras, COX, pAKT and pERK1/2,and upregulation of p53.

GRPR-expression in non-cancerous bronchial-epithelium is associated with the presence of lung-cancer in patients who never smoked or were former smokers[181]. In lung-cancer patients strong expression of GRPR in the tumor is more frequent in patients with advanced disease, or advanced-stages($p < 0.01$)[181].

In contrast to breast-cancers and prostate-cancer, there are only a few studies using the overexpression of BnRs on lung-cancer-cells to either image/target these cells with cytotoxic-agents. In one study a ^{99m}Tc -labeled-GRPR-agonist[45] imaged lung-cancer-xenografts in nude-mice(A549 cells). Good scintigraphic images with high tumor uptake to background ratios were obtained,leading to the proposal that this could be a useful imaging approach for detecting of non-small lung-cancer[45]. Heterodimeric-probes combining the RGD sequence which binds to $\alpha(v)\beta(3)$ integrins and a BnR-agonist coupled to ^{99m}Tc , demonstrated high uptake and the ability to image lung-cancer cell metastases in mice[182].

The overexpression of BnRs by lung-cancer can be used to target doxorubicin-loaded-lipid nanostructures(LN) conjugated to Bn-analogues[183]. *In vitro* cytotoxicity studies in NCI-H460-NSLC cells demonstrated post Bn-loaded-doxorubicin-NL-particles showed high transfection rates, 3-fold enhanced cytotoxicity and *in vivo* in xenografts demonstrated 2–5 fold greater cytotoxicity than controls[183].

There is only very limited data in human-lung-cancer patients on the ability of BnR-labeled-probes to image lung-cancers. In two studies involving 3 lung-cancer patients[130,177] using ^{99m}Tc -labeled-BnR-agonists,uptake by the tumors was seen(Table 1).

Both monoclonal antibody to the biological terminus of Bn(2A11) as well as the GRPR antagonist, RC-3095, have been infused into patients with lung-cancer and various malignancies[48,49]. In 13 patients with lung-cancer[49] the Bn-monoclonal antibody was well-tolerated and resulted in complete-remission in one patient and stable disease in 4 patients. The BnR-antagonist, RC-3095, was given to 25 patients with various malignancies[2 with SCLC], and also was well-tolerated, but no tumor responses were seen, however, the planned maximal doses could not be reached[48].

The 27 amino-acid peptide, GRP, is derived from a 148 amino-acid-precursor protein, preproGRP and increased levels of various precursor forms have been found in the plasma in patients with various tumors (prostate, neuroendocrine, medullary thyroid cancer, SCLC). In the case of patients with SCLC, numerous studies have reported that proGRP serum levels are frequently elevated [1,184–186]. In two studies [184,187] involving meta-analyses on 5146/6758 patients, 71% and 72% of patients with SCLC had elevated serum-proGRP levels; the specificity was 86% and 92% for SCLC, whereas in one study [184], 92% with other diseases/malignancies had low levels. In a number of studies serum-proGRP determinations had greater sensitivity and specificity in SCLC patients than other proposed tumor markers (NSE, CYFRA-21-1) [188]. Changes in serum-proGRP levels show a better correlation with changes in tumor size with treatment in patients with SCLC than changes in serum-NSE and have greater prognostic value than changes in NSE [188].

9. Bn-peptides-BnR: CNS/nervous-system tumors

BnRs (primarily-GRPR) are reported overexpressed by a number of CNS-tumors and some peripheral nervous-system tumors such as neuroblastomas [189]. Gliomas are a frequent primary tumor of the CNS (astrocytomas, ependymomas, oligodendrogliomas) and 85–100% possess GRPR [189,190]. Neuroblastomas, occurring at sites of the sympathetic nervous-system, are the most common solid tumor of infants/children, and GRP/GRPR are found in 80% of neuroblastomas. Furthermore, bombesin-related-agonists stimulate [189,191,192] and BnR-antagonists/GRP-antibodies, inhibit [36,38,189,191,192], the growth/proliferation of gliomas/neuroblastomas. A number of these studies show GRP-related-peptides function as autocrine-growth-factors in these tumors.

Silencing of GRP in neuroblastomas using siGRP induces apoptosis and it acts synergistically with chemotherapeutic agents (ectoposide, vincristine). Furthermore, silencing of GRPR in neuroblastomas not only reduces tumor-size; it decreases cell-proliferation; unregulates PTEN, an inhibitor of the PI3K/AKT-pathway; delays tumor-growth and diminished liver metastases *in vivo*.

