Targeting gastrin-releasing peptide receptors for cancer treatment

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Growth factor receptors play critical roles in cancer cell proliferation and progression. A number of such receptors have been targeted for cancer treatment by either a monoclonal antibody or a specifically designed small molecule to inhibit the receptor function. Bombesin/gastrin-releasing peptide receptors (BN/GRP-Rs) are expressed in a variety of cancer cells and have limited distribution in normal human tissue. Inhibition of BN/GRP-Rs has been shown to block small cell lung cancer growth *in vitro*. Early phase clinical trials targeting human GRP-R showed anti-cancer activity. This review will focus on the study of the distribution of BN/GRP-Rs in normal and malignant tissues, and various approaches to targeting BN-GRP-Rs for cancer diagnosis and treatment. *Anti-Cancer Drugs* 15:921–927 © 2004 Lippincott Williams & Wilkins.

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Introduction

The strategy of targeting growth factor receptors for cancer treatment has made significant progress in the last decade. The overexpression of growth factor receptors on malignant cells is usually a marker of a biologically aggressive tumor and is associated with a poor prognosis. These receptors are actively involved in cell proliferation, angiogenesis, local invasion, distant metastases and most are involved in inhibiting cell apoptosis as well. It is for these reasons that growth factor receptors are attractive targets for cancer treatment.

Bombesin (BN) is a 14-amino-acid peptide discovered in frog skin [1]. Gastrin-releasing peptide (GRP), a 27-amino-acid peptide, is the mammalian counterpart of BN. Both peptides are well conserved through evolution and differ only in one of the 10 carboxy-terminal amino acids. Neuromedin B (NMB), a 10-amino-acid peptide, is another member of the BN-like peptide (BLP) family (Table 1). BLPs are neuropeptides secreted by neuronal and endocrine cells. They play important physiologic roles in regulating smooth muscle contraction, release of hormones in the gastrointestinal tract, secretion of pancreatic enzymes and serve as neurotransmitters in the central nervous systems [2]. Four subtypes of BN/GRP receptors (BN/GRP-Rs) have been discovered: GRP receptor (GRP-R), neuromedin B receptor (NMB-R), and bombesin receptor subtype 3 (BB3) and subtype 4 (BB4). The natural ligand of BB3 has not been identified and BB4 is not expressed in mammalian tissue. BN binds to both GRP-R and NMB-R with high affinity as shown in Table 2 [3]. BN/GRP-Rs are coupled to G-protein via their intracellular domain and, thus, belong to the G-protein receptor superfamily. Upon the binding of BN/GRP-Rs multiple cellular signal transduction pathways are activated, including protein kinase C (PKC), mitogen-activated protein kinase (MAPK), tyrosine phosphorylation of focal adhesion kinase (FAK), paxillin, $p130^{cas}$ and calcium mobilization, resulting in cell proliferation and growth [4,5].

BLPs are critical in small cell lung cancer (SCLC) growth. It has been well documented that SCLC cells produce BLPs and overexpress BN/GRP-Rs [6]. This forms an autocrine or paracrine loop to promote SCLC growth and invasion. A number of investigators using different strategies to block either the BLPs or GRP-R have reported significant growth inhibition of SCLC *in vitro* as well as *in vivo* [7,8].

There are several approaches to targeting growth factor receptors. Monoclonal antibodies (mAbs) bind directly to their receptors and result in blockade of receptor function leading to tumor cell apoptosis. Small molecules can specifically inhibit receptor tyrosine kinases and arrest target cell growth. Antisense oligodeoxynucleotides

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Table 1 BN-like peptides

Peptide	Sequence
GRP	Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-Leu-Thr-Lys-Met-Tyr-Pro-Arg-**Gly-Asn**-His-**Trp-Ala-Val-Gly-His-Leu-Met**-NH_2
NMB	**Gly-Asn**-Leu-**Trp-Ala**-Thr-**Gly-His**-Phe-**Met**-NH_2
BN	Glu-Gln-Arg-Leu-**Gly-Asn-**Gln-**Trp-Ala-Val-Gly-His-Leu-Met**-NH_2

Table 2 Ligand binding affinity (K_i in nM) to human GRP-R or NMB-R in transfected cells [3]

Receptor	BN	GRP	NMB
GRP-R	1.4 ± 0.2	6.2 ± 1.3	437 ± 30
NMB-R	32 ± 3	5080 ± 770	8.1 ± 5.2

