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molecular pharmaceutics

# Targeted Therapy of Breast and Gynecological Cancers with Cytotoxic Analogues of Peptide Hormones

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Received April 30, 2007; Revised Manuscript Received June 18, 2007; Accepted July 6, 2007

**Abstract:** Gynecological cancers such as breast, ovarian, and endometrial carcinoma express receptors for luteinizing hormone-releasing hormone (LHRH), bombesin/gastrin-releasing peptide (BN/GRP), and somatostatin (SST). These tumors are therefore suitable candidates for targeted therapy with cytotoxic hybrid molecules consisting of a cytotoxic radical and a peptide hormone analogue as a carrier. These compounds have been shown to be more active and less toxic in vivo than nontargeted chemotherapy in models of various human cancers which express the respective receptors. The current review summarizes experimental and clinical findings with cytotoxic peptide hormone analogues of LHRH (AN-152 [AEZS 108], AN-207), BN/GRP (AN-215), and SST (AN-238) in breast, ovarian, and endometrial cancers.

**Keywords:** Targeted therapy; cytotoxic peptide analogues; AN-152 [AEZS 108]; AN-207; AN-238; AN-215; LHRH; somatostatin; bombesin

## 1. Introduction

The discovery of specific molecular characteristics of malignant cells prompted the development of a new class of drugs known as targeted therapeutics. The hypothesis of a "magic bullet" that could specifically eradicate cancers was conceived in 1898 by Paul Ehrlich but remained unexplored for many years. The new class of antitumor agents includes antibodies against surface structures on tumor cells, such as trastuzumab[1] (Herceptin), and hybrid molecules consisting of receptor specific ligands linked to toxins, radionuclides, or chemotherapeutic agents.[2] Specific, high-affinity receptors for peptide hormones expressed on malignant cells can be targeted by cytotoxic hormone analogues consisting of a peptide carrier linked to an antineoplastic agent.[2–4] A direct delivery of the cytotoxic radical to the malignant tissue results in a higher antitumor activity with a reduced systemic toxicity and can overcome chemoresistance of cancer cells. These conjugates consist of analogues of LHRH, BN, and SST [The abbreviations used are the following: bombesin (BN), luteinizing hormone-releasing hormone (LHRH), doxorubicin (DOX), multidrug resistance (MDR), multidrug resistance related protein (MRP), breast cancer resistance protein (BCRP), P-glycoprotein (Pgp), somatostatin (SST)] linked to DOX or its superactive derivative 2-pyrrolino-DOX (AN-201). On the basis of the presence of receptors for the above-mentioned peptide hormones in a variety of human cancers,

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**10.1021/mp0700514 CCC: $37.00** 
Published on Web 08/18/2007

***Table 1.*** Expression of Receptors for LHRH, BN/GRP and SST in Human Specimens of Breast, Endometrial, and Ovarian Cancers

| | breast | ovarian | endometrial |
|---|---|---|---|
| LHRH | >50% | 80% | 80% |
| BN/GRP | >60% | >70% | not determined |
| SST | 36% | 76% | 40% |

the synthesis and evaluation of targeted antitumor compounds was initiated by Schally and co-workers.[2–5]

After exploring various antineoplastic radicals and chemical linkages, it was decided to conjugate doxorubicin (DOX), the most widely used anticancer agent, to an analogue of LHRH to form cytotoxic LHRH analogue AN-152 (AEZS 108). Subsequently, a daunosamine-modified derivative of DOX (AN-201) was synthesized, which is 200–500 times more active in vitro than DOX. By linking this cytotoxic radical to analogues of LHRH, BN, and SST, cytotoxic hybrid molecules AN-207, AN-215, and AN-238 were created. It was subsequently demonstrated that these cytotoxic hybrid molecules powerfully inhibit growth of various experimental tumors.[3,5] The current review summarizes the experimental and clinical results, achieved with cytotoxic analogues of LHRH (AN-152 [AEZS 108], AN-207), BN (AN-215), and SST (AN- 238) in the treatment of mammary, ovarian, and endometrial cancer.

