

Expert Opinion

1. Introduction
2. GnRH receptors as molecular targets for cancer diagnosis and therapy
3. Cytotoxic GnRH derivatives
4. Radiolabeled GnRH derivatives
5. Somatostatin receptors as molecular targets for cancer diagnosis and therapy
6. Cytotoxic somatostatin derivatives
7. Somatostatin-based boron neutron capture therapy
8. Radiolabeled somatostatin derivatives
9. Conclusion
10. Expert opinion

Receptor-mediated tumor targeting based on peptide hormones

Gábor Mező[†] & Marilena Manea

[†]Eötvös Loránd University, Research Group of Peptide Chemistry, Hungarian Academy of Sciences, 1117 Budapest, Pázmány P. stny. 1/A, Hungary

Importance of the field: Tumor targeting with peptides is based on the discovery that receptors for many regulatory peptides are overexpressed in tumor cells, compared with their expression in normal tissues. Consequently, these peptides and their analogues can be used as carriers/targeting moieties for the preparation of diagnostic and therapeutic agents that have increased selectivity and decreased peripheral toxicity.

Areas covered in this review: Here an overview is given of the most relevant gonadotropin-releasing hormone (GnRH) and somatostatin derivatives, as well as of their applications in cancer diagnosis and therapy. For this purpose, recently published data in these areas (mostly articles published from 2000 to 2009) were reviewed.

What the reader will gain: In contrast to other regulatory peptides that stimulate the tumor growth, GnRH and somatostatin derivatives have inhibitory effect; therefore, they were used primarily for the preparation of various conjugates to be used in targeted chemotherapy, targeted radiotherapy, photodynamic therapy, boron neutron capture therapy and cancer diagnosis. Some of these conjugates have already found clinical applications, whereas others are now in preclinical and clinical trials.

Take home message: Tumor targeting with hormone peptides provides a basis for the development of new diagnostic and therapeutic approaches for cancer.

Keywords: cancer diagnosis and therapy, drug delivery systems, gonadotropin-releasing hormone, receptor-mediated tumor targeting, somatostatin

Expert Opin. Drug Deliv. (2010) 7(1):79-96

1. Introduction

Cancer is a leading cause of death and a major public health problem worldwide. According to the statistics of the World Health Organization, in 2007 cancer accounted for 7.9 million deaths (~ 13% of all deaths) all over the world. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030 [1] if no breakthrough in the therapeutic approaches occurs.

Since the middle of the last century, chemotherapy has been the main procedure for the treatment of advanced or metastatic cancer [2]. However, the application of free anticancer drugs has several drawbacks, such as the lack of selectivity, toxicity to normal cells, fast elimination from the blood circulation, and the acquired or intrinsic multi-drug resistance of cancer cells [3]. In the last two decades, cancer research has turned to a more selective, targeted approach, focused on the development of anticancer therapies with improved efficacy and reduced peripheral toxicity [4]. One of the most promising therapeutic strategies is based on the peptide receptors that are overexpressed in tumor cells, in comparison with their expression in normal tissues [3,5]. Many regulatory peptides (e.g., somatostatin,

informa
healthcare

Article highlights.

- Specific tumor targeting with peptides is based on the discovery that receptors for many regulatory peptides are overexpressed in tumor cells, compared with their expression in normal tissues.
- Gonadotropin-releasing hormone (GnRH) receptors are important molecular targets for cancer diagnosis and therapy.
- Cytotoxic GnRH derivatives are developed as drug delivery systems for targeted cancer chemotherapy.
- The application of radiolabeled GnRH derivatives is limited.
- High-affinity somatostatin receptors are expressed on the plasma membrane of tumor cells.
- Cytotoxic somatostatin derivatives are available for targeted cancer chemotherapy.
- Somatostatin-based boron neutron capture therapy is still in the *in vitro* testing phase.
- Radiolabeled somatostatin derivatives are used in targeted radiotherapy and tumor imaging.
- Tumor targeting with hormone peptides provides a basis for the development of new diagnostic and therapeutic approaches for cancer.
- GnRH and somatostatin analogues are promising tools to target radionuclides and antineoplastic agents to tumor cells.

This box summarises key points contained in the article.

gonadotropin-releasing hormone (GnRH), bombesin, gastrin-releasing peptide (GRP), neurotensin, substance P, vasoactive intestinal peptide (VIP) and cholecystikinin (CCK) have membrane-bound receptors on different types of tumor [5]. The receptors recognized by these hormone/neurotransmitter peptides are G-protein-coupled receptors; therefore, they can internalize into the cells after binding of their ligands. Consequently, a cytotoxic agent attached to the peptide can enter the tumor cell by a receptor-mediated way (Figure 1). The advantages of small peptides as carriers are their excellent tissue permeability (except crossing the blood–brain barrier), the lack of immunogenicity, high affinity to the receptors, minimal side effects, rapid clearance from the body, easy synthesis and chemical modifications as well as detailed and precise characterization. The only disadvantage is their rapid proteolytic degradation, which can be partially prevented by insertion of D-amino acids, reduced peptide bonds, *N*-methyl- or non-native amino acids, and so on. However, it has to be taken into account that regulatory peptides, which control and modulate the function of almost all key organs and metabolic processes, may also play prominent roles in cancer progression. Thus, peptide derivatives (agonists or antagonists) selected for drug delivery should have an inhibitory but not stimulatory effect on tumor growth [5,6]. Somatostatin and GnRH analogues have an inhibitory effect on tumor growth, whereas bombesin, GRP, VIP, CCK, and so on, stimulate it. Considering these aspects, the application of antagonistic derivatives as carriers in the case of the latest ones is suggested

by several authors [5,6]. However, others prefer agonists (e.g., in the case of bombesin) as drug targeting moieties owing to the observation that agonists induce rapid receptor–ligand internalization by tumor cells, which might be important in the case of targeted cancer chemotherapy [7,8].

Tumor targeting with peptides has found both diagnostic and therapeutic applications. In the case of cancer diagnosis and radiotherapy, a radioligand is usually attached to the regulatory peptide carrier by the aid of a chelator. A wide variety of chelating agents (e.g., diethylenetriaminepentaacetic acid [DTPA] and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid [DOTA]) have been developed for convenient radiolabeling of peptides. Radionuclides such as ^{99m}Tc , ^{111}In , ^{68}Ga , ^{123}I , ^{64}Cu and ^{18}F can be used for diagnosis and ^{90}Y , ^{177}Lu and ^{111}In for therapeutic purposes. Radionuclides used in cancer radiotherapy emit high energy β -rays or Auger electrons and have longer half-life (> 60 h) in comparison with the γ -ray or positron emitter radionuclides used as diagnostic tools [5,9].

Another therapeutic application of peptide-based tumor targeting is targeted cancer chemotherapy. In this case, cytotoxic drugs are attached to hormone/neurotransmitter peptides with the aim of increasing their selectivity and decreasing the peripheral toxicity (Figure 1).

The main difference between the conjugates containing radionuclides or drugs is that the antineoplastic agent should be released from the conjugate in order to obtain an efficient tumor growth inhibition, whereas in the case of radiopharmaceuticals this process is not necessary. Therefore, the insertion of pH-sensitive or enzymatic cleavable linkers between the drug and the peptide might be required for the antitumor activity of the cytotoxic conjugates [10,11].

The selection of the peptide to be used as a targeting moiety is based on the type of cancer to be treated. An important aspect of targeted cancer chemotherapy is that the receptor incidence and density must be different on tumoral tissues compared with normal ones. Therefore, VIP peptides seem not to be efficient for drug delivery compared with the others [5].

Here an overview is given of GnRH and somatostatin analogues that are widely used as carriers for radionuclides and antineoplastic agents.

2. GnRH receptors as molecular targets for cancer diagnosis and therapy

Type I and type II GnRH receptors (GnRH-IR and GnRH-IIR) have been identified among vertebrates, including mammals. GnRH-IR protein consists of 329 amino acids and it has no C-terminal cytoplasmic tail [12]. Therefore, GnRH-IRs do not desensitize rapidly and they internalize slowly [13]. The full-length GnRH-IIR have been found in marmoset and rhesus monkeys but not in humans [14,15]. The



GnRH-IIR has a cytoplasmic tail and undergoes rapid arrestin-dependent internalization and desensitization [16]. The mRNA expression of this full-length GnRH-IIR has also been shown by real-time polymerase chain reaction (RT-PCR) in human cancer cell lines [17]. However, it became evident that GnRH-IIRs have a frameshift and a premature stop codon and thus they are not functional. Therefore, it is believed that the GnRH-II signaling might also occur through

the GnRH-IR (Figure 2) [18]. By contrast, after knockout of GnRH-IR expression in endometrial and ovarian cancer cells, the antiproliferative effect of GnRH agonist Triptorelin ([D-Trp⁶]-GnRH) was abrogated, whereas the tumor growth-inhibitory effect of GnRH-II persisted [19]. Recent data of extensive research have shown that functional GnRH-IIR might occur on human cancer cells [20]. However, an intensive study of the role of GnRH-IIRs on tumor cells

Table 1. *In vivo* and *in vitro* antitumor effect of AN-152 and AN-207 conjugates.