There have been a few studies examining the ability of GRPR-overexpression to image gliomas in human-patients (Table 1) [193,194••,195,196]. In one study [193] of 15-patients with recurrent gliomas, a ⁶⁸Ga-labeled-BnR-agonist (interacting all three BnRs) was compared to ¹⁸F-FDG using dynamic PET-imaging. The ⁶⁸Ga-labeled-Bn-analogue identified gliomas in 70%, which was superior to FDG (40%), and when combined with the ¹⁸F-FDG-PET discriminated low from high-grade gliomas (Table 1) [193]. In a second study [194••], imaging results with a ⁶⁸Ga-labeled-Bn-agonist were performed in 4-normal volunteers for distribution and dosing parameters, as well as in 12-patients with gliomas. The ⁶⁸Ga-Bn-analogue [194••] was rapidly cleared, excreted primarily via the kidneys; and in the gliomas patients all lesions seen on MRI, were seen with ⁶⁸Ga-Bn-analogue (Table 1). These results [194••] led the authors to conclude this methodology could be used to target gliomas in the future. In a third study [195] in seven patients with recurrent gliomas, imaged with a ⁶⁸Ga-labeled-pan-BnR-agonist, the imaging data was compared to results of gene-array studies on the tumors for expression of BnRs (Table 1). There was a significant correlation of uptake data with GRPR-expression, but not with NMBR or BRS-3-expression [195]. The

authors proposed the quantitative analysis of this ligand's uptake by gliomas can be used to predict the GRPR-expression levels[195]. In the fourth study the ability of ^{68}Ga -labeled-Bn was compared to FDG-PET imaging in 9-glioma patients, to compare their abilities to distinguish between recurrence and malignant transformation(Table 1). In all 9-patients the combination of ^{68}Ga -Bn-PET imaging and ^{18}F FDG-PET imaging was able to detect a malignant transformation from recurrence, which is a critical differentiation in these patient's management.

10. Bn-peptides-BnR: Other tumors

A large number of other tumors also possess BnRs including: pancreatic-cancers/cell-lines(10–75% GRPR), head/neck-squamous-cell-cancers(100% GRPR), renal-cancers(38% GRPR), colon-cancer(76–100% GRPR,63% NMBR), intestinal-carcinoids(75% GRPR,46% NMBR), and bronchial-carcinoids(35% BRS-3)[23,197,198].

Detailed studies of the expression of GRPR in colon-cancer have provided some surprising results. Normal epithelial cells of the colon do not express GRP/GRPR, but colon-cancers highly express both[197]. The ectopic-expression of GRP/GRPR in colon-cancer is associated with improved survival, delayed recurrence, and fewer lymph node metastases[198]. Furthermore, the GRP/GRPR-expression is found primarily in well differentiated tumors, is associated with enhanced attachment to the extracellular matrix, increase colon-cancer cytolysis by activation of natural,killer lymphocytes and regulating heterochromatin-protein 1Hs β , leading to the proposal that it is acting primarily as a morphogen, rather than a growth-factor in these tumors[27,198].

In head/neck-squamous-cell-cancers GRP/GRPR have an autocrine-growth effect, and activation of GRPR results in activation of cellular matrix-metalloproteinase with release of EGFR-proligands, activation of human-rhomboid family-1-gene RHBDF, transactivation of EGFR in an Src-dependent manner and activation of MAPK[28•,42,199]. The combination of a EGFR-tyrosine, kinase inhibitor(erlotinib) and a GRPR-antagonist(PD2) results in a synergistic,antitumor effect[42], leading the authors to suggest that GRPR targeting could enhance the antitumor effects of EGFR-inhibition in patients with head/neck squamous-cell-cancer[42].

Recently, use of GRPR-agonists or BnR-antagonists(primarily-GRPR-antagonists) to inhibit the growth of a number of these tumors are reported[1, 2•,5,200] including colon-cancer either alone or with a cytotoxic-agent[37•,200]; pancreatic-cancer when used in combination with gemcitabine[40]; and hepatocellular-carcinoma cells.

