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**Review Article**

# Targeted cytotoxic somatostatin analogs: a modern approach to the therapy of various cancers

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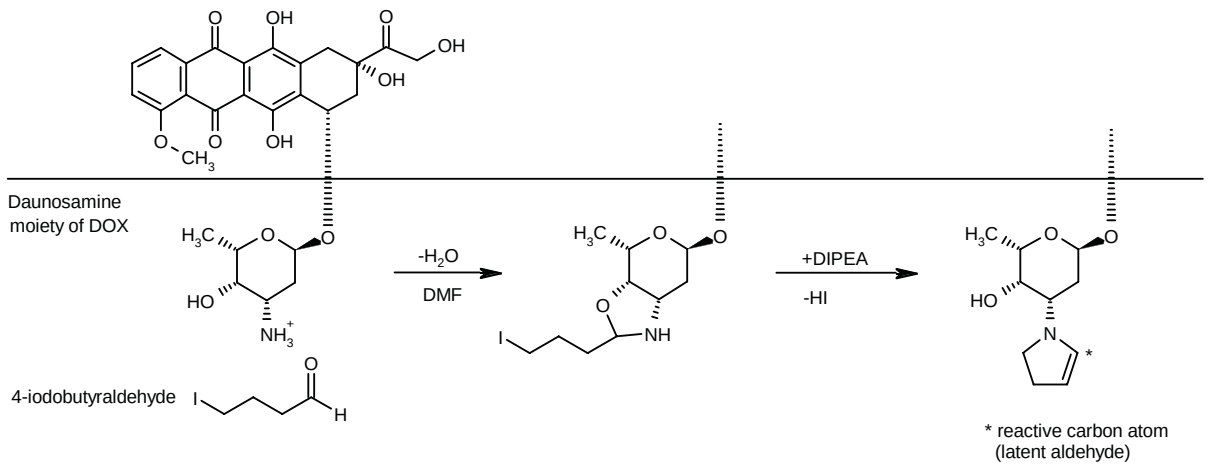
## Introduction

Somatostatin is a hormonal neuropeptide that was isolated from ovine and later from porcine hypothalami and characterized as an inhibitor of growth hormone secretion from the pituitary gland (1-3). In mammals, SST was found to exist in two active forms, a 14 amino acid peptide (SST-14) and an amino terminally extended version consisting of 28 amino acids (4, 5). Both forms of SST exhibit similar suppressive effects on a wide variety of cells and appear to be endogenous growth inhibitors (3-6). Because of the short plasma half-life of native SST (< 3 min), its clinical use is impractical. Therefore, more stable analogs with enhanced activities were synthesized to exploit the great therapeutic potential in this inhibitory peptide. As a result of intensive research, several stable SST analogs were developed including octreotide (Sandostatin; SMS 201-995), vapreotide (RC-160) and lanreotide (BIM-23014) (7-9). These analogs have a plasma half-life of approx. 120 min and are about 50 times more potent than SST in inhibiting growth hormone release from the pituitary. They are also more selective than native SST in suppressing insulin and glucagon secretion. Preclinical studies revealed that SST and its octapeptide analogs exert their effects through specific membrane receptors. Presently, 5 distinct subtypes ($sst_{1\text{-}5}$) have been cloned and characterized (10, 11). These receptors are widely distributed in normal and malignant tissues with cells often expressing more than one subtype (3-6). While SST-14 and SST-28 show similar high affinity to $sst_{1\text{-}5}$, the synthetic octapeptides such as RC-160 and RC-121, developed in our institute, and octreotide bind preferentially to $sst_2$ and $sst_5$, display moderate affinity to $sst_3$ and low affinity to $sst_1$ and $sst_4$ (11).

The receptors for SST on various neuroendocrine malignancies and many other solid tumors (3-6) could be used for targeted chemotherapy. Thus, we investigated the use of SST octapeptides as carriers capable of delivering cytotoxic agents specifically to cancerous cells, and during the late 1980s several such hybrid conjugates were designed in our laboratories (12, 13). One early analog, consisting of the antimetabolite methotrexate linked to carrier RC-121 (12), showed promising results in SST receptor-positive MIA PaCa-2 experimental pancreatic cancers in nude mice (13). In the last decade, a variety of SST radiotracer analogs have also been developed, including [$^{111}$In]-diethylenetriamine pentaacetic acid [(DTPA)-D-Phe$^1$]octreotide (Octreoscan) for scintigraphic use (14-17). Some of these analogs were demonstrated to be useful tools for the localization of SST receptor-positive tumors in patients (3, 14, 15). Subsequently, these and other SST analogs labeled with appropriate radionuclides such as $^{186}$Rhenium or $^{90}$Yttrium were also applied in cancer therapy. Encouraged by these results, we developed a series of novel targeted cytotoxic SST conjugates that consist of carriers RC-160 and RC-121 coupled to doxorubicin (DOX) or its superactive derivative, 2-pyrrolino-DOX (AN-201), which is 500-1000 times more potent *in vitro* than DOX (18-20). Very recently, an analog consisting of paclitaxel linked to octreotide has also been reported by others (21).