Tumor type	Receptor expression in specimens	Cell types that express GnRH-R	AN-152	AN-207
Breast	> 50	MCF-7 MDA-MB-231 MDA-MB-435 MX-1 T47-D HCC-70 ZR-75-1 MXT (mouse)	AN-152 > Dox* n.d. n.d. XXX AN-152 > Dox AN-152 > Dox AN-152 < Dox XXXX	AN-207 >> Dox XXXX [‡] XXX XXXXX n.d. n.d. n.d. XXXX
Ovarian	> 70	OV-1063 OVCAR-3 ES-2 EFO-21 EFO-27	XXXX XXXXX XX AN-152 > Dox AN-152 < Dox	XXX n.d. XXX n.d. n.d.
Endometrial	> 75	Ishikawa HEC-1A HEC-1B RC-95-2	AN-152 > Dox XXX XXXXX n.d.	n.d. XXX n.d. XXX
Prostate	> 85	LNCaP MDA-PCa-26 MDA-PCa-2b DU-145 C4-2 PC-82 LuCaP-35 R-3327-H (rat) R-3327-AT-1 (rat)	XXXX XXX XXX n.d. XXX (PSA inh.) [§] n.d. n.d. XXX XX	n.d. n.d. XXX XXXX n.d. XXX XXXX XXX n.d.
Colon	n.d.	HT-29 HCT-116 HCT-15 LoVo Colo-320DM	XX X XXXX XXX XXXX	XXX XXX XXXX XXX XX
Renal	80	A-498 ACHN 786-0	n.d. n.d. n.d.	XXXX XXXX XXX
Melanoma	100	MRI-H255 MRI-H187	n.d. n.d.	XXXX XXX
Lung	n.d.	NCI-H720	AN-152 > Dox	n.d.
Non-Hodgkin's lymphoma	100	RL HT	n.d. n.d.	XXXX XXXX
Hepatocellular	n.d.	SK-Hep-1	n.d.	XXXX
Oral	n.d.	KB	AN-152 > Dox	n.d.
Laryngeal	n.d.	HEp-2	AN-152 > Dox	n.d.

*AN-152 > Dox represents the comparison of the *in vitro* antitumor effect of free drug and its GnRH derivative conjugate.

[‡]*In vivo* studies: XXXXX: regression; XXXX: > 70% inhibition; XXX: 50 – 70% inhibition; XX: 25 – 50% inhibition; n.d.: not determined.

[§]PSA inh.: not the tumor volume, but PSA level was determined as a measure of the inhibitory effect.

The effects are not absolutely comparable because of different doses and treatment schedules used.

and of the mechanism of action of GnRH-II should be carried out. In addition to GnRH-IR, GnRH-IIR might be a good target for tumor therapy.

GnRH-R expression was identified on different tumors (breast, ovarian, endometrial, prostate, renal, brain, pancreatic, melanomas, non-Hodgkin's lymphomas, etc.) (Table 1) [21-23]. The limited number of GnRH-Rs in normal tissues provided a

basis for the development of diagnostic and therapeutic approaches of cancer (Figure 1).

3. Cytotoxic GnRH derivatives

Cytotoxic GnRH derivatives were initially developed by Schally's group after the mid-1980s. The first series of

conjugates contained agonistic (e.g., [D-Lys⁶]-GnRH; Glp-His-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH₂) or antagonistic (e.g., [Ac-D-Nal(2)¹,D-Phe(pCl)²,D-Pal³,Arg⁵,D-Lys⁶,D-Ala¹⁰]-GnRH) GnRH derivatives as carriers and cytotoxic moieties such as alkylating agents cisplatin and melphalan, antimetabolite methotrexate, DNA intercalating agents doxorubicin (Dox) and (2-hydroxymethyl)anthraquinone [21,24,25]. Based on the results of *in vitro* and *in vivo* studies using these compounds, more efficient conjugates were developed in which Dox or its analogue (2-pyrrolino-Dox; AN-201, which is 500 – 1000 times more potent than Dox) were attached to [D-Lys⁶]-GnRH [26]. In these compounds AN-152 (1) (Figure 3A) and AN-207 (2) (Figure 3B), the cytotoxic agents connected to glutaric acid by means of an ester bond (Dox-14-*O*-hemiglutarate), were conjugated to the ε-amino group of D-Lys⁶ of GnRH. The ester bond could be cleaved by carboxylesterases (CE), leading to the release of the free cytotoxic radicals. Cellular uptake studies showed that the conjugates were internalized in a receptor-mediated way by GnRH-R-positive cells (Figure 1A) but not by GnRH-R-negative cells (Figure 1B). The receptor-mediated internalization was also confirmed by blocking the receptors with the superagonist Triptorelin [27].

The antitumor action of AN-152 and AN-207 was studied intensively on numerous tumor types (Table 1). Their efficiency was tumor type-dependent, but in almost all cases AN-207 had higher antitumor activity. Significant *in vivo* tumor growth inhibition and the regression of several tumor types (e.g., ovarian, endometrial, breast and prostate cancers) were observed. These results have been reviewed extensively [3,21,28,29]. In the last few years, the strong inhibitory activity of the conjugates has been shown on more tumor types, such as renal, non-Hodgkin's lymphomas, melanomas, colorectal and hepatocellular carcinoma cell lines that expressed GnRH-Rs [22,23,30-32]. In most cases, the intravenous-administered conjugates were far less toxic and inhibited the growth of GnRH receptor-positive tumors better than the equimolar doses of free doxorubicin. Furthermore, AN-152 induces apoptosis in GnRH receptor-positive human ovarian and endometrial cancer cell lines without activating the MDR-1 (multi-drug resistance-1) efflux pump system [33].

Although the tumor growth inhibition effect of AN-207 was higher, AN-152 was the first cytotoxic GnRH derivative investigated in preclinical and clinical studies. In the Phase I clinical trial for ovarian and endometrial cancers (Æterna Zentaris, AEZS-108), AN-152 was well tolerated using escalated doses of 160 and 267 mg/m². Even at the highest dose, the hematological toxicity of AN-152 was low and the other non-hematological side effects were mild [34]. The Phase II clinical trial started in January 2008.

It is worth mentioning that the side effects of the conjugates were mainly related to the easy drug release by CE. In an early study, it was demonstrated that the half-life of AN-152 in mouse serum was ~ 20 min, whereas in human serum it lasted a longer time, with a half-life of 2 h [35]. This difference could

be explained by the CE levels in mouse and human sera (the CE level in mouse serum is ~ 10 times higher than in human serum). Therefore, to reduce the toxicity of the conjugate in many *in vivo* experiments, diisopropyl-fluorophosphate was used as a CE inhibitor. In this way the half-life of AN-152 in mouse serum was enhanced to ~ 70 min and the maximal tolerated dose (MTD) was increased from 200 to 2 × 400 nM. The hydrolyzed Dox and especially 2-pyrrolino-Dox had a non-receptor specific cytotoxic effect on rapidly proliferating cells, the main toxic side effect being the myelotoxicity. Another side effect might originate from the presence of GnRH-Rs on normal reproductive cells and gonadotroph cells. The temporary damage of gonadotrophs using AN-207 was recovered ~ 1 week after cessation of the treatment [36]. In the case of hormone-dependent tumors patients are usually hypophysectomized, thus this side effect of the cytotoxic GnRH derivatives is not harmful [37].

A natural isoform of GnRH, GnRH-III (Glp-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH₂), which is a weak GnRH agonist, has recently been used as a carrier in the authors' laboratories. This hormone peptide, isolated from sea lamprey (*Petromyzon marinus*) [38], specifically binds to the GnRH-Rs on cancer cells [39]. GnRH-III has antiproliferative activity on several tumor cell lines and 500 – 1000 times less potency in LH and FSH secretion both *in vitro* and *in vivo*, indicating its selective antitumor activity [40]. The modification of the side chain of Lys⁸ did not result in the loss of the antiproliferative activity of GnRH-III [41]. Furthermore, the absence of the free ε-amino group of lysine decreased the endocrine effect of the compounds [42]. Therefore, Lys⁸ might be used as a conjugation site for the attachment of cytotoxic drugs. Daunorubicin (Dau), an anthracycline derivative that differs from doxorubicin in the substitution at the C-14-position (H instead of OH) and has lower cardiotoxicity than Dox, was conjugated to GnRH-III via oxime (3) (Figure 3C) or hydrazone (4) bonds (Figure 3D). As the oxime bond is relatively stable under physiological conditions, a GFLG tetrapeptide spacer known to be cleaved by lysosomal enzymes, especially by Cathepsin B which is overexpressed in tumor cells [41,43,44], was inserted between the drug and the hormone peptide. Ester bond-containing Dox-GnRH-III conjugates were also prepared. *In vitro* cytostatic effect of the conjugates was determined by MTT assay on MCF-7 human breast cancer and HT-29 human and C26 murine colon carcinoma cell lines [25,41]. The results were compared with the IC₅₀ values of the free drugs and AN-152. All compounds were more potent on breast cancer cell lines; however, there were no significant differences between their antitumor effects on colon cancer cells. The ester bond-containing conjugates had similar antitumor activity as the free drugs and one order of magnitude higher activity than that of the conjugates containing hydrazone or oxime bonds.