Only a few studies have investigated the possibility of using the overexpression of BnR's on these tumors for imaging/targeted-delivery of cytotoxic-agents. A $^{99\text{m}}\text{Tc}$ -labeled-BnR-pan-agonist imaged pancreatic-cancer-cells in xenografts with a 4-fold higher uptake in the tumor-cells than normal cells[201]; a ^{68}Ga -labeled-BnR-agonist showed enhanced uptake into the pancreatic-cancer cell-lines-AR42J compared to a ^{68}Ga -RGD-labeled-ligand for $\alpha\text{v}\beta 3$ -integrin binding[202]; a $^{99\text{m}}\text{Tc}$ -labeled-Bn-agonist imaged colonic-cancer xenografts[203] and a study[103] in which the tumor uptake/cytotoxicity of a ^{188}Re -labeled-

liposomal-Bn-agonist with or without doxorubicin loading in pancreatic-cancer(AR42J cells) xenografts was compared. Treatment with the probe with the doxorubicin resulted in greater survival times and when compared with a probe without ^{188}Re but with doxorubicin, provided evidence the co-delivery of the ^{188}Re and doxorubicin had antitumor effect[103].

A Bn-analogue coupled to a derivative of doxorubicin(AN-215) has been used in number of in vitro and tumor xenografts studies of different cancers[renal[204], endometrial, pancreatic, ovarian] and reported to have enhanced cytotoxicity over non-Bn-conjugated - doxorubicin.

Bn-radiolabeled-agonists were used in two human-studies of patients with colonic tumors(Table 1)[130,205]. In one study involving 13-patients(7-proven rectal cancer, 6-suspected), using a $^{99\text{m}}\text{Tc}$ -labeled-Bn-agonist,16/17-colorectal-cancer locations were identified(sensitivity-94%)but it was also positive in two/6 nontumor-lesions(1-Crohns disease, 1-polyp with dysplasia) resulting in a specificity of 64%(Table 1). In another study[130] $^{99\text{m}}\text{Tc}$ -labeled-Bn-imaging was performed in 5-patients with colorectal-cancer,and the radioisotope was rapidly taken up by the tumors(Table 1).

11. Conclusions

Bombesin-related-peptides are synthesized by many common tumors and overexpression of their receptors(especially-GRPR) is well documented in many tumors, including some of the most frequent causing death and needing new, novel, antitumor approaches(breast, prostate, lung, pancreas, CNS). While considerable progress has been made on the cellular signaling cascades involved in mediating the effects of activation of these receptors on growth/proliferation/differentiation of various tumors, and it is well-established that in many tumors these peptides have an autocrine-growth effects, in other tumors(colon) this is less clear, and a role as a morphogen has been proposed. There still remains controversy in the exact role that overexpression of this family of receptors play in these tumor's pathogenesis/pathophysiology. Nevertheless, numerous preclinical studies show in animal models as well as in vitro studies, that inhibition of BnR's in these tumors either alone or in combination with various cytotoxic-agents, can have marked antitumor effects. Furthermore, the overexpression of these receptors in these tumors in many preclinical studies and more recently in human-studies(especially breast/prostate-cancer) (Table 1) show promising results for imaging these tumor as well as a promising approach for tumoricidal therapy using targeted-delivery of cytotoxic-compounds via the BnR. The coupling of this targeted approach with nanoparticles-loaded with cytotoxic-agents recently, appears particularly of interest as a novel, therapeutic approach for these malignancies.

12. Expert Opinion

Despite many management/therapeutic advances a number of common tumors such as cancer of the breast, prostate, lung, and CNS, are still a frequent cause of death and new treatment approaches are needed. New approaches for both their early detection, assessment of disease-extent initially, detection of early-recurrences and new approaches for the treatment of advanced-disease, would all be important advances. There is increased

recognition that many of these tumor not only synthesize and release neuropeptides that function as growth-factor/differentiating factors in these tumors, often in an autocrine-fashion, because the tumors also very frequently overexpress the receptors for these same neuropeptides.

This is particularly true, as reviewed in this article, for the Bn-family of peptides [gastrin-releasing-peptide(GRP), neuromedin-B(NMB)] and for their receptors(GRPR,NMBR). The orphan receptor BRS-3, for which the ligand is unknown, is also included in this family of receptors[5]. Studies reviewed in this article demonstrate that manipulation or targeted use of this family of receptors has the potential to provide new, novel approaches that could be used in each of the phases of management outlined above. Manipulation of the growth/differentiating effects of activation of these receptors in various tumors by various BnR-ligands (particularly BnR-antagonists, siRNA, antibodies,etc.) either alone or coupled with other cytotoxic-agents, in preclinical studies has marked antitumor effects. Furthermore, as reviewed in this article, it is increasingly appreciated that the frequent overexpression of the BnR-family of receptors has the potential to allow sensitive imaging of these tumors allowing early detection, detection of recurrence, better assessment of tumor-extent initially, as well as when coupled to cytotoxic-agents(radioisotopes, chemotherapeutic agents, other cytotoxic-agents) to be used for targeted-therapy. One of the principal limitations of many forms of effective cytotoxic-agents in *in vitro* studies *in vivo*, is the problem of how to selectively delivery these agents to enhance their cytotoxicity as well as to reduce their side-effects, and it is increasing recognized the overexpression of the BnR-family of receptors could be used to address this problem.