## 2. Cytotoxic Analogues of LHRH, BN, and SST

**2.1. Cytotoxic Analogues of LHRH (AN-152[AEZS 108], AN-207).** DOX-14-O-hemiglutarate is conjugated to [D-Lys$^6$]LHRH to form the cytotoxic analogue AN-152 (AEZS 108).[5] A daunosamine-modified derivative of DOX, 2-pyrrolino-DOX (AN-201), which is about 200 times more active than DOX, was also developed and coupled to [D-Lys$^6$]LHRH to form analogue AN-207.[5] High-affinity binding of AN-152 [AEZS 108] and AN-207 to LHRH receptors on rat pituitary and human breast cancer cells and specimens was demonstrated.[5,6] The mechanism of action of AN-152 [AEZS 108] is depicted in Figure 2 (see Figures 1 and 2).

**2.2. Cytotoxic Analogues of Bombesin (AN-215).** Specific receptors for bombesin-like peptides, such as the gastrin releasing peptide receptor (GRPR), the neuromedin B receptor (NMBR), and the bombesin receptor subtype 3 (BRS-3), have been found in various human cancer specimens and cancer cell lines.[7–11] The cytotoxic analogue of

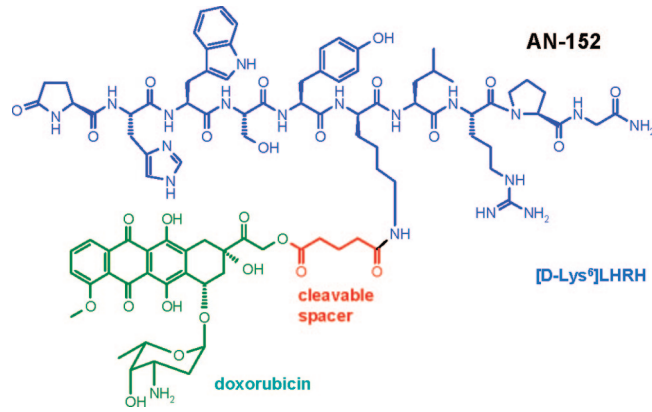


***Figure 1.*** Cytotoxic analogue of LHRH AN-152 [AEZS 108]. The LHRH analogue [D-Lys$^6$ ]LHRH is linked to doxorubicin through a cleavable spacer.

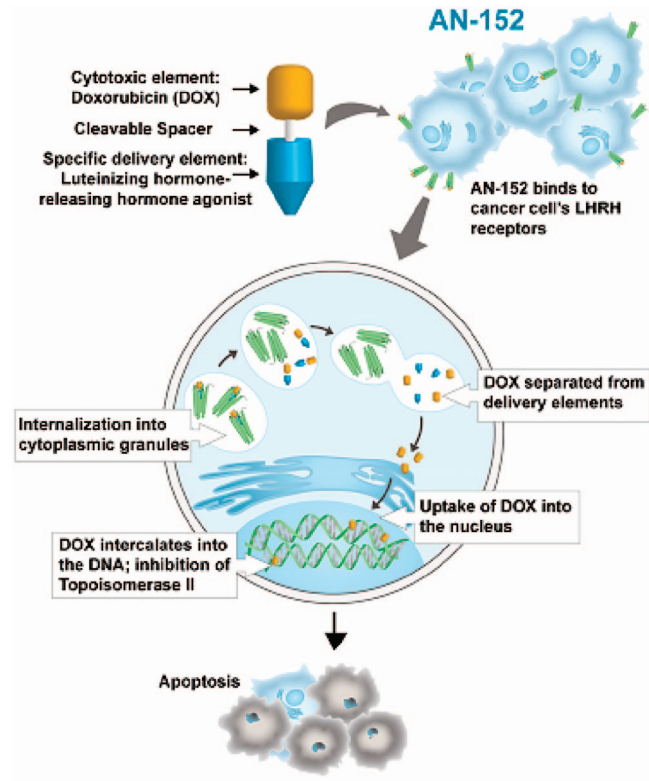


***Figure 2.*** Mechanism of action for cytotoxic LHRH analogue AN-152 [AEZS 108]. It binds to the LHRH receptor with the [D-Lys$^6$]LHRH moiety, is internalized and cleaved in the cytoplasm-free DOX, and subsequently enters the nucleus.