**Scheme 1: Synthesis of 2-pyrrolino-DOX**




This review describes the design, synthesis and mechanism of action of our targeted cytotoxic SST analogs. We also tried to compile and interpret experimental oncological results from the perspective of possible clinical application.

## Design and synthesis

### *Cytotoxic radicals*

Our selection of DOX as the active chemotherapeutic radical for targeted cytotoxic conjugates is based on the fact that after more than 3 decades, DOX is still one of the most widely used anticancer agents, with the broadest spectrum of antitumor activity (22). Although the exact mechanism of action of DOX is still not fully understood, it has been established that its antiproliferative activity is mainly due to DNA intercalation and inhibition of topoisomerase II, which in turn leads to DNA strand breaks in dividing cells (23). This explains why DOX shows some selectivity towards cancerous cells and also accounts for certain side effects such as myelosuppression. The main dose-limiting toxicity of DOX, however, is not myelotoxicity but cardiomyopathy, which is believed to be due to the generation of free radicals mediated by the quinone moiety of the anthracycline molecule in the presence of iron ions (24). Thus, chronic exposure to > 450 Mg/m$^2$ cumulative dose of DOX was found to overwhelm the heart's weak antioxidant defenses, leading to irreversible life-threatening damage of cardiac muscles. DOX was also shown to kill malignant cells without entering the nucleus, through binding to membrane phospholipids (25). In addition to toxic effects, the clinical efficacy of DOX is further impeded by intrinsic or acquired multidrug resistance of cancerous cells (26).

To overcome these drawbacks, thousands of DOX derivatives have been synthesized and tested, including 2-pyrrolino-DOX (AN-201). AN-201 can be readily formed by reacting DOX with a 5- to 10-fold excess of 4-iodobutyraldehyde in dimethylformamide (Scheme 1). The conversion of DOX takes place within minutes and is virtually 100% (19). AN-201 was shown to be 500-1000 times more potent than DOX *in vitro*. The high *in vitro* and *in vivo* antiproliferative activity of some daunosamine-modified DOX derivatives, even against DOX-resistant cell lines, was attributed to their ability to "alkylate" an amino group of a guanine base in close vicinity to the DNA intercalation site (19, 27, 28). Such derivatives were shown to be non-cross-resistant with DOX, and possibly due to their low maximum tolerated doses, noncardiotoxic (27, 28). Not surprisingly, the dose-limiting toxicity of these strong DNA intercalating and crosslinking agents is due to the damage to bone marrow (20, 28).

A modern approach developed by us to lower the toxicity and enhance the efficacy of these very potent anticancer agents is based on the targeting of their respective conjugates to specific receptors for peptide hormones such as luteinizing hormone-releasing hormone (LH-RH) (29), bombesin/gastrin releasing peptide (30) and SST (18) present on cancerous cells (20).

### *Cytotoxic SST conjugates*

The outcome of targeted chemotherapy greatly depends on the chemical modifications used to construct the new hybrid entity. It is essential to preserve the ability of the carrier peptide portion to bind specifically to receptors on target tumor tissue and to retain the cytotoxicity of the anticancer agent. Ideally, the conjugate should be stable and inactive in the circulation, with the cytotoxic agent being released in an active form only in the tumors (20).