Supporting the viability of this approach are recent results using radiolabeled-somatostatin-analogues to both image neuroendocrine-tumors as well as for targeted-delivery of cytotoxic-agents to these tumors using peptide-receptor radiotherapy(PRRT)[50,206]. Numerous recent studies demonstrated that coupling various radioisotopes such as ^{68}Ga to somatostatin-analogues, which have high-affinity for somatostatin-receptors(sst1-5), which are almost invariably overexpressed by well-differentiated, neuroendocrine-tumors, with *in vivo* detection using positron-emission tomography(PET-scanning), is a highly sensitive method to detect these tumors[50,206]. In fact, recent studies demonstrate it not only has high specificity, but is more sensitive than any other imaging modality both for detection of the primary tumor, the extent of metastases and for early tumor-recurrences in patients with neuroendocrine-tumors[50,206]. When these somatostatin-analogues are coupled to cytotoxic-radioisotopes such as ^{177}Lu or ^{90}Y trium, numerous studies demonstrated they allow targeted-delivery of the cytotoxic-probes to the malignant, neuroendocrine-tumor resulting in tumor cytotoxicity effecting its growth with acceptable safety[50,206]. The efficacy of this approach was recently further supported by preliminary results of a double-blind, prospective study comparing treatment with ^{177}Lu -somatostatin-analogues to unlabeled-somatostatin-analogues in patients with advance small intestinal,neuroendocrine-tumors(NETTER-1)[51].

As reviewed in this study and in other recent papers[2,12,16,32], the potential therapeutic value of using the overexpressed BnR's to delivery cytotoxic-agents to the tumor is not limited to radioisotopes and can include coupling to a diverse range of cytotoxic-

agents(chemotherapeutic agents, cytotoxic-toxins such as marine-toxins or diphtheria-toxin, mitochondria-disruptive-peptides, agents that activate the immune system against the tumor, to photosensitizing-agents, to siRNA), and especially when coupled using nanoparticle-technology, can allow enhanced delivery of a broad spectrum of potential therapeutic agents.

Because of the widespread occurrence of BnR overexpression in a number of common neoplastic tumors, this family of receptors, particularly the GRPR, is being increasingly used to explore innovative approaches to image these tumors, as well as to target them with cytotoxic agents using the overexpression of the BnRs and specific BnR ligands to accomplish this. With the promising results with a number of these approaches reviewed in this paper, it is likely a similar approach will be used with other G-protein coupled receptors overexpressed in other tumors.

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Reference annotations

* Of interest

** Of considerable interest

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Highlights

1. Bombesin (Bn)-related peptides are frequently ectopically synthesized and secreted by numerous common tumors.
2. The Bn family of receptors [BnRs],(NMBR [BB1],GRPR [BB2],BRS-3 [BB3]) is one of the receptor classes most frequently ectopically expressed or over-expressed by many common neoplasms (breast, prostate, CNS tumors, lung, pancreas, colon).
3. The presence of both ectopically expressed receptors and synthesis of Bn-ligands results in autocrine-growth/proliferative/differentiating effects on many of these tumors whose disruption can lead to therapeutic effects.
4. Numerous recent studies *in vitro*, preclinical studies using tumor xenografts and increasingly in human-disease, report the use of various approaches to use the overexpression of BnR's to image the primary tumor, tumor-extent and recurrences and show promise. These are reviewed in depth for the common tumors listed above overexpressing BnRs.
5. Similarly the overexpression of BnRs in these tumors, particularly breast and prostate, are being used to target novel cytotoxic-agents to the tumors and show promise. Progress in this area is also reviewed and summarized.

Table 1

Studies in human using radiolabeled bombesin analogs.