The *in vivo* toxicity study on BDF-1 mice indicated high tolerability of oxime bond-linked GnRH-III(Dau=Aoa-GFLG) conjugate. This conjugate had an MTD > 54 μmol

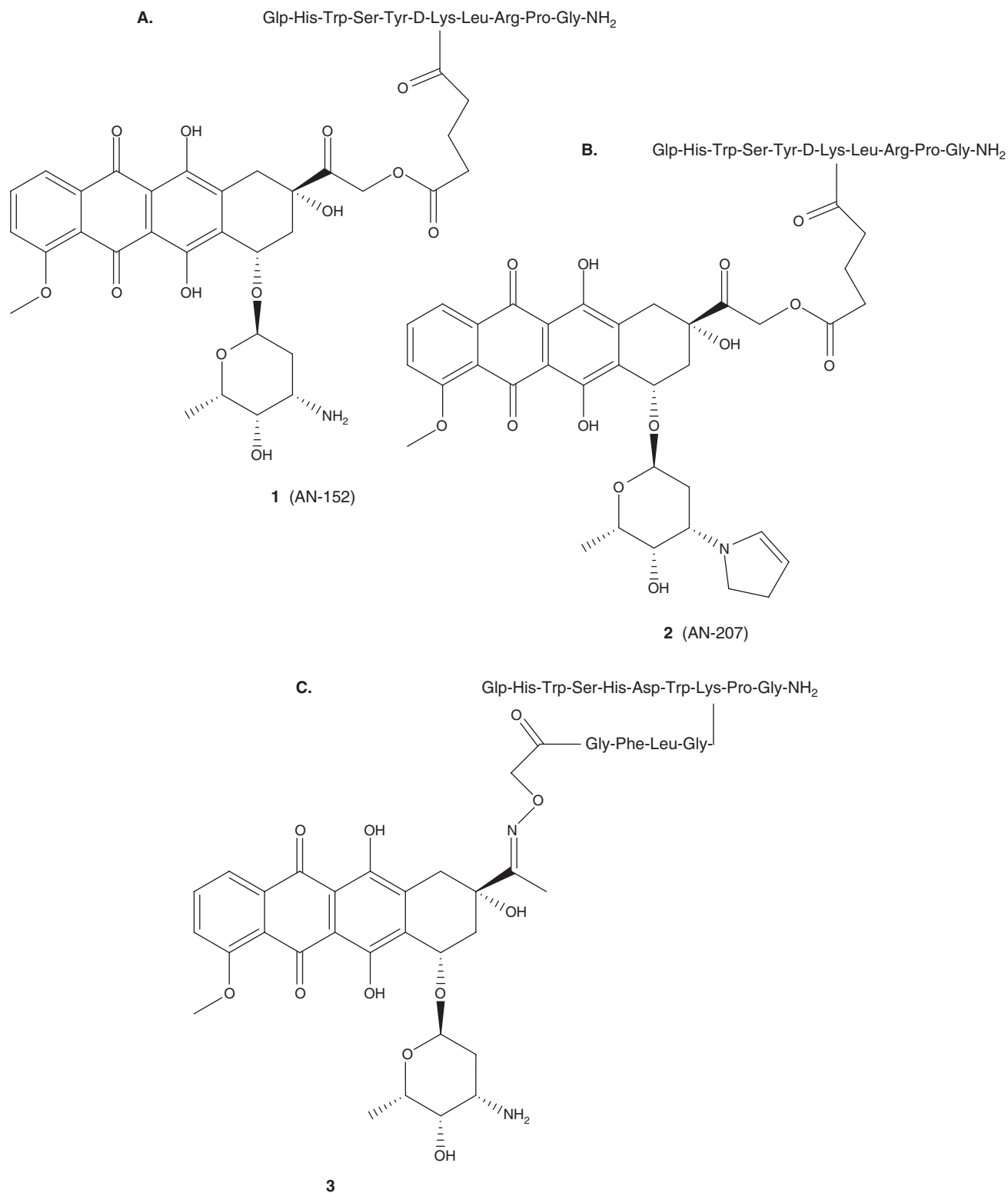


Figure 3. Chemical structures of cytotoxic GnRH derivatives: ester bond-linked doxorubicin-[D-Lys⁶]-Gn RH (A) and 2-pyrrolino-doxorubicin-[D-Lys⁶]-GnRH (B), oxime bond containing daunorubicin-GnRH-III (C), hydrazone bond-linked daunorubicin-GnRH-III (D) and of a radiolabeled GnRH derivative, D-Lys⁶(Ahx-[¹⁸F]FBOA)-GnRH (E).

Ahx: ϵ -Aminohexanoic acid; FBOA: *p*-Fluorobenzyloxime acetyl.

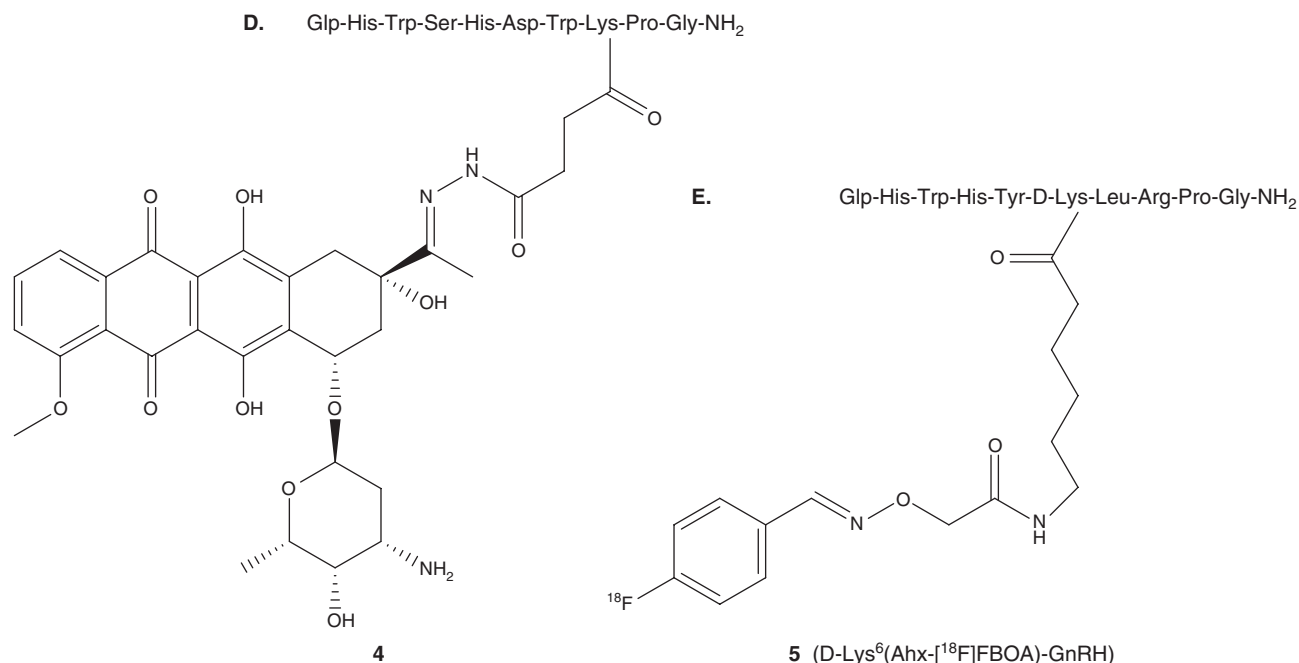


Figure 3. Chemical structures of cytotoxic GnRH derivatives: ester bond-linked doxorubicin-[D-Lys⁶]-Gn RH (A) and 2-pyrrolino-doxorubicin-[D-Lys⁶]-GnRH (B), oxime bond containing daunorubicin-GnRH-III (C), hydrazone bond-linked daunorubicin-GnRH-III (D) and of a radiolabeled GnRH derivative, D-Lys⁶(Ahx-[¹⁸F]FBOA)-GnRH (E). (Continued)

Ahx: ϵ -Aminohexanoic acid; FBOA: *p*-Fluorobenzyloxime acetyl.

Dau-content in the conjugate per kilogram using intraperitoneal administration. The *in vivo* antitumor activity of the conjugate was evaluated on C26 colon cancer-bearing mice and the tumor growth inhibition was 20 – 46% depending on the dose and on the time schedule. Similar results were obtained after the administration of the free Dau. However, the survival of the animals treated with the conjugate increased by 15 – 40% compared to the controls, whereas the animals treated with the free drugs died earlier [41].