bombesin AN-215 consists of 2-pyrrolino-DOX (AN-201),[4] covalently linked to a bombesin antagonist carrier octapeptide Gln-Trp-Ala-Val-Gly-His-Leu-$\psi$($CH_2$–NH)-Leu-$NH_2$ (RC-

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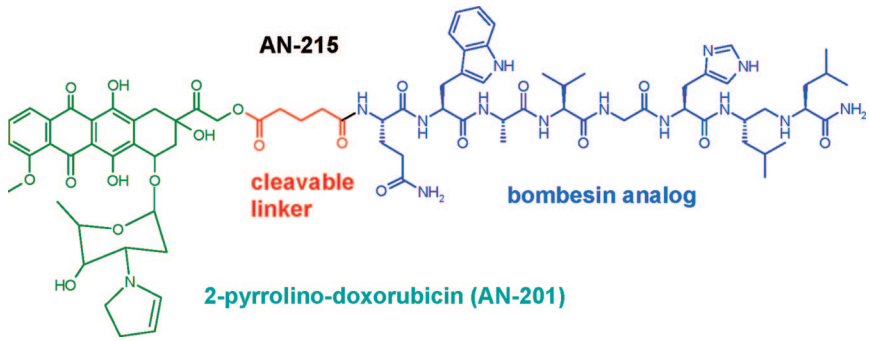


***Figure 3.*** Cytotoxic analogue of bombesin AN-215, which consists of 2-pyrrolino-DOX (AN-201),[4] covalently linked to a bombesin-like carrier octapeptide Gln-Trp-Ala-Val-Gly-His-Leu-$\psi$($CH_2$–NH)-Leu-$NH_2$ (RC-23094).

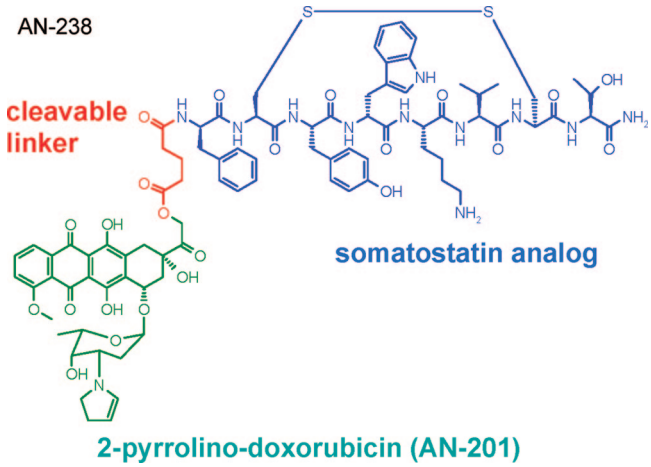


***Figure 4.*** AN-238 is an SST analogue, which consists of the SST carrier octapeptide RC-121 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-$NH_2$) covalently linked to 2-pyrrolino-DOX (AN-201).

23094)[12] (Figure 3). AN-215 shows high affinity to GRPR and retains the antiproliferative activity of its cytotoxic moiety AN-201.