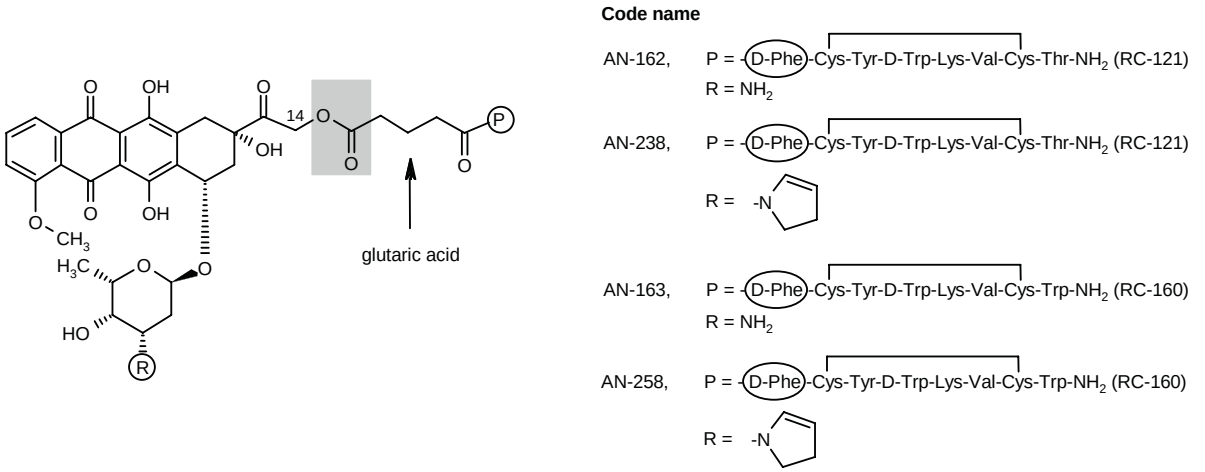



Fig. 1. Molecular structure of cytotoxic SST conjugates AN-162, AN-163, AN-238 and AN-258. Cytotoxic radicals DOX (R = $NH_2$) and AN-201 (R = 2-pyrrolino) are linked through a glutaric acid spacer to the amino terminal D-Phe residues (circled) of SST octapeptide carriers RC-121 and RC-160. The ester bond between the 14 OH group of the cytotoxic radical and glutaric acid is indicated by a shaded area. P denotes the peptide carrier. (Modified with permission from Proc Natl Acad Sci USA 2000, 97: 829-34. Copyright National Academy of Science 2000.)

Early tests with SST octapeptide analogs indicated that certain modifications at the amino terminal can be done without a severe loss of binding affinity (12, 13, 17). Thus, we linked DOX and AN-201, which are fairly large molecules, to the amino terminal of RC-160 and RC-121 to form cytotoxic SST hybrids. Binding assays demonstrated that in spite of the bulkiness of DOX, the conjugates AN-162 and AN-163 containing DOX displayed an affinity to receptors for SST on membrane preparations of rat pituitary in the nanomolar range (18). Analogs AN-238 and AN-258 containing AN-201 had $IC_{50}$ values of 23.8 nM and 80.1 nM, respectively. However, $IC_{50}$ values for AN-238 were found to be between 1 nM and 10 nM on a variety of tumor membranes. The conjugates were also able to effectively inhibit growth hormone-releasing hormone or forskolin-induced growth hormone release at nanomolar concentrations, as determined in perifused rat pituitary cell system *in vitro* (18).

Various chemical linkages have been used to couple DOX to peptidic or other carriers without loss of its antineoplastic activity. These include the sodium periodate oxidation followed by reductive alkylation at the daunosamine sugar moiety (31), the use of spacer arms that are acid sensitive such as *cis*-aconitic acid (32) and maleimidocaproyl hydrazone (33), or enzyme sensitive like the tetrapeptides ALAL (34) and GPLG (35). Self immolative spacers based on 1,4- and 1,6-elimination (36) or trimethyl lock lactonization (37) were also used with success. Because 14-*O*-esters of DOX are relatively stable (38), and 14-*O*-glutaryl esters can be also used to link daunosamine-modified DOX derivatives such as AN-201 to free amino groups on carriers, we synthesized *N*-Fmoc-DOX-14-*O*-hemiglutarate and 2-pyrrolino-DOX-14-*O*-hemiglutarate (18). These DOX derivatives were then coupled to the amino terminal of [Lys(Fmoc)$^5$]-RC-121, followed by deprotection to form AN-162 containing DOX or superactive analog AN-238 incorporating radical AN-201, respectively. The corresponding cytotoxic conjugates with carrier RC-160 (AN-163 and AN-258) were prepared similarly (18) (Fig. 1).

## Mechanism of action

Although tissue distribution studies with our cytotoxic SST analogs have not been completed at present, it is assumed that these cytotoxic hybrids can accumulate in both normal and cancerous cells bearing receptors for SST, as demonstrated with radioactive SST analogs (14, 15, 20). After binding and internalization of the SST conjugate (40), the cytotoxic radical may be released inside the cells by nonspecific carboxylesterase enzymes (EC.3.1.1.1), which are ubiquitous (39). It is also possible, however, that the cytotoxic radical is released, in part, after binding but before internalization. This mechanism could still produce a higher concentration of the radical in SST receptor-positive tissue and, in addition, a "bystander effect", affecting SST receptor-negative neighboring cells. As a result of the bystander effect, it is very difficult to demonstrate targeting of these conjugates *in vitro*. Despite such difficulties, recently we were able to show a more selective toxicity of AN-238 towards receptor-positive cells in mixed populations of SST receptor-negative and receptor-positive cells, using cell line-specific microsatellite markers for semiquantitative analysis (41, 42).