Study#	Year	No. Pts	Patients (Pts) studied	Isotope	Peptide	Imaging technique	Results	Ref. N
1	2000	26	Cancers of breast (n=13), prostate (n=5), colorectum (n=5), lung (n=2), gastrinoma (n=1)	^{99m} Tc	Bn	SPECT and planar scintigraphy	23 of the 26 cancers showed enhanced uptake of tracer.	[130]
2	2000	13	13 (6 suspected +7 known to have rectal cancer)	^{99m} Tc	"[Leu ¹³]Bn" [Cys ⁰ -Aca ¹ , Bn(2-14)]	SPECT and planar scintigraphy	Cancer detected in 11/13 and 2 false positives. 5/5 positive LN's detected. Results confirmed by pathologic evaluation. After 60 min all radiopeptide in intestine.	[205]
3	2002	5	3 normal; 1 prostate cancer , 1 SCLC			SPECT and planar scintigraphy	Visualize both tumors, radioactivity accumulation in liver, kidneys and thyroid Tumor uptake higher than with ^{99m} Tc sestamibi alone.	[177]
4	2002	5	5 suspicious for breast cancer			Planar scintigraphy	100% cancer and LN's visualized. Radioactivity accumulated in liver, kidneys and thyroid Tumor/breast uptake ratio higher than with ^{99m} Tc sestamibi.	[131]
5	2003	5	Biopsies from 5 suspicious for breast cancer			Biopsy withy Imaging / X-ray	48/48 biopsies high, 19/21 intermediate and 2/8 low radioactivity uptake positive for cancer.	[132]
6	2003	10	10 suspected and 1 proven with prostate cancer			SPECT and planar scintigraphy	100% cancer and LN's visualized. Results confirmed by pathologic evaluation. Detection of the LN's better than with MRI.	[178]
7	2004	14	14 pts positive for prostatic lesions			SPECT and planar scintigraphy	100% cancer and LN's visualized. Results confirmed by pathologic evaluation while ¹¹¹ In-Octreotide only detected 2/3 cases.	[179]
8	2008	11	11 (3 with proven breast cancer and 8 with possible cancer)	^{99m} Tc	[Lys ³]Bn	SPECT and planar scintigraphy	Predominant renal clearance. Pts with breast cancer showed asymmetrical uptake by breast tissue, with higher accumulation in pts with breast cancer.	[207]

Study#	Year	No. Pts	Patients (Pts) studied	Isotope	Peptide	Imaging technique	Results	Ref. N
9	2000	10	4 pts with bone metastases with androgen-resistant prostate cancer + 6 suspected breast carcinoma	^{99m} Tc	"RP527", 5-Ava-Bn(7-14)	SPECT and planar scintigraphy	Hepatic and renal clearance non blood accumulation. Radioligand uptake in 1/4 prostate cancer bone metastases and 4/6 breast cancer metastases and affected LN's.	[133]
10	2001	6	6 healthy subjects			Study of dosimetry	Hepatobiliary and renal clearance, non blood accumulation. Low uptake by brain, myocardium, lungs, breast and testes. Possible radiotracer for the supradiaphragmatic region and favorable dosimetry for SPECT.	[208]
10	2008	14	14 pts (9 suspected breast carcinoma+5 tamoxifen-resistant bone metastasized breast carcinoma)			Planar scintigraphy	8/9 suspected pts tumor and LN's were detected. 0/5 resistant pts detected.	[134]
11	2015	6	All had breast cancer	^{99m} Tc	RGD-Bn compared to 99mTc-RGD2	SPECT/CT scintigraphy compared to 99mTc-RGD2	99mTc-RGD-Bn and 99mTc-RGD2 detected 6/6	[128]
12	2015	126	Suspected breast cancer	^{99m} Tc	RGD-Bn	SPECT/CT scintigraphy compared to ultrasound	99mTc-RGD-Bn PET/CT compared to ultrasound: sensitivity: 93.5 vs 82%; specificity-79 vs 76%; PPV -87 vs 83%; NPV 89 vs 74%; accuracy- 88 vs 76%, thus higher sensitivity and NPV than ultrasound for primary and axillary- LN's	[76]
13	2014	8	All prostate cancer(2- only primary, 6 metastatic; 2- no prior hormone treatment)	^{99m} Tc	Demobesin 4	SPECT and planar scintigraphy	Positive in the 2 pts with no prior hormone treatment, negative in 6 others with previous hormone treatment	[176]
14	2013	8	All prostate cancer	^{99m} Tc	Aca-Bn(7-14)	SPECT /CT scintigraphy	No disease detected but ligand was unstable with only 20% intact after 30 min	[175]
15	2014	33	All had palpable breast lumps and ? breast cancer. (12-proven proven breast cancer	^{99m} Tc	Bn	SPECT /CT scintigraphy	Sensitivity was 100%, specificity-66%, NPV-100%, PPV-63% and accuracy-76% for differentiating malignant from benign breast lesions	[129]
16	2007	17	17 GIST pts	⁶⁸ Ga	"BZH3" [D ¹³ Tyr ⁶ βAla ¹¹ , Thr ¹³ , Ni ¹⁴] Bn(6-14)	PET scans comparing ⁶⁸ Ga-BZH3 to ¹⁸ F-FDG	FDG discovered 25/30 lesions, BZH3 8/30. Tumor uptake is lower with BZH3 than with FDG. In 1 case the lesion was	[209]