**2.3. Cytotoxic Analogues of Somatostatin (AN-238).** AN-238 is a SST analogue, which consists of the SST carrier octapeptide RC-121 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-$NH_2$) covalently linked to 2-pyrrolino-DOX (AN-201) (Figure 4). This analogue fully retains the cytotoxic activity of the radical and the receptor binding affinity of the peptide carrier[13] and has been shown to significantly inhibit the growth of various tumors that express SST receptor subtypes 2a, 3, and 5 ($sst_{2a,3,5}$).[14–18]

## 3. Results and Discussion

**3.1. Experimental Studies on Breast Cancer with Cytotoxic Analogues.** Breast cancer is the most common malignancy and ranks second as a cause of cancer-related deaths among women. In 2005, about 40 000 women in the United States are expected to die from breast cancer.[19] The great majority of the patients succumb to this disease not because of their primary cancer, but because of metastases. Despite the use of endocrine therapy, systemic chemotherapy, and novel approaches such as treatment with trastuzumab (Herceptin) humanized antibody against the epidermal growth factor receptor 2 (HER-2), metastatic disease remains generally incurable with a median survival time of 2–3 years. Therefore, it is mandatory to develop novel, more effective

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treatment strategies with low toxicity for the treatment of breast cancer.

Cytotoxic analogues of LHRH are good candidates for the treatment of mammary cancer, as the corresponding receptor is found in >50% of human breast cancer specimens. Thus, AN-152[AEZS 108] was tested in nude mice bearing LHRH receptor positive human MX-1 breast cancers Accordingly, treatment with AN-152(AEZS 108) significantly ($P < 0.05$) inhibited the growth of human MX-1 breast cancers xenografted into nude mice, while the unconjugated radical doxorubicin was ineffective. Additionally, the expression of mRNAs for HER-2 and HER-3 and the levels of HER-2 and HER-3 proteins were also significantly reduced by the treatment with AN-152(AEZS 108).[20] In a further experimental study, a single iv injection of AN-207 significantly inhibited growth of MDA-MB-231 estrogen independent breast cancers xenografted into nude mice, while the administration of equimolar doses of the cytotoxic radical AN-201 did not shown any significant effect.[21] In both studies, toxic side effects such as leukopenia were less pronounced in the groups of animals which had been treated with AN-152 (AEZS 108) and AN-207 than in animals treated with nonconjugated cytotoxic radicals.[20,21]

Since somatostatin (SST) receptors are expressed in about 36%[22] of human breast cancers, the effects of targeted cytotoxic somatostatin analogue AN-238 were studied in three human breast cancer models.[16] The models included estrogen-independent MDA-MB-231 and MX-1 and estrogen-sensitive MCF-7-MIII tumors. High-affinity SST receptors and mRNA for both $sst_2$ and $sst_5$ subtypes were found in all three tumor lines. Nude mice bearing xenografts of these cancers were injected iv with 250 nmol/kg doses of cytotoxic radical AN-201, cytotoxic analogue AN-238, or the unconjugated mixture of AN-201 and SST analogue RC-121. Significant inhibition of growth of MDA-MB-231, MX-1, and MCF-7-MIII tumors was observed 1 week after injection of a single dose of cytotoxic analogue AN-238. The volume of MDA-MB-231 tumors remained significantly decreased 3 weeks after treatment. The volumes and weights of MCF-7-MIII tumors continued to be significantly reduced 60 days after therapy with AN-238. AN-238 also caused complete regression of MX-1 tumors in 5 of 10 animals, which remained tumor-free 60 days after treatment. In contrast, after treatment with cytotoxic radical AN-201, MDA-MB-231, and MCF-7-MIII, tumors grew steadily and the regression of MX-1 tumors was only transitory in most animals. Toxicity of AN-201 was more pronounced than that of AN-238, as measured by animal deaths, loss of body weight, and leukopenia. Expression of SST receptors was not significantly affected by treatment with AN-238.[16]

The presence of specific binding sites for BN/GRP in human breast cancer specimens was first established by Schally and coworkers,[7] and it has been recently demonstrated that the GRP receptor, which binds BN with high affinity, is expressed in 29 of 46 human invasive ductal breast carcinomas.[23] In addition, the lymph node metastases from GRPR positive tumors were all found to remain GRPR positive, whereas in surrounding lymphoreticular tissue no receptor expression could be found.[23] Primary breast cancers and their lymph node metastases were sucessfully detetcted using GRPR scintigraphy in several clinical studies.[24–26] These findings suggest BN/GRP receptors on breast cancer as a potential target for the therapy with cytotoxic bombesin analogues.