(ASOs) can interfere with the translation of receptor mRNA and decrease the receptor expression on the cell surface. Radiolabeled peptides can target receptors on tumor cells for imaging purposes and cytotoxicity. Bispecific molecules (BsMol) can function as a bridge between a target molecule on cancer cells and immune effector cells to mediate specific tumor cell destruction [9]. Several mAbs have been approved by the Food and Drug Administration (FDA) for cancer treatment. Two mAbs recently approved for the treatment of colon cancer are cetuximab (Erbitux; ImClone Systems), which targets human epidermal growth factor receptor (EGF-R), and bevacizumab (Avastin; Genentech), which targets vascular endothelial growth factor receptor. Transtuzumab (Herceptin; Genentech) targets human epidermal growth factor 2 (Her2/*neu*) and has been used in the treatment of breast cancer for several years. These mAbs can mediate the destruction of target tumor cells by inducing complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity. Several small molecules targeting receptor tyrosine kinases have been approved for cancer treatment, including imatinib (Gleevec; Novartis), targeting BCR–ABL tyrosine kinase for the treatment of chronic myeloid leukemia and gastrointestinal stroma tumor, and gefitinib (Iressa; AstraZeneca), targeting EGF-R for the treatment of advanced non-SCLC.

Distribution of BN/GRP-Rs in human malignancy and normal tissue

Since the cloning and characterization of BN/GRP-Rs, we have learned more about the expression of these receptors in a variety of human malignancies. The first human malignancy found to express BN/GRP-Rs was lung cancer. Corjay *et al.* reported the detection of mRNA for GRP-R in four of seven SCLC cell lines, four of 13 NSCLC cell lines and two of two carcinoid cell lines [10]. The mRNA for NMB-R was detected in three of seven SCLC cell lines and two of 13 NSCLC cell lines [10]. Toi-Scott *et al.* studied 20 SCLC and 13 NSCLC cell lines; mRNA for GRP-R and NMB-R was detected in the majority of these cell lines [11]. Siegfried *et al.* reported the presence of mRNA for GRP-R in nine of 14 NSCLC cell lines, and NMB-R in all 14 cell lines [12]. Intracellular BLPs were detected in almost all SCLC cell lines studied [13]. Binding sites on the SCLC cell surface are relatively low, as detected by conventional assays, likely due to the rapid internalization of these receptors upon the high-affinity binding of BLPs produced by these SCLC cells. Two groups reported studying BN/GRP-Rs in human SCLC tissue samples by detecting their mRNA in approximately 30% of the cases [14,15].

GRP-R can be detected in breast cancer cell lines [16] and tumor specimens from breast cancer patients. Halmos *et al.* reported the detection of GRP-R in 33 of 100 cases of newly diagnosed breast cancer tissues using radiolabeled [^{125}I-Tyr4]BN [17]. Using a similar binding assay, Gugger and Reubi reported the detection of GRP-R in 29 of 46 cases of invasive ductal carcinomas, in 11 of 17 ductal carcinomas *in situ*, in one of four invasive lobular carcinomas and in one of two lobular carcinomas *in situ* [18]. The density of the receptor on tumor cells varied from 417 to 20 082 d.p.m./mg of tissue [18]. Reubi *et al.* reported the detection of BN/GRP-Rs in 41 of 57 cases of breast cancer by use of radiolabeled ^{125}I-[D-Tyr^6, β-Ala^{11}, Phe^{13}, Nle^{14}]BN(6–14), which bound to all three subtypes of BN/GRP-R [15].

BN/GRP-Rs are frequently expressed on prostate cancer cells. Sun *et al.* reported the expression of GRP-R in 50 of 80 cases of prostate cancer tissues [19]. They also studied the expression of mRNA in 22 cases of prostate cancer, of which 20 expressed GRP-R mRNA, three expressed NMB-R mRNA and two expressed BB3 mRNA.

Using a radiolabeled binding assay, Markwalder and Reubi detected the expression of GRP-R in all 30 cases of prostate cancer [20]. They reported the density of GRP-R was significantly high in the area of malignant cells (5241 ± 927 d.p.m./mg of tissue), compared to the area of benign prostate hyperplasia (201 ± 31 d.p.m./mg of tissue). Two prostate cancer cell lines, PC-3 and DU-145, expressed a high number of BN/GRP-Rs on their cell surfaces, ranging from 19 000 to 44 000 receptors/cell [21]. The mRNA for both GRP-R and NMB-R was detected in both cell lines as well [22].