*In vivo*, the release of the radicals can also occur in the bloodstream before the targeting is completed, impeding the efficacy of the therapy. In fact, the plasma half-life of the ester bond appears to play a key role in the

efficiency of this type of targeting (18, 43). A longer half-life apparently allows a more complete targeting and produces a higher concentration of the conjugate in SST receptor-positive normal and cancerous tissues. A possible damage to receptor-positive normal cells seems to be less harmful than the myelosuppression caused by a non-targeted cytotoxic agent, and consequently, a better targeting is accompanied by a lower toxicity. Thus, we found a much lower toxicity of cytotoxic SST analog AN-238 in rats in which the half-life of the ester bond is > 60 min, compared with its cytotoxic radical AN-201 (44). In contrast, the toxicity of the conjugate was found to be only slightly lower in mice in which the half-life of the ester bond is ~ 20 min (20). We demonstrated in mice that by extending the plasma half-life of the ester bond from 20 to 70 min by an esterase inhibitor, the same tolerance pattern can be produced as in rats (42, 43). Receptor-mediated action of AN-238 was also demonstrated in mice with suppressed esterase activity by blocking the SST receptors with an excess of carrier RC-121 prior to administration of AN-238 at a high dose. Such a dose is tolerated when the receptors are available (42, 43), but despite the prolonged half-life, AN-238 was lethal in this model, indicating that when receptors are occupied a toxic dose of cytotoxic radical is eventually released by hydrolysis in the circulation. Others also reported that efficient targeting of DOX conjugates can be achieved with plasma half-lives ranging from 0.2-24 h, the optimal being about 2 h (37). Because the half-life of the DOX-14-*O*-glutaryl ester bond in human plasma *in vitro* is approx. 2 h, the mouse model with pharmacological inhibition of esterase activity can also serve as a more accurate preclinical model to predict possible therapeutic effects in a clinical setting (43). In view of the fact that the plasma half-life of our carrier SST octapeptides is also about 2 h, the use of 14-*O*-glutaryl esters of DOX or its daunosamine-modified derivative AN-201 appears to be a reasonable choice.

## Therapeutic application

Recent developments in SST receptor subtype determination and a better understanding of the mechanism of the antiproliferative action of SST octapeptide analogs helped to identify patients with tumors who may benefit from SST hormonal therapy (6). However, with the exception of growth hormone producing pituitary adenomas and certain neuroendocrine tumors such as carcinoids, the results of clinical treatment with SST analogs are not satisfactory (45). Most of these tumors eventually escape from SST analog treatment (45). Extensive experimental and clinical experience with radionuclide SST analogs in neuroendocrine and other SST receptor-positive tumors indicate that by targeting radiopharmaceuticals to tumor cells, better response rates could be achieved (15, 16, 46, 47). Because chemotherapy is one of the main modalities for the treatment of various cancers, especially metastatic and inoperable, the presence of $sst_2$, $sst_5$ or $sst_3$ on a wide variety of tumors and their vasculature (3, 20, 48-50) provides a firm rationale for introducing a new therapeutic approach based on targeting chemotherapeutic agents to these receptors (3, 20). Latest results of our studies with AN-238 and AN-162, consisting of carrier RC-121 linked to AN-201 or DOX, respectively, demonstrate that these targeted cytotoxic SST analogs are more effective and less toxic than their corresponding cytotoxic radicals even in experimental tumor models expressing a relatively low density of SST receptors (20, 48).

### *Prostate cancer*

Prostate cancer is the most common noncutaneous malignancy in men. The prognosis of patients with androgen refractory prostate cancer is very poor, and no effective treatment exists at present (3, 51). Although the normal prostate gland expresses $sst_2$, this subtype seems to vanish during cancerous transformation (52). Nevertheless, high affinity binding of radiolabeled RC-160 could be demonstrated on 50 of 80 (65%) primary prostate cancer specimens (53) and we found the expression of $sst_2$ on 14% and $sst_5$ on 64% of 22 samples tested (53). Successful scintigraphic imaging of metastatic lesions with Octreoscan also indicate that although the density of receptors for SST on metastases of prostate cancer may be low, it could be adequate for targeted therapy with cytotoxic SST analogs (54).

The antitumor efficacy of the targeted cytotoxic SST analog AN-238 was first evaluated on the very aggressive androgen-independent Dunning R-3327-AT-1 prostate carcinoma in Copenhagen rats. At a well-tolerated dose of 300 nmol/kg, AN-238 produced a > 80% decrease in tumor weight and significantly decreased tumor burden 4 weeks after therapy. In contrast, the cytotoxic radical AN-201 showed only a weak effect at 110 nmol/kg and killed 9 of 10 animals at 115 nmol/kg within 12 days. mRNA for rat $sst_2$ was identified in Dunning R-3327-AT-1 tumors, and high affinity binding of AN-238 to tumor membrane preparations was characterized with an $IC_{50}$ value of 1.63 ± 0.33 nM (Table I) (44).