Rahimipour *et al.* applied [D-Lys⁶]-GnRH and [D-Glp¹,D-Phe²,D-Trp³,D-Lys⁶]-GnRH (an antagonistic peptide) to increase the selectivity of protoporphyrin IX (PpIX) as a photosensitizer for photodynamic therapy [45]. In both cases, PpIX was conjugated to D-Lys⁶ by means of amide bonds. The conjugates bound to the GnRH-Rs with lower affinity than the parent peptides, but their selective and efficient phototoxicity compared with the unconjugated PpIX could be demonstrated.

Drug delivery systems based on GnRH targeting moieties were developed by Dharap *et al.* Camptothecin (CPT) was connected to Glp-His-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-EA (EA: ethylamide) through a polyethylene glycol (PEG) chain. CPT was attached to the cysteine by means of an ester bond and then Cys-CPT was conjugated to NHS-PEG-VS (NHS: *N*-hydroxysuccinimide; VS: vinylsulfone) by a thioether bond. After that, the GnRH derivative was attached to the PEG conjugate by means of an amide bond. PEG increased the solubility and bioavailability of the conjugate. The

GnRH-PEG-CPT conjugate showed higher cytotoxicity and apoptosis induction than the free CPT or PEG-CPT [46]. These results indicated the efficiency of receptor-mediated endocytosis for selective drug targeting over the simple diffusion or direct endocytosis [47]. Moreover, *in vivo* experiments did not show any disturbance of the reproductive functions of female mice. GnRH-PEG-CPT conjugate accumulated in tumor cells but, similarly to the free CPT and PEG-CPT, caused overexpression of *BCL-2* and *BCL-XL* genes and thereby increased the cellular antiapoptotic defense. To overcome this drawback, a multifunctional targeted proapoptotic drug delivery system was designed. This new conjugate contained a BH3 antiapoptotic peptide (MGQVGRQLAIIGDDINRRY) beside the GnRH derivative, CPT and PEG. The conjugation of the three components to PEG was performed by inserting a multifunctional citric acid spacer that allowed simultaneous binding of several copies of different active components to one polymeric carrier [48,49]. It is important to note that BH3 peptide could not enter the cells without any targeting moiety [50]. The conjugate containing all four components induced the greatest suppression of the antiapoptotic gene expression and the highest cytotoxic and apoptotic effects in A2780 human ovarian carcinoma cell line as compared with the conjugates CPT-PEG-BH3 and GnRH-PEG-CPT or their physical mixture. The conjugate also showed antitumor activity on A2780/AD multi-drug resistant human ovarian carcinoma cell line, but the effect was lower than in case of non-resistant cell lines.

4. Radiolabeled GnRH derivatives

Except radioiodinated compounds, only a few data have been published so far about the development of other radiolabeled GnRH derivatives. Barda *et al.* prepared a ^{99m}Tc -labeled GnRH derivative as a potential single photon emission computed tomography (SPECT) tracer [51]. However, the insertion of the radiometal via backbone cyclization of the peptide led to a dramatic loss of the binding affinity. Schottelius *et al.* investigated ^{68}Ga - and ^{18}F -labeled GnRH analogues [52]. Ga was incorporated by the aid of DOTA, while for the insertion of F, *p*-fluorobenzyloxime acetyl (FBOA) moiety was applied. DOTA was attached directly to the ϵ -amino group of D-Lys in [D-Lys⁶]-GnRH, whereas in case of the fluor-containing derivative, either β -alanine or ϵ -aminohexanoic acid (Ahx) were inserted between the peptide and the radioligand. In the *in vitro* binding experiments, radioiodinated Triptorelin (^{125}I -[D-Trp⁶]-GnRH) was also used. The compound radiolabeled with ^{68}Ga had very low binding capacity, whereas the binding of fluorinated derivatives was four to six times lower than the radiolabeled Triptorelin. Compared with Triptorelin, the internalization of D-Lys⁶(Ahx-[^{18}F]FBOA)-GnRH (5) (Figure 3E) into EFO-27 ovarian carcinoma cells was $86 \pm 16\%$, whereas the β -Ala-derivative showed only $42 \pm 3\%$. There was no significant internalization observed in case of the Ga-labeled GnRH derivative. These data suggested that the lipophilicity of the compound that modified the lysine side chain and the distance between the peptide backbone and the radiolabeled moiety had significant influence on the binding capacity of the conjugate to the receptor and on its internalization properties. However, the biodistribution of the conjugates in OVCAR-3 ovarian tumor-bearing mice indicated no significant tumor localization ($\sim 0.5\%$) of radiolabeled compounds. The results showed a very low level of GnRH-R expression on tumor cell surfaces, suggesting that the GnRH-R system is not a suitable target for peptide-receptor imaging using radiolabeled GnRH analogues. This is in contrast to the effective tumor growth inhibition by cytotoxic GnRH derivatives. The contradiction might be explained by the observations that GnRH-R protein is inefficiently processed by the cells under physiological conditions and it is retained at the site of production [53]. A proportion of intracellular receptors might traffic to the cell surface during stimulation, thus providing a rationale for the successful application of GnRH analogues for suppressing the tumor growth [54].

Considering these data, one can conclude that GnRH-Rs represent a highly interesting target for pharmacological interventions; however, many open questions have to be answered in the future.

5. Somatostatin receptors as molecular targets for cancer diagnosis and therapy

It has been shown that somatostatin and its analogues can inhibit tumor growth. Their action is mediated by specific,

high-affinity somatostatin receptors located on the plasma membrane of the tumor cells. Up to now, five human somatostatin receptor subtypes ($\text{sst}_1 - \text{sst}_5$) have been cloned and partially characterized [5,9]. The most frequent receptor subtypes on tumor cells are sst_2 and sst_5 . On ligand binding, sst_3 and sst_2 internalize more efficiently than sst_1 or sst_5 . Somatostatin receptors were detected in a low amount in human brain and in many peripheral tissues such as pancreas, gut, thyroid, adrenal and kidney. Therefore, during the application of somatostatin analogues for targeted therapy, the unwanted side effects should be carefully investigated. As both somatostatin-14 and somatostatin-28 bound with high affinity to all receptor subtypes, more selective derivatives to sst_2 and sst_5 were developed for tumor therapy, one of the most efficient compounds being octreotide (OCT, Sandostatin®, Sandoz), a cyclic octapeptide alcohol (D-Phe-c(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-ol) [55].

6. Cytotoxic somatostatin derivatives

In the mid-1980s, similarly to GnRH derivatives, intensive studies of new somatostatin analogues were carried out by Schally's group. An analogue of octreotide (H-D-Phe-c(Cys-Tyr-D-Trp-Lys-Val-Cys)-Thr-NH₂, RC-121) [28,56] was found to be efficient, especially for drug targeting. An initial study of a conjugate in which methotrexate was linked to the N terminus of RC-121 indicated the tolerance of the carrier for the amino terminal modifications. Later on, Dox and pyrrolino-Dox (AN-201) were conjugated to RC-121, resulting in AN-162 (6) (Figure 4A) and AN-238 (7) (Figure 4B) derivatives [57]. The conjugates that fully retained the cytotoxicity of the drugs and the receptor binding affinity of the peptide carrier showed strong growth inhibition of various tumors (Table 2) that express sst_2 , sst_3 and sst_5 (breast, renal, ovarian, lung, colorectal, pancreatic tumors) [3,6,58,59]. AN-238, similarly to the AN-207 GnRH analogue, was very effective intravenously at ~ 200 nmol/kg doses corresponding to 0.4 – 0.45 mg/kg. The estimated human doses would be ~ 22.5 mg/50 kg. In mice, AN-238 showed no or low toxicity up to a dose of 400 nmol/kg. The toxic side effects might be related to the release of AN-201 resulting from the cleavage of the ester bond by carboxylesterases. The toxicity occurred mostly on the fast dividing cells, such as gastrointestinal or myeloid cells, resulting in a transient fall in the white blood cell (WBC) count and body weight. As the esterase activity is much lower in humans than in mice, the hematotoxicity of AN-238 is expected to be reduced in patients, as demonstrated in case of the GnRH derivative. In addition, no significant nephrotoxicity was observed under the treatment with AN-238.