The expression of either BN/GRP-Rs or its mRNA was detected in other cancer cell lines including glioblastoma [23], endometrial cancer [24] and melanoma [25]. In other human malignancies, BN/GRP-Rs were found in the gastrointestinal (GI) tract [26–28], colon [29,30], pancreas [31,32], kidney [33], ovary [34], squamous cell carcinoma of the head and neck [35], and neuroblastoma

Table 3 Expression of the three subtypes of BN/GRP receptor in human cancer cell lines

Tumor type	Receptor type	Surface binding[a]	mRNA[b]	Reference
SCLC	GRP-R,	10/10	17/20	[6,11]
	NMB-R		11/20	
	BB3		5/20	
NSCLC	GRP-R	7/9	20/27	[11,12]
	NMB-R		18/27	
	BB3		1/13	
Breast	GRP-R	3/8	1/1	[16]
Prostate	GRP-R	4/5	3/3	[21,22]
	NMB-R		2/2	
Pancreas	GRP-R	1/4	3/4	[32]
	NMB-R		4/4	
Stomach	GRP-R	4/5	1/3	[26]
	NMB-R		3/3	
	BB3		0/3	
Duodenum	GRP-R	4/6	1/3	[28]
Colon	GRP-R	5/9	9/9	[6,11]
Kidney	GRP-R	2/2	4/4	[33]
Head and neck	GRP-R	NA	14/14	[35]
Ovarian	GRP-R	2/2	2/2	[34]
	NMB-R		2/2	
	BB3		1/2	
Endometrium	GRP-R	3/4	NA	[24]
Glioblastoma	GRP-R	11/13	NA	[23]
Neuroblastoma	GRP-R	4/4	2/2	[36]
Melanoma	GRP-R	1/4	4/4	[25]

[a]Number of positive cell lines versus number of tested cell lines measured by either a radiolabeled BN or functional study.
[b]Number of positive cell lines versus number of tested cell lines measured by RT-PCR.
NA: not available.

[36] tumor cell lines and tissues. Table 3 summarizes the study of three subtypes of BN/GRP-Rs in human cancer cell lines, by either a radiolabeled binding assay for cell surface receptor or RT-PCR for mRNA expression. Table 4 summarizes published studies of BN/GRP-R expression in primary tumors.

In contrast, the distribution of BN/GRP-Rs in normal human tissue is not well known. The lack of a mAb to human BN/GRP-Rs for immunohistochemical staining limited the study of the distribution of BN/GRP-Rs in normal human tissue. Radiolabeled BN was commonly used to detect the BN/GRP binding sites in animals such as mouse, rat and guinea pig. The expression of BN/GRP-Rs was detected in gastric mucosa, pancreatic acinar cells, smooth muscle cells of the GI, pituitary cells, and the central and peripheral nervous system. Xiao *et al.* studied the expression of GRP-R mRNA in normal human tissues. They reported the detection of GRP-R mRNA in pancreas, stomach, adrenal cortex and brain. There was no expression of GRP-R mRNA in small intestine, colon, lung, prostate, thymus, thyroid gland, adrenal medulla, liver, kidney, heart, skeletal muscle and ovary [37]. Recently, Sano *et al.* reported a similar expression profile of GRP-R mRNA in normal human tissues [38]. They also found NMB-R mRNA in brain, spinal cord, testis and stomach, but not in intestine and uterus. The expression of BB3 mRNA was mainly found in testis, pituitary gland and brain [38].

Table 4 BN/GRP-Rs expression in human tumors

Tumor type	No. cases	No. positive cases	Reference
Lung			
small cell	7	2	[14]
	9	7	[15]
carcinoid	26	9	[15]
Breast	57	41	[15]
	100	33	[17]
	71	44	[18]
Prostate	12	12	[15]
	80	50	[19]
	30	30	[20]
	12	12	[22]
Pancreas	26	2	[31]
GI	29	16	[15]
	26	22	[27]
Colon	21	5	[30]
Kidney	16	6	[15]
Ovarian	22	19	[34]
Head and neck	25	25	[35]
Neuroblastoma	33	24	[36]

In summary, the expression of GRP-R is much more commonly found in human malignancies than in normal human tissues. It is not clear whether the expression of GRP-R signals the aggressive biologic behavior of these cancers or is just a tumor marker.