Similarly, impressive results were obtained in nude mice bearing androgen-independent PC-3 human prostate cancers. In two separate studies, nude mice with subcutaneously grown PC-3 tumors were treated with a single dose of 200 nmol/kg or two consecutive injections of 150 nmol/kg of AN-238. In both experiments, AN-238 reduced final tumor volumes, tumor weights and tumor burden by more than 60%. Histological examination of tumors revealed that the effect of AN-238 is mainly due to a significant increase in the number of cells undergoing apoptosis. This is important because the growth of prostate cancer is associated with a low rate of apoptosis rather than a high rate of mitosis. In accord with the known chemoresistance of androgen refractory prostate cancer, AN-201 was ineffective and more toxic. In the metastatic model of PC-3, treatment with AN-238 at

*Table I: SST receptor expression and efficacy of targeted chemotherapy with AN-238 containing AN-201 in various experimental cancers.*

| Tumor models | mRNA[a] $sst_2$, $sst_3$, $sst_5$ | Binding of $^{125}$I-RC-160 $K_D$ (nM) | $B_{max}$ (fmol/mg prot.) | Binding of AN-238 $IC_{50}$ (nM)[b] | Effect of AN-238 on tumor volume | Effect of AN-201 on tumor volume |
|---|---|---|---|---|---|---|
| **Prostatic** | | | | | | |
| Dunning R-3327-AT-1 | +[c] ND –[c] | 9.0 | 229.9 | 1.63 | 40% inhibition at 115 nmol/kg<br>80% inhibition at 300 nmol/kg | 35% inhibition at 110 nmol/kg<br>Lethal at 115-125 nmol/kg |
| PC-3 | | | | | | |
| subcutaneous | (+) ND + | ND | ND | ND | 62 and 74% inhibition at 2 x 150 and 200 nmol/kg, respectively | No effect, 3 of 7 mice died at 200 nmol/kg dose |
| orthotopic | (+) ND + | ND | ND | ND | 77% inhibition at 2 x 150 nmol/kg | 34% inhibition at 2 x 150 nmol/kg |
| metastases | (+) ND (+) | ND | ND | ND | 0 of 6 mice developed metastases | 4 of 6 mice developed metastases |
| **Renal** | | | | | | |
| SW-839 | + ND – | 8.46 | 491 | 7.38 | 67% inhibition at 3 x 150 nmol/kg | 27% inhibition at 3 x 150 nmol/kg |
| 786-0 | | | | | | |
| subcutaneous | – ND + | 4.33 | 378 | 2.04 | 78% inhibition at 3 x 150 nmol/kg | 40% inhibition at 3 x 150 nmol/kg |
| orthotopic | ND ND ND | ND | ND | ND | 87% inhibition at 3 x 150 nmol/kg | 35% inhibition at 3 x 150 nmol/kg |
| metastases | ND ND ND | ND | ND | ND | 1 of 7 mice developed metastatses | 5 of 6 mice developed metastases |
| CAKI-1 | – ND – | no binding | no binding | ND | No effect at 2 x 150 nmol/kg | No effect at 2 x 150 nmol/kg |
| **Breast** | | | | | | |
| MCF-7MIII | + ND + | 2.9 | 270 | ND | Lasting regression at 250 nmol/kg | No effect at 250 nmol/kg |
| MDA-MB-231 | + ND + | 7.3 | 946 | ND | Initial regression at 250 nmol/kg | No effect at 250 nmol/kg |
| MX-1 | + ND + | 7.9 | 292 | ND | 5 of 10 mice cured, no deaths | 1 of 10 cured, 1 death |
| **Brain** | | | | | | |
| U-87-MG | | | | | | |
| subcutaneous | + ND – | 9.77 | 835 | 3.67 | 82% inhibition at 150 nmol/kg<br>30% shrinkage at 2 x 150 nmol/kg | 35% inhibition at 150 nmol/kg<br>32% inihibition at 2 x 150 nmol/kg |
| orthotopic | ND ND ND | ND | ND | ND | Significantly prolonged survival | No significant effect |
| **SCLC** | | | | | | |
| H-69 | + ND (+) | ND | ND | ND | 55% inhibition at 200 nmol/kg<br>73% inhibition at 3 x 150 nmol/kg | 38% inhibition at 200 nmol/kg<br>33% inhibition at 3 x 150 nmol/kg |
| **non-SCLC** | | | | | | |
| H-157 | – – –<br>+[d] , | 8.66 | 574 | ND | 91% inhibition at 200 nmol/kg<br>1 of 7 mice died<br>83% inhibition at 2 x 150 nmol/kg | 20% inhibition at 200 nmol/kg<br>3 of 7 mice died<br>2 x 150, not tested |
| **Ovarian** | | | | | | |
| UCI-107 | + (+) + | 11.1 | 375.5 | 3.39 | 61% inhibition at 2 x 150 nmol/kg | 34% inhibition at 2 x 150 nmol/kg |
| with esterase inhibitor | | | | | 70% inhibition at 2 x 400 nmol/kg | 1 x 400 nmol/kg was lethal |

[a]+ present, (+) weak expression, – not detectable; [b]$IC_{50}$ is defined as the concentration of AN-238 causing a 50% inhibition of $^{125}$I-RC-160 binding to tumor membranes; [c]mRNA for rat SST receptor subtype; [d]mRNA for mouse SST receptor subtype; ND = not determined.