In the past few years, it has been shown that AN-238 is efficient on human endometrial carcinomas that have not responded well to chemotherapy [60]. Chemoresistance mediated by membrane transporters, such as multi-drug resistance (MDR-I) glycoprotein, can be avoided by targeted therapy. AN-238 not only induced tumor growth inhibition

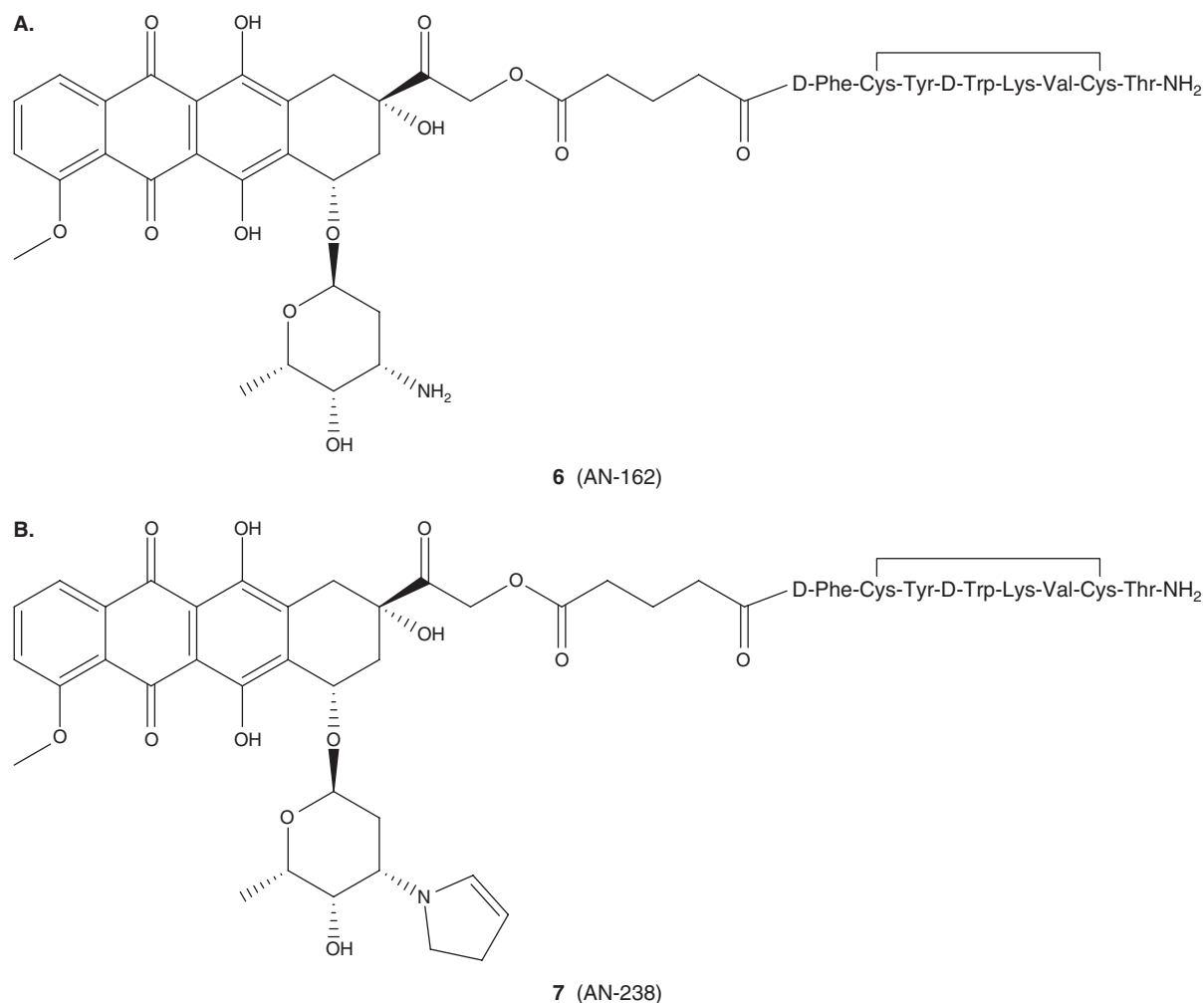


Figure 4. Chemical structures of cytotoxic somatostatin derivatives in which doxorubicin (A) and 2-pyrrolino-doxorubicin (B) were attached via ester bond to an octreotide analogue; ester bond-linked paclitaxel-octreotide (C) and carbamate containing camptothecin-somatostatin derivative conjugate (D).

(50 – 65%) of endometrial carcinomas, but also the expression of MDR-I protein during the treatment was much lower than in case of the treatment with AN-201.

Lung cancer is one of the tumor types that can be treated better by targeted therapy based on somatostatin derivative carriers than by using cytotoxic GnRH derivatives. About 80% of all lung cancers are classified as non-small cell lung cancer (NSCLC). Therefore, the presence of somatostatin receptors in NSCLCs to a greater extent than in normal cells makes them good targets for cytotoxic somatostatin derivatives [61,62]. AN-238 was found to be effective on H69 (SCLC) and H157, H838 (NSCLCs) [63,64]. However, large-scale synthesis of an AN-238 is still in progress. Therefore, AN-162 (Æterna Zentaris, AEZS-124) was used for preclinical studies, and it has recently been investigated for the inhibition of NSCLCs [65]. All studied NSCLCs (A549, H460, H838 and H1299) expressed *sst*₁, *sst*₂ and *sst*₄. Strong expression of mRNA for *sst*₅ was found in A549 and H460 and it was marginal in

H838, whereas *sst*₃ was present in H460 and H1299. A549, H460 and H1299 are resistant to doxorubicin, thus AN-162 was more effective than the free drug on proliferation inhibition *in vitro*. The best results (~ 17% cell viability at 50 µM) were obtained in the case of H460 cells that express all the receptor subtypes. A less significant difference between Dox and AN-162 was observed in the case of H1299 cells, which do not express *sst*₅. In Dox-sensitive H838 cells, both Dox and AN-162 showed significant proliferation inhibition at much lower concentrations. *In vivo* studies showed ~ 70% tumor growth inhibition on H460 xenografted nude mice, and ~ 55% on H1299 xenografted ones. Upregulation of apoptotic genes by the treatment with AN-162 was also indicated. Similar results were obtained on tumor growth inhibition of the MDA-MB-231 estrogen-independent human breast cancer cell line xenografted into nude mice [66].

The treatment of hepatic cancer cells with a combination of two or three cytotoxic conjugates (AN-207 and AN-238 with

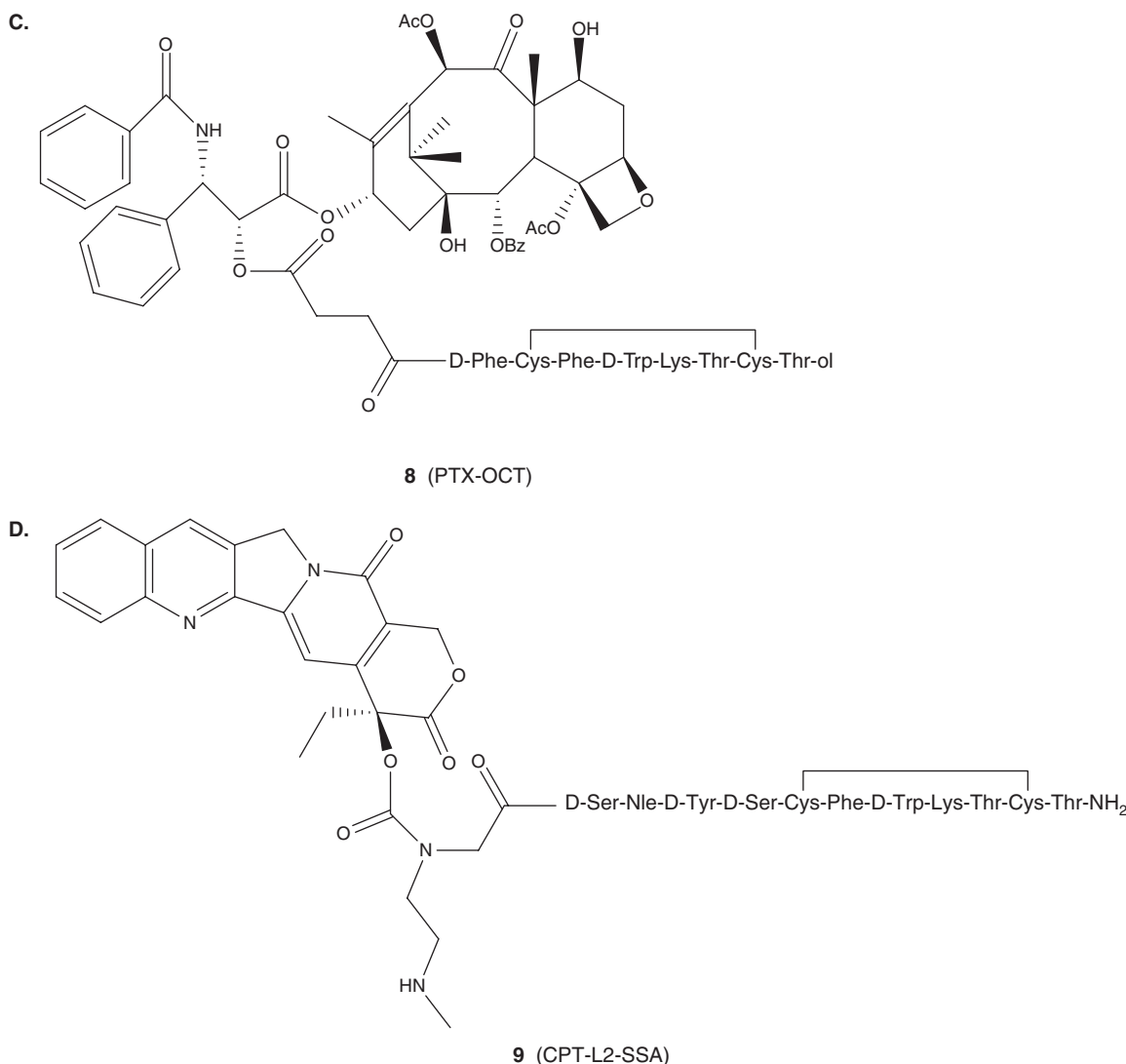


Figure 4. Chemical structures of cytotoxic somatostatin derivatives in which doxorubicin (A) and 2-pyrrolino-doxorubicin (B) were attached via ester bond to an octreotide analogue; ester bond-linked paclitaxel-octreotide (C) and carbamate containing camptothecin-somatostatin derivative conjugate (D). (Continued)

an extra bombesin-Dox conjugate; AN-215) resulted in higher *in vitro* tumor growth inhibition in comparison with their separate administration [32].