Experimental therapy and clinical applications

BN/GRP antagonists

Synthetic BN/GRP antagonists are designed to bind to human GRP-R with high affinity and block the receptor-activated signal transduction pathways. Numerous BN/GRP antagonists have been synthesized and tested. Different classes of BN/GRP antagonists and their biological functions are reviewed elsewhere [39]. Two promising BN/GRP antagonists, RC-3095 [D-Tpi^6, Leu$^{13}\psi$(CH_2NH)-Leu^{14}]BN(6–14) and RC-3940-II [Hca^6, Leu$^{13}\psi$(CH_2NH)-Tac^{14}]BN(6–14), were developed and reviewed by Schally *et al.* [40]. Both antagonists can significantly inhibit the growth of a variety of cancer cells *in vitro* as well as *in vivo*. In animal studies, mice were implanted with human SCLC, breast, or prostate cancer cells. RC-3095 or RC-3940-II was injected s.c. daily for 4 weeks. The treatment resulted in a significant reduction of tumor volume. The authors also reported the significant decrease of mutant p53 protein production, decrease of cell surface expression of GRP-R and EGF-R, and decreased mRNA levels for GRP-R, ErbB-2/Her-2, c-*jun* and c-*fos* oncogenes in cancer cells treated with both antagonists [8,41,42].

Ashwood *et al.* reported on the first non-peptide GRP-R antagonist, PD176252, (3-(1H-Indol-3-yl)-*N*-[1-(5-methoxy-pyridin-2-yl)-2-cyclohexyl-methyl]-2-methyl-2-[3-(4-nitro-phenyl)-ureido]-propionamide). It bound to both GRP-R and NMB-R with high affinity [43]. Moody *et al.* reported that PD176252 inhibited 50% of the colony formation of lung cancer cells at a low concentration of

0.2–0.3 μM [44]. In animal studies, mice bearing human lung cancer received daily injection of PD176252, resulting in a significant reduction of the tumor volume by 50% after the 4-week treatment. The advantage of a non-peptide antagonist is, of course, its resistance to proteases found in the circulation.

A mouse mAb against BLPs (2A11) was developed by Cuttita *et al.* [6]. This mAb 2A11 showed high-affinity binding to both exogenous and endogenous BLPs, and neutralized their biologic function. In a phase I clinical trial, 12 SCLC patients received an escalating dose of 2A11, from 1 to 250 mg/m^2 given i.v. once a week for 4 weeks. There was no significant toxicity from the antibody infusion, and no tumor response was observed either [45]. In a phase II clinical trial, 13 SCLC patients received an i.v. infusion of mAb 2A11 at 250 mg/m^2/dose, once a week for 4 weeks; there was one case of complete response and four cases of stable disease by radiographic criteria [46].

BN/GRP peptide antagonists and non-peptide antagonists are small molecules that can potentially penetrate into tumor tissues and provide a prolonged local inhibition of solid tumor growth. The major disadvantage of synthetic BN/GRP antagonists is their short biologic half-life, due to rapid degradation and clearance from the circulation.

Drug-linked or radiolabeled BN/GRP analogs

Peptides and antibodies can be used as carriers to deliver a drug, radionuclide or toxin to targeted cells. Growth factor receptors are attractive targets for such an approach. Ideally, such a receptor should be expressed in high density on the cell surface, and a peptide should have a high-affinity binding to the target. Several anticancer drugs have been linked to BN analogs and have been studied for cancer treatment. Nagy *et al.* developed a cytotoxic peptide, AN215, consisting of the BN antagonist RC-3094, and a prodrug of doxorubicin (DOX), 2-pyrrolino-DOX [47]. AN215 was able to bind to GRP-R with high-affinity. The anticancer activity of AN215 was evaluated in mice bearing a variety of BN/GRP-R expressing human cancers [48]. Human prostate cancer was xenografted into nude mice. AN215 (150 nM/kg) was given i.v. once a week for 3 weeks. Control mice received either unconjugated drug or the BN/GRP antagonist RC-3094. At the end of treatment, tumor volume was reduced by 69% in mice that received AN215, compared to 22% in mice that received unconjugated drug and 24% in mice received RC-3094 alone. There was no significant toxicity observed in mice that were treated with AN215 [49].

Safavy *et al.* constructed a cytotoxic peptide consisting of BN(7–13) and the anticancer drug paclitaxel. This conjugate retained high-affinity binding to BN/GRP-Rs and had significant cytotoxicity on human NSCLC cells *in vitro* [50]. Moody *et al.* recently reported the construction of a camptothecin–BN conjugate, CPT-L2-BA3 [51]. It bound to all three subtypes of BN/GRP-R, functioned as a full agonist, was internalized rapidly into cytoplasm, where it was metabolized, and released the free drug camptothecin inside the cytoplasm. The compound effectively inhibited the growth of human NSCLC cells *in vitro* [51].