Thus, the antitumor effect and the toxicity of AN-215 was evaluated in five human breast cancer cell lines xenografted into nude mice. All five cell lines expressed BN/GRP receptors, and AN-215 significantly ($P < 0.05$) inhibited tumor growth in all models, while its cytotoxic radical AN-201 had no significant effect in four models. In MX-1 tumors, AN-201, although it inhibited tumor growth, displayed a significantly weaker antitumor effect than AN-215. The effect of AN-215 was nullified by a blockade of BN/GRP receptors. AN-215 induced less toxic side effects in the animals than cytotxic radical AN-201. A low or no induction of multidrug resistance proteins MDR-1, MRP-1, and BCRP occurred after treatment with AN-215.[27] These data show that cytotoxic analogues AN-152 (AEZS 108), AN-207, AN-215 and AN-238 strongly inhibit experimental breast cancers, which express the corresponding receptors with low toxic side effects and might thus provide a new treatment modality for women with mammary carcinoma.

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**3.2. Experimental Studies on Ovarian Cancer with Cytotoxic Analogues.** Ovarian cancer is the most frequent cause of death from gynecological cancer in the United States.[28] Present therapies fail mainly because of the advanced stage of the disease at the time of the diagnosis.[29] Advanced epithelial ovarian cancer is currently treated by cytoreductive surgery and paclitaxel- and platinum-based chemotherapy. Yet after an initial response,[30] most of the patients eventually experience disease recurrence.[31] This underlines the importance of establishing new strategies for the treatment of ovarian cancer.

Receptors for luteinizing hormone-releasing hormone (LHRH) are found in nearly 80% of human ovarian cancers. Therefore, Schally and co-workers investigated the effects of AN-152 (AEZS 108) on the growth of LHRH receptor-positive OV-1063 and LHRH receptor-negative UCI-107 human epithelial ovarian cancers xenografted into nude mice.[32] The growth of OV-1063 ovarian tumors, as based on reduction in tumor volume, was inhibited significantly 4 weeks after treatment with AN-152 (AEZS 108), while equimolar doses of free doxorubicin did not cause significant growth inhibition and caused substantial mortality. The growth of UCI-107 ovarian cancers was not inhibited by AN-152 (AEZS 108) indicating that LHRH receptors are necessary for the antitumor action of AN-152 (AEZS 108).[32] Similar results were obtained in OV-1063 ovarian cancers with AN-207[33] and with AN-152 (AEZS 108) and AN-207 in ES-2 human ovarian cancers.[34,35] A further study investigated the cytocidal effects of AN-207 and cytotoxic radical AN-201 on the LHRH receptor-positive ES-2 ovarian cancer cells and LHRH receptor-negative UCI-107 ovarian cancer cells was by semiquantitative polymerase chain reaction (PCR) amplification of microsatellite markers.[36] To investigate targeting, ES-2 cells were cocultured with UCI-107 cells, treated with 10 nM AN-207 or AN-201 for different times. Genomic DNA was extracted for microsatellite analyses using different markers. Semiquantitative analyses of the intensity of the alleles that correspond to each cell line indicated that AN-207 was selectively targeted to ES-2 cells, while AN-201 showed no selectivity for either cell line. These results extend the previous findings that AN-207 can be targeted to cancers that express receptors for LHRH.

Specific binding sites for SST were detected in 76% of human specimens of ovarian cancers and in xenografts of OV-1063 and UCI-107 human ovarian cancer lines. The mRNA for somatostatin receptor subtype (SST) 1 was detected in 65% of the ovarian cancer specimens, while the incidence of $sst_{2a}$, $sst_3$, and $sst_5$ was 65%, 41%, and 24%, respectively. Both ovarian cancer cell lines also expressed mRNA for these four subtypes. The presence of these SST receptor subtypes in human ovarian cancers allows the use of SST analogues and their radionuclide and cytotoxic derivatives for the diagnosis and treatment of this malignancy.[37]