It has been shown that Paclitaxel (PTX) has high antitumor activity owing to its ability to promote tubulin assembly into microtubules, but it has a low cell specificity. Therefore, it was modified by succinic acid on its 2'-OH functional group and attached by means of an amide bond to the N terminus of octreotide [67]. This linkage allows the drug release by carboxyl-esterases, regenerating the free 2'-OH, which appears essential for tubulin binding [68]. MCF-7 human breast cancer cell lines expressing *sst*₁, *sst*₂, *sst*₄ and *sst*₅ but not *sst*₃, as well as CHO (Chinese hamster ovary) cells that have a very low level of somatostatin receptor (196 fmol/mg) were used to study the biological activity of the PTX-OCT (8) conjugate (Figure 4C). In the presence of PTX or PTX-OCT (10^{-6} , 10^{-5} and 10^{-4} M),

the percentage of MCF-7 cells containing tubulin bundles was similar (67, 83 and 92% or 65, 81 and 93%, respectively). However, on CHO cells, only PTX was significantly active. A large amount of octreotide could antagonize the cellular uptake of the conjugate but not of PTX, suggesting the difference between the cellular uptake mechanisms of the compounds (receptor-mediated versus passive diffusion). The viability of the MCF-7 cells incubated for 24 h with the conjugate or with the free PTX was similar (46 ± 2.7 and $41 \pm 3.3\%$, respectively). These results indicate that PTX-OCT conjugate is highly selective to the cells containing somatostatin receptors in comparison with the cells that express low levels of the receptors.

Recently, two PTX molecules were attached to the Lys-modified octreotide (2PTX-OCT) and the conjugate was tested on human NSCLC tumors [69,70]. Higher *in vitro*

Table 2. *In vivo* and *in vitro* antitumor effect of AN-238 and other cytotoxic somatostatin derivative-conjugates.

Tumor type	Receptor expression in specimens	Cell types that express SSTR	AN-238	Others
Breast	sst2, sst5 sst2, sst5 sst2, sst5 sst2a, sst4, sst1, sst5	MX-1 MDA-MB-231 MCF-7-MIII MCF-7	XXXXX* XXX XXX n.d.	XXXX (AN-162) PTX-OCT ~ PTX
Ovarian	sst2a, sst3, sst5	UCI-107 OVCAR-3 ES-2	XXX XXX XX	
Endometrial	sst2a, sst5 sst2a, sst5 sst2a, sst5	HEC-1A RL-95-2 AN3CA	XXX XXX XXX	
Prostate	sst2a, sst5 sst2a, sst5 sst2a, sst5 sst2a, sst5	MDA-PCa-2b C4-2 DU-145 PC-3 R-3327-AT-1	XXX (PSA) [§] XXX (PSA) XXXX XXXX XXXX	
Colon	sst2, sst5 sst5 sst2, sst5, sst3	HCT-15 HT-29 HCT-116	XXXX XXXX XXXX	
Renal	sst2a sst5	SW-839 786-0	XXX XXXX	
Melanoma	sst1-sst5	MRI-H255 MRI-H187	XXX XXX	
Lung	sst2, (sst5) sst2a, (sst5) sst5, sst3, sst1, (sst2) (sst2a) sst1, sst2a, sst3, (sst5) sst2a, sst4, sst1, sst5 sst2a, (sst5) sst2 sst2a, sst5, sst3, sst1 sst2a, sst4, sst1, sst3	H838 (NSCLC) H157 (NSCLC) H727 H720 KRJ-I A549 (NSCLC) H69 (SCLC) H345 (SCLC) H460 (NSCLC) H1299 (NSCLC)	XXX XXXX AN-238 < AN-201 AN-238 < AN-201 AN-238 = AN-201 n.d. XXX AN-238 < AN-201 n.d. n.d.	XXX (2Ptx-OCT) AN-162 < Dox [‡] CPT-L1(L2)-SSA AN-162 < Dox CPT-L1(L2)-SSA XXX (AN-162) XXX (AN-162)
Gastric	sst2a, sst5, sst3 sst2a, sst5, (sst3) sst2a, sst5, (sst3)	AGS Hs 746T NCI-N87 MKN-45	XXX XXXX XXX AN-238 < AN-201	AN-162 < Dox
Pancreatic	sst3, sst5 sst3, (sst2), (sst5) sst3, (sst2), (sst5) sst3, sst2, (sst5) sst2, sst5 sst3, sst2, (sst5)	SW-1990 Panc-1 Capan-1 Capan-2 CFPAC-1 Mia-PaCa-2	XXXX XXXX XXXX XXX XXXX XX	
Non-Hodgkin's lymphoma	sst1-sst5 sst1-sst5	RL NL	XXXX XXXX	
Hepatocellular	sst2, sst3, sst5 sst2, sst3, sst5 sst2, sst3, sst5	SK-Hep-1 HepG2 Hep3B	XXXX AN-238 > AN-201 AN-238 > AN-201	
Brain	sst2 sst2 sst2	IMR-32 neuroblastoma U-118MG glioblastoma U87-MG glioblastoma	n.d. XX XXXXX	CPT-L1(L2)-SSA [¶] < XX (AN-162)

**In vivo* studies: XXXXX: regression; XXXX: > 70% inhibition; XXX: 50 – 70% inhibition; XX: 25 – 50% inhibition; n.d.: not determined.

[‡]AN-162 > Dox represents the comparison of the *in vitro* antitumor effect of free drug and its GnRH derivative conjugate.

[§]PSA inh.: not the tumor volume, but PSA level was determined as a measure of the inhibitory effect.

[¶]CPT was more active than CPT-L1-SSA and CPT-L2-SSA.

The effects are not absolutely comparable because of different doses and treatment schedules used.

and *in vivo* antitumor activity of 2PTX-OCT than that of PTX-OCT and free PTX was clearly demonstrated. Furthermore, its toxicity indicated by the white blood cell count was significantly lower than that of the free PTX. The antitumor effect of 2PTX-OCT (3×150 nmol/kg) resulted in the same inhibition on H838 xenografted nude mice (60.7%) as observed by Schally's group using AN-238 (~ 60%) [64].

Fuselier *et al.* conjugated camptothecin as a cytotoxic agent to a somatostatin derivative D-Ser-Nle-D-Tyr-D-Ser-c(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-NH₂. The N-terminal linking motif allowed the attachment of large groups without significant loss of receptor recognition of the cyclic somatostatin analogue [71]. CPT, which has antitumor activity by inhibiting the topoisomerase I, contains a hydroxyl group as a conjugation site, similarly to Dox and PTX. However, in this study a carbamate was applied as a linker because of its high stability in plasma compared with the commonly used ester-type linkage. The idea was that the cytotoxic agent should be masked until the internalization of the conjugate. Basic conditions or enzymatic attack result in the nucleophile-assisted release of the free camptothecin. Carbamates are metabolized by P450 enzyme that is upregulated in human cancer cells [72,73]. The half-life of the prepared compounds was between 18 and 106 h in rat serum, depending on the type of carbamate linker. The highest cytotoxic activity on human neuroblastoma IMR-32 cells was obtained in the case of CPT-*N*-methyl-aminoethyl-Gly-D-Ser-Nle-D-Tyr-D-Ser-c(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-NH₂ (CPT-L1-SSA) (9) (Figure 4D) (IC₅₀ = 54.2 ± 6 nM). Furthermore, the antitumor activity of the conjugate was evaluated on small cell lung cancer cells [74]. The conjugate bound to the NCI-H69 homogenate one order of magnitude better than its non-methylated version (CPT-aminoethyl-Gly-D-Ser-Nle-D-Tyr-D-Ser-c(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-NH₂ [CPT-L2-SSA]). Significant *in vitro* cytotoxicity of the conjugate at 0.3 – 1 μ M concentration was obtained on NCI-H69 and NCI-H345 cell lines that have mRNA for sst₂ receptors.