Radiolabeled peptides to target cell surface receptors have been studied in several clinical trials in Europe. The somatostatin receptor has certain similarities with the BN/GRP-R, and has been targeted for cancer diagnosis and treatment. Octreotide is a synthetic somatostatin analog and can be conjugated with ^{111}In or ^{90}Y. Waldherr *et al.* completed a phase II clinical trial using [^{90}Y]octreotide in 41 patients with progressive neuroendocrine tumors. The treatment consisted of four i.v. injections of [^{90}Y]octreotide at a dose of 6000 MBq/m^2, given once every 6 weeks. The overall response rate was 24%, including one case of complete response [52].

Radiolabeled BN/GRP analogs have been explored for their diagnostic and therapeutic use in GRP-R-expressing tumors. Since BN/GRP-Rs are rapidly internalized upon ligand binding, radiolabeled BN analogs have the advantage of a higher target to background ratio, tumor specific uptake, increased accumulation inside tumor cells and rapid clearance from the circulation. Van de Wiele *et al.* reported their results in a clinical study using a ^{99m}Tc-labeled GRP analog ([^{99m}Tc]RP527) in 10 patients with prostate or breast cancer [53]. All patients had histology-proven metastases. They reported a positive uptake of [^{99m}Tc]RP527 in four of six cases of both primary breast cancer and metastatic to lymph nodes. However, low tracer uptake was observed in normal breast tissue. Positive uptake of [^{99m}Tc]RP527 was seen only in one of four cases of prostate cancer [53]. Scopinaro *et al.* reported a study of 10 patients with elevated PSA using combination of ^{99m}Tc-labeled BN, transrectal ultrasonography, computed tomography, magnetic resonance imaging and biopsy. Positive uptake in prostate fossa was detected in two of 10 cases as early as 1 min after the injection of ^{99m}Tc-labeled BN. Eight patients with positive uptake of ^{99m}Tc-labeled BN were later confirmed to have prostate cancer. Two patients with negative uptake were found to have benign prostate adenoma [54], Other radionuclides, including ^{90}Y, ^{188}Re, ^{111}In and ^{177}Lu, have been conjugated to BN analogs by different investigators, and were studied in animal models bearing human prostate, breast and pancreatic cancer; results are reviewed elsewhere [55]. All of these studies support the further exploration of radiolabeled BN/GRP analogs to target BN/GRP-Rs for cancer diagnosis and treatment.

Antisense therapy

ASOs are designed to bind to mRNA and interfere with the translation of mRNA to protein synthesis. The majority of ASOs are approximately 18–22 nucleotides in length (mer). Several ASOs targeting tumor-associated proteins have been studied in clinical trials.

The PKC family is actively involved in the regulation of the cell cycle and proliferation. Activation of PKC is common in NSCLC and other malignancies. Affinitak is a 20mer ASO targeting a member of the PKC family, PKC-α. In early phase clinical trials with a variety of human cancers, Affinitak showed encouraging anticancer activity. In a phase III trial, chemotherapy-naïve NSCLC patients were randomized to receive either a standard chemotherapy consisting of paclitaxel and carboplatin or in combination with Affinitak for six cycles. A total of 616 patients were enrolled in the trial. A survival analysis of 256 patients who completed the prescribed course of treatment showed a median survival of 17.3 months for patients who received standard chemotherapy plus Affinitak versus 14.4 months for patients who received six cycles of standard chemotherapy alone [56].

BCL-2 is a potent anti-apoptosis protein and is over-expressed in a variety of human malignancies. The neutralization of BCL-2 can potentially restore the normal cell apoptosis process and inhibit uncontrolled tumor growth. Genasense is an 18mer ASO targeting BCL-2 protein. In a phase I clinical trial, 21 patients with non-Hodgkin's lymphoma received daily s.c. injection for 14 days with escalating doses of Genasense from 4.6 to 195.8 mg/m^2/day. No significant toxicity was observed at doses up to 110.4 mg/m^2/day. Of 16 assessable patients, a decreased production of BCL-2 protein was observed in seven patients [57]. Several phase III clinical trials are ongoing with Genasense in combination with standard chemotherapy.