Study#	Year	No. Pts	Patients (Pts) studied	Isotope	Peptide	Imaging technique	Results	Ref. N
17	2008	9	9 low grade glioma pts			PET scans comparing ⁶⁸ Ga-BZH3 to ¹⁸ F-FDG	seen with BZH3 and not with FDG. 6/6 pts with increase with BZH3 and FDG uptake had malignant transformation. 2/2 with decreased BZH3 and no FDG uptake had malignant transformation.	[196]
18	2011	15	All had recurrent gliomas			PET scans comparing ⁶⁸ Ga-BZH3 to ¹⁸ F-FDG	10/15 positive on ⁶⁸ Ga-BZH3 and 6/15 of FDG PET. Discriminat analysis of the FDG and ⁶⁸ Ga-BZH3 kinetic could distinguish low from high grade in all cases.	[193]
19	2012	7	All had recurrent gliomas			PET/CT Scanning correlate results with gene array data	BB2 and BB3 had the highest expression levels in the gliomas. The compartment parameter k1 correlated with the expression of BB2(=0.89) while k3 reflecting internalization did not show a correlation. Therefore the expression seen on the array study could be predicted from the kinetics.	[195]
20	2013	14	All prostate cancer	⁶⁸ Ga	Bay86-7548(also called RM2)	PET/CT Scanning	Sensitivity was 88%, specificity-81%, and accuracy-83% for primary tumor; and 70% for detection of metastatic LN's.	[174]
21	2015	7	All prostate cancer		⁶⁸ Ga-RM2 compared to ⁶⁸ Ga-PSMA	PET/CT Scanning	45 different metastatic lesions were seen in the 7 patients with ⁶⁸ Ga-PSMA positive in all and ⁶⁸ Ga-RM2 positive in 43/45, missing two lesions both in the same patient	[173]
22	2015	17	8- Breast cancer and 9= prostate cancer	⁶⁸ Ga	SB3 (DPhe ⁶ Leu-NHEt ¹³]Bn(6-13)	PET/CT Scanning	4/8 pts with breast cancer and 5/9 with prostate cancer showed pathological uptake on PET/CT	[62]
23	2016	16	4 healthy volunteers and 12 glioma pts	⁶⁸ Ga	Aca-Bn(7-14)	PET/CT Scanning	⁶⁸ Ga Bn scanning identified gliomas in all patients and identified all gliomas seen by CT or MRI. In normal volunteers, ⁶⁸ Ga-Bn was rapidly cleared via the kidneys and urinary tract.	[194]

Study#	Year	No. Pts	Patients (Pts) studied	Isotope	Peptide	Imaging technique	Results	Ref. N
24	2014	4	All prostate cancer	⁶⁴ Cu	PEG4-DPhe,Gln,Trp,Ala,Val,Gly,His,Sta,LeuNH2	PET/CT Scanning	3 of 4 tumors visualized with high contrast (ratio>4) and 1 of the 4 with moderate contrast (ratio=1.9).	[171]
25	2015	10	All prostate cancer(5-primary, 5-recurrence)	¹⁸ F	BAY 864367	PET/CT Scanning	5/10 pts showed tumor	[172]

Abbreviations; Br-bombesin, LN-lymph node; pts-patients; Tc-technetium; Ga-Gallium; BAY 864367 (3-cyano-4-18F-fluorobenzoyl-Ala(SO3H)-Ala(SO3H)-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH2)(GRPR Antagonist); PPV-positive predictive value; NPV-negative predictive value; Demobesin 4- N4-Pro-Gln-Arg-Tyr-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Nle-NH2; BAY86-7548(same as RM2)=DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH2 (GRPR Antagonist);