CPT was also attached to the same somatostatin analogue via a cleavable carbamate group but using a different peptide linker sequence (D-Lys-D-Tyr-Lys-D-Tyr-D-Lys) [75]. This conjugate retained the potent biological activity and had comparatively low toxicity [76]. Its efficiency against PC-3 prostate cancer cell invasion was also demonstrated [77]. The CPT-somatostatin vector conjugate inhibited the expression of MMP-2 and MMP-9, but not the expression of MMP-3 and MMP-10 (MMP: matrix metalloproteinase) in PC-3 tumor cells. MMP-2 and MMP-9 have higher expression levels in tumor cells than in normal tissues and their expression correlates with the metastatic potential of the tumor cells [78]. CPT and the conjugate, but not the somatostatin analogue, inhibited the expression of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins at cell surfaces, tumor progression and metastasis, leading to the conclusion that CPT was responsible for this effect.

All these data show that the conjugation of CPT to somatostatin analogues increases the water solubility of

CPT, lowers the toxicity and increases the selectivity. The specific antitumor and antiangiogenic effects depend on the tumor cells that overexpressed somatostatin receptors.

7. Somatostatin-based boron neutron capture therapy

Boron neutron capture therapy (BNCT) is based on the nuclear capture and fission reactions that occur when non-radioactive ¹⁰B is irradiated with low energy (0.025 eV) thermal neutrons to produce ¹¹B in an unstable form, which undergoes instantaneous nuclear fission to produce α -particles and recoiling ⁷Li nuclei. These high linear energy transfer particles have a range of 5–9 μ m, thereby restricting their destructive effects to only those cells containing ¹⁰B. If ¹⁰B could be accumulated selectively in tumor tissues, which subsequently could be irradiated with thermal neutrons, the nuclear reaction products would specifically destroy the tumor DNA. The presence of 10⁹ (1.67×10^{-15} mole) boron atoms per cell is generally required to achieve an antitumor effect. The target cells that efficiently bind the somatostatin conjugates contain several hundred femtomols (~ 10⁻⁹ – 10⁻¹⁰ mole) of receptor per milligram membrane protein (~ 10⁷ cells) that might allow the uptake of the required amount of *closo*-borane cluster derivative for efficient BNCT. For this purpose, [Tyr³]-octreotate (D-Phe-c(Cys-Tyr-D-Trp-Lys-Thr-Cys)-Thr-OH) coupled with a borane cluster was prepared and functionally studied [79,80]. [Tyr³]-octreotate is an octreotide analogue with carboxyl group at the C terminus and Tyr in position 3 instead of Phe, which shows higher binding affinity and internalization rate [5,81]. A correlation was found between the binding affinity and the length of the inserted spacer between the borane cluster (10 boron atoms) and the octreotate [80]. However, the attachment of a higher number of *closo*-borane clusters (20 boron atoms altogether) in the conjugate had a negative influence on the affinity of the peptide. The binding affinity of the best compound (10) (Figure 5A) was 2 – 3 nM (IC₅₀) on sst₂, but also some binding was observed on sst₃, sst₄ and sst₅ receptor subtypes. This range was similar to that observed in the case of radioligand-labeled somatostatin derivatives. Results of *in vivo* studies have not been published yet; however, the promising *in vitro* binding affinity of the conjugates to the somatostatin receptor-containing cells (Chinese hamster ovary carcinoma cells [CHO-K1] and Chinese hamster fibroblasts [CCL39]) may open a new direction for BNCT.

8. Radiolabeled somatostatin derivatives

Targeted radiotherapy of tumors overexpressing somatostatin receptors was shown to be a promising cancer therapeutic approach [5,9]. Although ¹¹¹In-DTPA-octreotide (OctreoScan®, Mallinckrodt Medical) has become the most widely used tracer for somatostatin receptor scintigraphy [82,83], in the case of radiotherapy ⁹⁰Y-DOTA-Tyr³-octreotide (⁹⁰Y-DOTATOC, 11) (Figure 5B) has been the most frequently

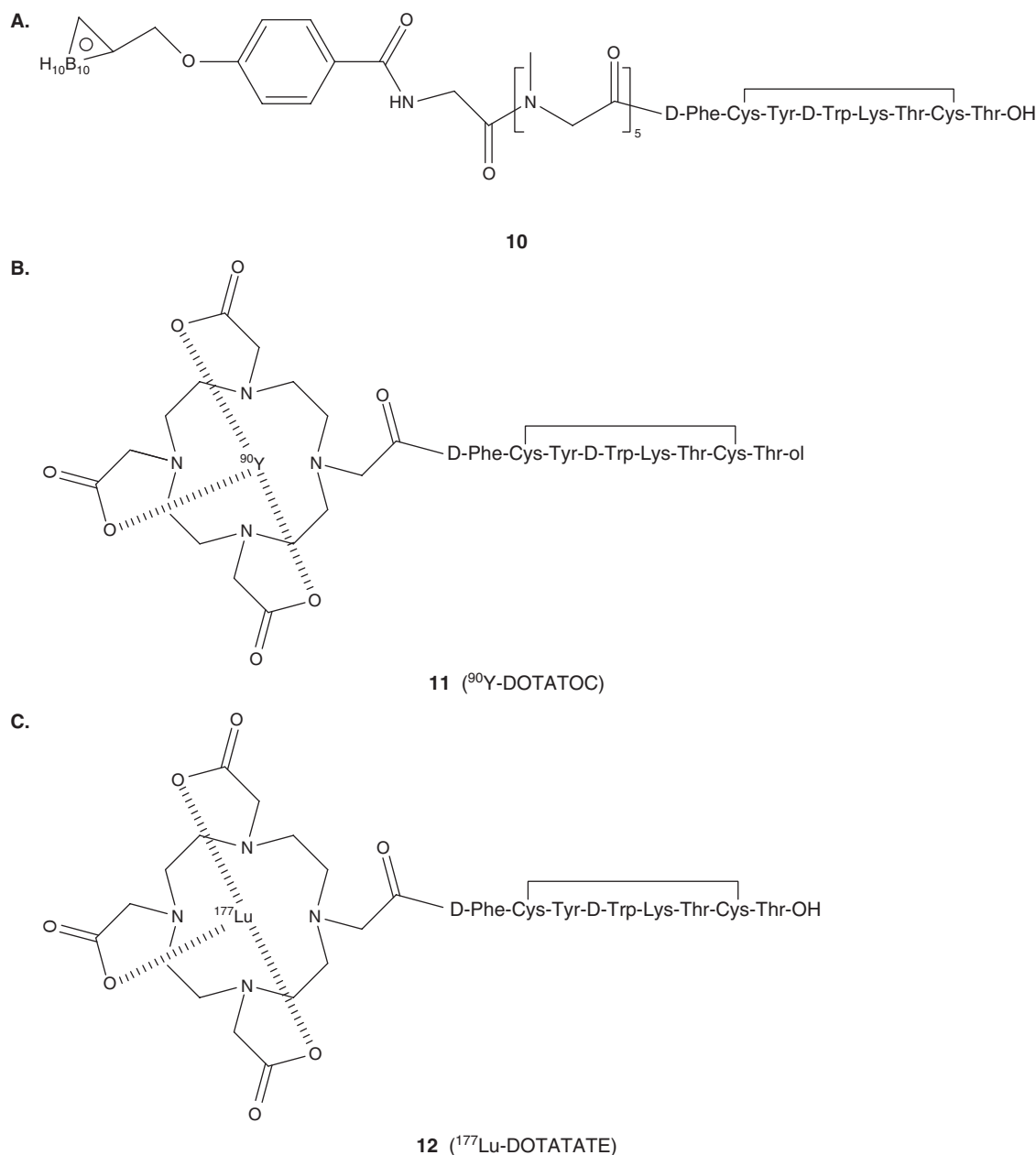


Figure 5. Chemical structures of a somatostatin conjugate for boron neutron capture therapy (A) and of radiolabeled somatostatin derivatives: ⁹⁰Y-DOTA-Tyr³-octreotide (B) and ¹⁷⁷Lu-DOTA-Tyr³-octreotide (C).