Langer *et al.* designed and constructed three ASOs targeting GRP-R at different base pairs. Human SCLC cell lines were exposed to three ASOs in combination. Using a receptor-binding assay, the authors reported a maximal decrease of GRP-R expression by 60% when all three ASOs were used. Using a calcium-releasing assay, the authors reported a maximal decrease of GRP-R by 75% [58].

BsMol

We have constructed several BsMol targeting BN/GRP-R and immune effector cells. One BsMol (mAb22 × Lys^3-BN) was constructed with a BN/GRP-R agonist, Lys^3-BN and mAb22, an antibody that is directed to the high-affinity immunoglobulin γ Fc receptor (FcγRI). FcγRI is a potent cytotoxic trigger molecule expressed on human monocytes, macrophages and activated neutrophils. Its expression is regulated by a number of cytokines including interferon-γ, granulocyte colony stimulating factor and interleukin-10. The working hypothesis was that the BsMol could redirect immune effector cells to target tumor cells and mediate specific cytotoxicity. Using cytokine-activated monocytes and neutrophils isolated from normal donors, the BsMol, mAb22 × Lys^3-BN, mediated the lysis of 60–98% of targeted SCLC cells from different cell lines. The efficacy of cell lysis was dependent on the effector to target cell ratio and the activity of immune effector cells from each individual donor [59].

To further explore the clinical application of the BsMol, humanized mAb22 (H22) and F(ab′)$_2$ fragments of H22 were conjugated with a synthetic BN/GRP-R antagonist, (D-Trp^6-Leu^{13}-ψ[CH$_2$NH]Phe^{14})BN(6–14) [60]. These BsMol could bind specifically to SCLC cells and mediate the lysis of target cells [9,61]. Recently, we constructed a new BsMol using a simplified chemical conjugation method. A synthetic BN/GRP antagonist, (D-Phe^6, Leu-NHEt^{13}, des-Met^{14})BN(6–14) [62], containing an additional cysteine at the N-terminus was conjugated to H22. This BsMol, H22 × Antag 2, retained the same binding capacity to target SCLC cells and mediate specific cytotoxicity. We combined the targeted immunotherapy with two commonly used anti-cancer drugs, cisplatin and etoposide, to further increase the therapeutic efficacy. We observed a significant increase in SCLC cell cytotoxicity *in vitro* when the immunotherapy was combined with standard chemotherapy [63]. A similar approach has been explored in targeting the CD3 molecule on T cell surface so as to induce T cell-mediated cytotoxicity of SCLC cells. In our pilot study, SCID/NOD mice bearing human SCLC were treated with a BsMol, OKT3 × Antag2, in combination with human T cells. Control mice were treated with human T cells and un-conjugated OKT3. After 5 weeks, five of eight mice which had received the combined treatment (BsMol ± T cells) had no macroscopic tumor growth, while all control mice developed visible tumors (unpublished data).

Conclusions

Strategies of cancer treatment have evolved in the last two decades. While conventional chemotherapy and radiation remain the primary modalities of advanced cancer treatment, recent efforts have been focused on targeted therapy to reduce toxicity, to enhance specific cancer cell killing and to improve the quality of life for cancer patients. BN/GRP-Rs are attractive targets for cancer treatment since they are frequently expressed on cancer cells and have limited distribution in normal tissue. We have reviewed the different approaches to targeting BN/GRP-Rs. The antibody-based approach has made great progress, as seen in recent clinical trials, and has become an integral part of the treatment of breast

and colon cancer, and non-Hodgkin lymphoma. The advantage of a mAb lies in its capacity to directly mediate target cell killing, prolonged biologic half-life, ease of administration (usually once 1–2 weeks) and minimal toxicity. The generation of a human mAb to human BN/GRP-Rs would be a welcome development. In the absence of a mAb, a BsMol such as we have described might have therapeutic effects. The major disadvantages of this method include the cumbersome production and quality control of the chemical conjugation process, and the variability of individual immune activity. Small molecules such as receptor antagonists and ASOs should be further explored in well-controlled clinical trials. They suffer the disadvantages such as the susceptibility to degradation in circulation, poor cellular uptake in cancer tissues and rapid clearance *in vivo*. Radiolabeled and drug-linked BLPs have shown some promise in animal studies and early clinical trials. Their disadvantages include a cumbersome production process, transportation and storage, and the practical issues involved in using such products in hospitals and clinics.

The BN/GRP-R family may be an important target for cancer therapy. We hope this review will stimulate some interest in further drug development targeting these growth factor receptors that are widely expressed on human cancer cells.

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