150 nmol/kg given twice had an even greater effect on the weight of orthotopically grown tumors, producing a 77% reduction. In addition, no retroperitoneal or distant metastases could be observed 4 weeks after the initiation of therapy with AN-238. In contrast, cancer spread was detected in all control animals and 4 of 6 mice treated with an equimolar dose of AN-201 (55). The presence of mRNA for $sst_2$ and $sst_5$ was found in all PC-3 tumor samples, the levels of mRNA for $sst_5$ being much higher in primary orthotopic and subcutaneous tumors.

*Renal cell carcinoma*

Renal cell carcinoma (RCC) often remains occult during the progression of the disease and is diagnosed mainly at an advanced stage, when metastatic spread cannot be prevented by surgical intervention. The prognosis for metastatic RCC is dismal because of its resistance to both chemotherapy and radiotherapy (41).

Because more than 70% of RCCs express high affinity binding sites for SST and its bioactive analogs (56), we tested the effects of AN-238 on $sst_2$-positive SW-839, $sst_5$-positive 786-0 and SST receptor-negative CAKI-1 human RCC lines in nude mice (41). High affinity binding of AN-238 was found on membrane preparations of SW-839 and 786-0 characterized by $IC_{50}$ values of 7.38 ± 0.68 and 2.04 ± 0.25 nM, respectively (Table I). While no significant antitumor effect of AN-238 was observed on CAKI-1 xenografts, the growth of SW-839 and 786-0 tumors was inhibited significantly. Even more striking results were obtained with orthotopically grown 786-0 metastatic RCC. Three of 7 mice treated with 3 i.v. injections of AN-238 at a dose of 150 nmol/kg biweekly were found to be tumor-free 6 weeks after the initiation of treatment, and in 3 other animals the tumor mass was < 30 mg. In 1 mouse, a relatively large tumor mass (320 mg) was found which did not express SST receptors, as evidenced by a radioreceptor assay. The mean tumor weight in the group treated with AN-238 was 55.3 ± 44.3 mg, representing an 87% reduction compared with controls, which measured 414.2 mg ± 41.0. More importantly, in the AN-238-treated group only the mouse with the large receptor-negative primary tumor developed lymphatic metastases. In contrast, metastases were observed in 5 of 6 animals in both the control and the AN-201-treated groups. In all these studies, AN-201 was again ineffective and more toxic than AN-238, indicating that the accumulation of the drug in tumors by targeting may overcome the chemoresistance of RCCs and alleviate the toxicity (41). A selective cytotoxic effect of AN-238, but not of AN-201, on $sst_5$-positive 786-0 cells could also be demonstrated *in vitro* when these cells were mixed with SST-negative CAKI-1 cells (41).

*Breast cancer*

Over half a million breast cancer cases are reported worldwide each year. Although an early detection by screening mammography significantly improves the cure rate, estrogen-independent metastatic breast cancer still poses an enormous challenge to oncologists, especially in patients with chemoresistant tumors (57). The presence of SST octapeptide-preferring subtypes on human breast cancer specimens has been thoroughly investigated by several research groups (57). While a nonhomogeneous receptor distribution was demonstrated by *in situ* hybridization and receptor autoradiography in 28 human breast cancer specimens (58), immunocytochemical staining of 33 primary breast tumors with polyclonal anti-$sst_2$ antibodies showed that in 85% of the specimens receptor immunoreactivity was uniformly present in nearly all cancerous cells (59). Consequently, the patients with breast cancer could benefit from treatment with analogs such as AN-238, which may release the cytotoxic radical, in part, in the interstitial space of tumor tissue affecting both receptor-positive and neighboring cells by bystander effect.

To investigate the efficacy of SST receptor targeting in human breast cancer, nude mice bearing xenografts of estrogen-sensitive MCF-7-MIII, estrogen-independent MDA-MB-231 and MX-1 (also DOX-resistant) tumors were treated with a single i.v. injection of AN-238 or AN-201 at 250 mnol/kg (57). In the MCF-7-MIII model, 3 of 8 tumors showed a regression, even 60 days after the administration of AN-238, while all tumors grew steadily in the group treated with AN-201. In the MDA-MB-231 model, 4 of 13 tumors showed an apparent initial regression after treatment with AN-238 that lasted for about 2 weeks. AN-201 again had no effect on tumor growth. Both AN-201 and AN-238 showed a very strong inhibition on the growth of MX-1 tumors. After 60 days, 5 of 10 animals in the AN-238-treated group and only 1 of 9 mice in the AN-201 group were tumor free. Because chemoresistance is a major problem in the management of breast cancer in patients, it is important to point out that AN-238 is active on DOX-resistant tumor cells. In all 3 experiments, AN-201 was again more toxic as assessed by animal deaths and white blood cell counts (57).

*Brain tumors*

Glioblastomas represent the most common form of primary brain tumors and are considered incurable. Low grade glioblastomas express $sst_2$ (60) and radionuclide SST analog therapy in patients with such tumors shows promising results (46).