Accordingly, the toxicity and antitumor effects of AN-238 in nude mice bearing UCI-107 human ovarian tumors were investigated. In vivo, the volume and weights of UCI-107 tumors treated with AN-238 were decreased by more than 60% ($P < 0.05$) compared with controls. Cytotoxic radical AN-201 or the unconjugated mixture of AN-201 with carrier RC-121 had no significant effects on tumors and were toxic.[38]

As more than 70 % of ovarian cancer specimens express receptors for BN/GRP[39] cytotoxic analogue of BN AN-215 was tested in vivo in four human ovarian cancer cell lines.[40] ES-2, SKOV-3, OV-1063, and UCI-107 human ovarian carcinomas were tested positive for BN/GRP receptors. Accordingly, AN-215 significantly inhibited tumor growth in three of four cell lines ($P < 0.05$) and prolonged the survival of nude mice bearing intraperitoneal ES-2 xe-

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nografts. Cytotoxic radical AN-201, the unconjugated mixture of BN antagonist RC-3095 with cytotoxic radical AN-201, RC-3095 alone or AN-215 after the blockade of the BN/GRP receptors, had no significant effects and appeared to be more toxic. Expression of BN/GRP receptors remained unchanged after repeated treatment cycles with AN-215.

The aim of a further study was to investigate the effect of treatment of experimental ovarian cancers with targeted cytotoxic analogues as single compounds and in combination.[41] AN-215 alone at 200 nmol/kg and its combination with AN-238 at one-half of the dose were able to inhibit the growth of UCI-107 tumors. A combination of AN-238 with AN-207 at 50% of the dose strongly suppressed the proliferation of ES-2 and OV-1063 ovarian tumors. Cytotoxic radical AN-201 was toxic and had no significant effect on tumor growth. Because ovarian cancers tend to acquire chemoresistance, in the same study, real-time PCR was used to measure the mRNA expression of multidrug resistance protein 1, multidrug resistance-related protein 1, and breast cancer resistance protein after treatment. Low or no induction of multidrug resistance protein 1, multidrug resistance-related protein, and breast cancer resistance protein occurred after treatment with AN-238, AN-215, and the combination of AN-238 with AN-207 or AN-215. These results demonstrate that a therapy with cytotoxic analogues such as single agents and combinations is effective and nontoxic and does not induce chemoresistance rapidly.[41]

**3.3. Experimental Studies on Endometrial Cancer with Cytotoxic Analogues.** Endometrial carcinoma is the most common neoplasm of the female genital tract, accounting for nearly one-half of all gynecologic cancers in the Western world. It is estimated that approximately 40 000 new cases of endometrial cancer are diagnosed annually in the USA and about 7000 deaths occur from this disease. Endometrial carcinoma is thus the fourth most common malignancy and the eighth leading cause of cancer related death in women. The lifetime risk of developing endometrial cancer is about 2–3%. Primary surgery, followed by radiation, is the most widely accepted treatment for endometrial cancer. In the case of late-stage or recurrent disease, systemic hormonal treatment with progestagens or combination chemotherapy are added. Early stage endometrial cancer has a good prognosis, with 5-year survival rates of 87% and 76% for FIGO stages I and II, respectively.[42] However, the 5-year survival rate falls to 59% (FIGO III) and 18% (FIGO IV) in patients with late-stage disease.[42] The overall survival is poorest in patients with recurrent endometrial cancer (7.7%).[43] These statistical findings underline the importance of developing new therapeutic modalities for late-stage and recurrent endometrial cancer.

About 80% of human endometrial carcinomas express receptors for LHRH.[44,45] Thus, cytotoxic LHRH analogues could be used for targeted therapy of this malignancy, and it could be first demonstrated in HEC-1B endometrial cancers in vivo that AN-152 effectively inhibits tumor growth without toxic side effects.[46] A further study demonstrated the efficacy of AN-152 (AEZS 108) in a mouse model of human HEC-1A endometrial tumors cancers.[47] In the same study, it was also shown that AN-207 significantly suppresses the proliferation in vivo of HEC-1A ($P < 0.01$) and RL-95-2 ($P < 0.05$) cancers.[47] Cytotoxic radicals DOX and AN-201 had no effect. mRNA for LHRH receptors and LHRH-receptor protein and high affinity binding sites for LHRH were demonstrated on tumors of both cell lines.

In a recent study, $sst_{2a}$ and $sst_3$ were detected in 43% and 39% of human endometrial cancer specimens, respectively.[48] The presence of SST receptor subtypes $sst_{2a}$ and $sst_3$ was also demonstrated in three human endometrial cancer cell lines (HEC-1A, RL-95-2, and AN3CA).[49] Accordingly, AN-238 significantly ($P < 0.05$) inhibited the growth of these tumors, while cytotoxic radical AN-201 had no effect. Blockade of SST receptors suppressed the effects of AN-238.[49] In all experiments, AN-238 led to a weaker induction of multidrug resistance protein MDR-1 than AN-201. No major induction of multidrug resistance proteins MRP-1 and BCRP occurred after treatment with AN-238 and AN-201.[49]

Since receptors for BN/GRP were detected in AN3CA, KLE, HEC-1A, and Ishikawa human endometrial cancer cells in vitro,[50] an experimental study was designed to investigate the efficacy of targeted chemotherapy with AN-215 in three human endometrial cancer cell lines in vivo. Nude mice bearing HEC-1A, RL-95-2, and AN3CA cancers were treated

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with AN-215 and its cytotoxic radical (AN-201). The expression of BN/GRP receptors was demonstrated on all three tumor models.[51] AN-215 significantly ($P < 0.05$) inhibited the growth of HEC-1A, RL-95-2, and AN3CA tumors.[51] The cytotoxic radical AN-201 was ineffective. AN-215 caused a weaker induction of MDR-1 in HEC-1A and RL-95-2 cancers. MRP-1 and BCRP were not induced by AN-215 and AN-201.[51]

**3.4. Clinical Findings.** A phase I clinical study assessed dose limiting toxicities, maximum tolerated dose (MTD), and pharmacokinetics of AN-152 (AEZS 108) given once every 3 weeks in patients with gynecologic and breast cancers.[52] Patients with tumors proven immunohistochemically to be LHRH-R positive were eligible if prior therapy did not exceed 70% of the recommended maximum lifetime dose for doxorubicin. Doses of AN-152 (AEZS 108) were doubled starting at 10 mg/m$^2$ until side effects occurred. Seventeen patients entered the study and received AN-152 by intravenous infusion over 2 h at dosages of 10, 20, 40, 80, 160, and 267 mg/m$^2$. Infusion of AN-152 (AEZS 108) was well tolerated at all dosages, without supportive treatment. The cytotoxic LHRH analogue AN-152 was stable in human plasma, a prerequisite for LHRH receptor-mediated uptake by tumor tissue. Preliminary pharmacokinetic analyses showed dose-dependent plasma levels of AN-152 (AEZS 108) and only minor release of doxorubicin. A dose of 267 mg/m$^2$ of AN-152 (AEZS 108), given once every 3 weeks, was recommended for phase II trial. Evidence of therapeutic activity was demonstrated, as disease stabilization and remission at 160 and 267 mg/m$^2$ dose levels of AN-152 (AEZS 108).[52]

## 4. Conclusion

Gynecological cancers such as ovarian and endometrial and mammary carcinomas express receptors for LHRH, BN/GRP, and SST in a high percentage of cases. They are therefore suitable candidates for targeted therapy with cytotoxic analogues of LHRH, bombesin, or somatostatin targeted to these receptors. Experimental evidence shows that AN-152 (AEZS 108), AN-207, AN-215, and AN-238 are highly active in experimental models of breast, endometrial, and ovarian cancers. Results of a phase I trial with AN-152 in gynecological cancers expressing receptors for LHRH showed low toxicity and early indications of antitumor activity. AN-152 (AEZS 108) will now enter phase II trials.

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