Because U-87 MG human glioblastomas express receptors for SST, we tested AN-238 in both subcutaneous and orthotopic models in nude mice (61). Animals with large (> 500 $mm^3$) subcutaneous xenografts were treated with a single injection of AN-238 or AN-201 at 150 nmol/kg i.v. Nineteen days later, the group injected with AN-238 showed an 82% tumor growth inhibition compared with controls ($p$ = 0.00168). This tumor inhibition could be reduced to 37% by blocking of SST receptors with a high dose of RC-160 given prior to treatment with

AN-238. AN-201 was ineffective. In the same study, mice bearing very large tumors (approx. 900 $mm^3$) were given 2 injections of AN-238 or AN-201 at doses of 150 mnol/kg. Nineteen days after the first injection, tumors shrank by 30% in the AN-238-treated group, while AN-201 again had no effect on the progression of tumor growth. In both studies, AN-201 was also more toxic. In an additional study, the effects of AN-238 at 150 mnol/kg were compared to those of its DOX-containing counterpart AN-162 (Fig. 1) at 13,750 nmol/kg, representing an equitoxic dose. While the relatively large tumors treated with AN-162 grew steadily, AN-238 virtually arrested tumor growth. In the second part of the experiment, animals with tumors that had already been treated unsuccessfully with AN-162 received AN-238 or a second dose of AN-162. While tumors in the AN-162-group continued to grow, AN-238 caused an impressive shrinkage (61). These results are in agreement with the fact that brain tumors are resistant to therapy with DOX. We also tested the efficacy of AN-238 injected into the tail vein of mice bearing orthotopically grown U-87 MG tumors. Again, AN-238 produced a significant prolongation of survival time compared with controls, indicating that the tumor blood-brain barrier may be penetrable for the cytotoxic SST analog AN-238.

AN-238 bound to the receptors present in U-87 MG tumors with high affinity ($IC_{50}$ = 3.67 ± 0.46, Table I) (61) and $sst_2$ was determined as the receptor subtype by mRNA analysis.

*Small and non-small cell lung carcinomas*

Small cell lung cancer (SCLC) constitutes only about 20% of all lung cancers, but most cases are already metastatic at the time of diagnosis. Although chemotherapy can be used for treatment, the long-term survival rate is low. SCLC is of neuroendocrine origin and, accordingly, a high percentage of primary tumors and metastatic lesions can be localized in patients with Octreoscan scintigraphy (15). Other types of lung cancer, classified together as non-SCLC, can also be visualized by Octreoscan in patients, but the receptors for SST were found only in peritumoral tissue (blood vessels, immune cells, *etc*.) and not on tumor cells (15).

To test the merits of SST receptor targeting in SCLC, we injected nude mice bearing H-69 SCLC xenografts (~ 350 $mm^3$ in size) with a single dose of AN-238 or AN-201 at 200 nmol/kg or twice with 150 nmol/kg i.v. AN-238 produced a > 50% inhibition of tumor growth with both treatment schedules and was significantly ($p$ <0.05) more effective than AN-201, which was also more toxic (62). H-69 tumors bound radiolabeled RC-160 and expressed high levels of mRNA for $sst_2$ and lower levels for $sst_5$. In contrast, we could not find mRNA for $sst_2$, $sst_5$ or $sst_3$ in H-157 non-SCLC tumors and in cultured cells, but radioreceptor assays indicated that 4 of 5 control tumors showed specific binding of radiolabeled RC-160. Treatment with AN-238 at a dose of 200 nmol/kg also produced a very impressive 91% growth inhibition compared with controls. Therapy with an equimolar dose of AN-201 again did not produce significant antitumor effect and was more toxic than AN-238. Because tumor vasculature from the host could be the target for AN-238, we evaluated the RNA extract from the control tumor specimens for mRNA for the mouse $sst_2$ and $sst_5$ subtypes and found a strong expression of the former.

These results are the first examples of a successful targeted therapy based on SST receptors in tumor vasculature in an experimental model in which tumor cells themselves are SST receptor-negative (62). Similarly impressive results were obtained when the endothelial cells of angiogenic vessels were targeted with a different peptide-DOX conjugate that specifically homes in on tumor blood vessels (63).

*Pancreatic cancer*

One of the natural target organs of SST is the pancreas and, accordingly, this organ expresses high levels of SST receptor subtypes $sst_2$ and $sst_5$ (64). Hormone producing endocrine pancreatic tumors also express these subtypes, and administration of SST analogs is a widely accepted treatment for these types of malignancy (3-6). With the exception of some insulinomas, most endocrine pancreatic cancers can be visualized and treated with radionuclide SST analogs (15, 16). However, the cancer of the exocrine pancreas seems to be totally different from endocrine tumors with regard to SST receptor expression. Although there are some conflicting reports on this subject, SST binding sites appear to be poorly or not at all detectable on many of these cancers. Loss of $sst_2$ mRNA expression and lack of effect of SST analog therapy (64, 65) also demonstrate the difference between exocrine and endocrine pancreatic cancers. Presently there is no treatment for advanced metastatic pancreatic adenocarcinoma and the median survival time of patients with this malignancy is about 2-5 months (65).

Because some studies indicated a binding of a radionuclide SST octapeptide to pancreatic cancers in patients (66), and the expression of mRNA for $sst_5$ and $sst_3$ was also reported (64), we tested AN-238 on human pancreatic cancer lines xenografted in nude mice that express these subtypes (67). We were able to demonstrate very good tumor inhibition with AN-238, while AN-201 was ineffective (details of this study will be published soon).

Loss of $sst_2$ expression in pancreatic cancers could be connected with the proliferation of cells. To test this theory, PC-1.0 cells, originating from chemically induced ductal pancreatic cancers in Syrian golden hamsters, were stably transfected with the $sst_2$ gene to produce PC-1.0/$sst_2$ cells (67). It was demonstrated that after orthotopic injection in hamsters, PC-1.0/$sst_2$ cells grew significantly more slowly than control PC-1.0 cells. When animals with $sst_2$-expressing tumors received an i.v. injection of AN-238 at 100 mnol/kg, tumor growth was further

slowed down compared with untreated PC-1.0/$sst_2$ tumors. In contrast, AN-238 had no effect on SST receptor-negative PC-1.0 tumors, demonstrating the importance of receptors for the efficacy of the targeted therapy (67).

*Epithelial ovarian cancer*

Treatment options currently available for recurrent epithelial ovarian cancer do not provide satisfactory results (42). In search for better therapeutic modalities, we demonstrated using OV-1063 experimental human ovarian cancer model in nude mice that receptors for LH-RH, which are present on 80% of ovarian cancers, can be utilized for targeted therapy. In these studies we also showed that UCI-107 tumors, that are devoid of LH-RH receptors, do not respond to treatment with cytotoxic LH-RH analogs (20). Most recently, we reported that 13 of 17 (76%) surgical specimens of human epithelial ovarian cancer exhibited high affinity binding sites for radiolabeled RC-160 ($K_D$ = 6.55 nM and $B_{max}$ = 575.4 fmol/mg). The incidence of mRNA expression for $sst_2$, $sst_3$ and $sst_5$ on these specimens was 65%, 41% and 24%, respectively. Two human ovarian cancer cell lines, OV-1063 and UCI-107, were also identified as SST receptor-positive (68).

Because LH-RH receptor-negative ovarian cancers may express receptors for SST, as in the case of UCI-107, we tested AN-238 in this model xenografted into nude mice (42). After 2 i.v. injections of AN-238 at 150 mnol/kg, given 7 days apart, final tumor weights were reduced by 67.3% compared with controls. Treatment with the same dose of AN-201 produced only a slight but nonsignificant effect. A similar degree of myelotoxicity was observed in surviving mice after both agents, but 2 of 8 animals died in the group treated with AN-201 while none died in the AN-238 group. Binding assays demonstrated that AN-238 displays high affinity binding to tumor membrane fractions *in vitro*, characterized by an $IC_{50}$ value of 3.39 ± 0.74 (42) (Table I).

When starting tumors were 50% larger than in the first study, a comparable growth inhibition (70%) was achieved with AN-238 after pharmacological suppression of serum carboxylesterase activity. Following injection of 400 nmol/kg of AN-238, 2 of 9 animals died, apparently due to inadequate inhibition of carboxylesterase. Two weeks later, a second injection was given at the same dose and no deaths occurred. AN-201 under the same conditions killed all animals within 4 days after 1 injection.

*Other cancers*

Presently, ongoing investigation of the efficacy of SST receptor-targeted chemotherapy on gastric and colon cancers as well as melanomas, in which AN-162 also showed very promising results, indicate that this therapeutic approach may find an application in a broad variety of cancers.

## Conclusions

The results of preclinical studies and clinical trials with SST octapeptides and their radionuclide analogs indicate that the receptors for these peptides are present on a wide variety of cancerous cells. Although the SST receptor concentration on prostatic, renal, ovarian or pancreatic cancers appears to be much lower than on neuroendocrine cancers, it still should be high enough to produce significant responses to chemotherapy with targeted cytotoxic SST analogs. This targeted chemotherapy should be associated with a higher efficacy and lower toxicity than systemic chemotherapy. The presence of receptors for SST on the endothelial cells of newly formed tumoral vasculature and peritumoral veins may also serve as targets for cytotoxic SST conjugates, further broadening the scope of this type of therapy.

Although additional experimental work with cytotoxic SST hybrids is necessary before the initiation of clinical trials, the results summarized in this review indicate that analogs such as AN-238 or AN-162 should result in major improvements in the clinical management of various cancers that are presently treated with systemic chemotherapeutic agents.

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