used clinically for >15 years [84,85]. In the last decade, numerous compounds were developed in order to increase the receptor selectivity and/or bioactivity of the radiolabeled somatostatin derivatives. The modifications were carried out on the peptide sequence or by changing the radionuclides and/or chelator. Insertion of Ga into DOTATOC markedly improved the sst₂ binding affinity and the *in vivo* tumor imaging [86-88]. Recent data also showed the high efficiency of ^{99m}Tc-EDDA/HYNIC-TOC for radioimaging (EDDA: ethylenediamine-*N,N'*-diacetic acid, HYNIC: 6-hydrazinopyridine-3-carboxylic acid) [89-91]. The treatment

of neuroendocrine gastroenteropancreatic tumors with ¹⁷⁷Lu-DOTA-Tyr³-octreotate (¹⁷⁷Lu-DOTATATE, 12) (Figure 5C), which had high affinity to sst₂, resulted in complete or partial tumor remission in 30% of patients in comparison with 10 – 30% in the case of ⁹⁰Y-DOTATOC treatment [92]. ¹⁷⁷Lu-DOTATATE also showed lower and mostly transient side effects (e.g., nephrotoxicity) and milder bone marrow suppression than ⁹⁰Y-DOTATOC. However, owing to the higher adsorbed dose but the lower tissue penetration range of ¹⁷⁷Lu than of ⁹⁰Y, the ¹⁷⁷Lu-DOTATATE may be more effective for smaller tumors. The combination of

the two compounds might be also beneficial [92,93]. Incorporation of multiple DOTA chelators into the [Tyr³]-octreotate was described as a possible way to increase the efficacy [94]. Recently, it has been shown that the gemcitabine pretreatment upregulated the somatostatin receptor expression and acted as a radiosensitizer. The uptake of ¹⁷⁷Lu-DOTATOC was 1.5 – 3 times greater than that of untreated control cells [95]. Furthermore, the replacement of β -emitting ¹⁷⁷Lu (E_{\max} = 0.5 MeV) in DOTATOC by α -emitting ²¹³Bi (E_{\max} = 5.87 MeV, half-life 45.6 min) increased the effectivity 3.4 times [96]. The short-distance energy deposition of α -ray (~ 0.1 mm) might be very active for inhibiting the tumor growth without side effects in the case of efficient tumor localization. Moreover, this high energy radiation depresses enzymatic DNA repair mechanisms.

The usefulness of somatostatin antagonists for tumor targeting has been demonstrated in the last few years. Somatostatin receptor-selective antagonists did not trigger receptor internalization, yet they were excellent tumor markers [97,98]. Antagonists are more hydrophobic and chemically more stable than agonists, resulting in longer duration of action and possible stabilization in the lipid-rich environment of the receptors. The strong receptor–ligand interaction and the slow internalization in the case of antagonists may provide a longer-lasting accumulation of the radioligand-labeled antagonist conjugate on tumor cells. A series of highly sst₂-selective somatostatin antagonists for radiotargeting were prepared [99]. The two most promising compounds were DOTA-*p*NO₂Phe-c[D-Cys-Tyr-D-Aph(Cbm)-Lys-Thr-Cys]-D-Tyr-NH₂ and DOTA-Cpa-c[D-Cys-Aph(Hor)-D-Aph(Cbm)-Lys-Thr-Cys]-D-Tyr-NH₂ (Cpa: 4-Cl-phenylalanine; Aph: 4-aminophenylalanine; Cbm: carbamoyl; Hor: L-hydroorotyl) with high binding affinities (IC_{50} < 1 nM). These results suggest that somatostatin antagonists as targeting moieties are suitable for radiotherapy.

9. Conclusion

Tumor targeting with hormone peptides provides a basis for the development of various cancer diagnostic and therapeutic approaches (targeted chemotherapy, targeted radiotherapy, photodynamic therapy and BNCT). The increased selectivity and decreased peripheral toxicity of such systems have already been demonstrated on different types of tumor. Moreover, increased solubility and bioavailability can be obtained by the application of these conjugates. Both somatostatin- and GnRH-containing conjugates are effective for targeting antineoplastic agents; however, the advantages of somatostatin analogues in targeted radiotherapy have been presented clearly. The first most active anticancer drug–peptide hormone conjugates are now in preclinical or clinical trials. However, it has to be taken into account that the side effects are mostly related to the low stability in serum of peptide drug conjugates, which leads to early drug release and consequently to ‘nonspecific’ toxicity. Furthermore, the presence of

hormone receptors (especially somatostatin receptors) on normal cells might prevent the clinical application of some compounds. Therefore, the application of targeting moieties with increased specificity for the peptide receptors expressed on cancer cells and the application of linkers/spacers between the peptide and the drug that are stable in serum might improve the selectivity and efficacy of hormone–drug conjugates in targeted chemotherapy.

10. Expert opinion

Targeted chemotherapy represents a promising approach for the treatment of cancer. One possibility to enhance the selectivity and to decrease the peripheral toxicity of the well-known antineoplastic agents as well as radionuclides is the attachment to hormone peptides that bind to their receptors overexpressed on tumor cells. However, only a few of these hormone peptides (GnRH and somatostatin) inhibit the tumor growth by receptor-mediated signal transduction pathway, the others stimulate the cell proliferation. Although the hormone peptide–drug conjugates are used at concentrations lower than the hormone dose required for tumor growth inhibition, the peptides with inhibitory effect should be preferentially used as targeting moieties for the preparation of drug delivery systems. Furthermore, the application of agonist derivatives instead of antagonists (preferred in the case of stimulatory peptides) might have a further benefit of faster receptor–ligand internalization. The internalization may be more important in the case of delivery of antineoplastic agents that have an intracellular site of action, whereas the intracellular delivery of radionuclides might not be necessary, especially in the case of β -emitters. However, the internalization of somatostatin agonist-bound radionuclides may lead to longer residence times of radionuclides in the tumor cells.

There are many promising data that confirm the efficiency of targeted therapy based on hormone peptides as targeting moieties. However, their receptors on cancer cells are limited, and their distribution on the cell surface might be heterogeneous. Furthermore, some receptors desensitize under continuous exposure to the hormone conjugate treatment. Research in the future should be focused on increasing the efficacy of the targeted therapeutic approaches. Several strategies to be pursued are the following.

(1) The combination of conjugates containing different targeting moieties. In this case different hormone receptors can be targeted. The same or different anticancer drugs can be attached to the selected hormone peptides. The latter combination might have the benefit that the drugs have different sites of action, resulting in high toxic effects on tumor cells.

(2) The development of conjugates in which two antineoplastic agents are coupled to a single hormone peptide. In this case, there should be an appropriate distance between the drugs and the targeting peptide in order to avoid the significant decrease of receptor binding affinity of the

hormone. The number of drug molecules in a conjugate could be increased by the aid of multifunctional linkers.

(3) The combination of radionuclides and anticancer drugs in one conjugate or physical mixing of two different conjugates with the aim of combining radiotherapy and chemotherapy.

(4) The development of targeted nanoparticles containing encapsulated drugs by modifying their surface with hormone peptides.

It has been shown that the most efficient hormone–drug conjugates prepared so far have fairly low stability in serum. Therefore, the linkage and/or spacer between the components should be optimized further. The improvement of the serum stability of the conjugates as well as the efficient drug release might increase the therapeutic window and the efficacy of the drug targeting.

Further development of carriers, especially in the case of GnRH, is required in order to prepare more potent compounds. Peptide derivatives with low endocrine but high antitumor activity are good candidates.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. World Health Organization (WHO). Available from: <http://www.who.int/cancer/en/>, Fact sheet Nr. 297, February 2009
2. Frei E III, Antman KH. Combination chemotherapy, dose, and schedule. In: Holland JR, Bast RC Jr, Morton DL, Frei E III, Kufe DW, Weichselbaum RR, editors, *Cancer medicine*. 4th Edition. Baltimore: Williams and Wilkins; 1997. p. 817-37
3. Schally AV, Nagy A. Chemotherapy targeted to cancers through tumoral hormone receptors. *Trends Endocrinol Metab* 2004;15:300-10
4. Magrath T. Targeted approaches to cancer therapy. *Int J Cancer* 1994;56:163-6
5. Reubi JC. Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr Rev* 2003;24:389-427
- A comprehensive review of receptors of hormone/neurotransmitter peptides as molecular targets for cancer diagnosis and therapy.
6. Schally AV, Nagy A. Cancer chemotherapy based on targeting of cytotoxic peptide conjugates to their receptors on tumors. *Eur J Endocrinol* 1999;141:1-14

7. Moody TW, Mantey SA, Pradhan TK, et al. Development of high affinity camptothecin-bombesin conjugates that have targeted cytotoxicity for bombesin receptor-containing tumor cells. *J Biol Chem* 2004;279:23580-9
8. Moody TW, Sun L-CH, Mantey SA. In vitro and in vivo antitumor effects of cytotoxic camptothecin-bombesin conjugates are mediated by specific interaction with cellular bombesin receptors. *J Pharm Exp Ther* 2006;318:1265-72
9. Okarvi SM. Peptide based radiopharmaceuticals and cytotoxic conjugates: potential tools against cancer. *Cancer Treat Rev* 2008;34:13-26
- A comprehensive overview of peptide-based radiopharmaceuticals and cytotoxic conjugates, as well as their potential applications in cancer diagnosis and therapy.
10. Ulbrich K, Etrych T, Chytil P, et al. HPMA copolymers with pH-controlled release of doxorubicin: in vitro cytotoxicity and in vivo antitumor activity. *J Control Release* 2003;87:33-47
11. Shiose Y, Ochi Y, Kuga H, et al. Relationship between drug release of DE-310, macromolecular prodrug of DX-8951f, and cathepsins activity ins tumors. *Biol Pharm Bull* 2007;30:2365-70
12. Neill JD, Musgrove LC, Duck LW. Newly recognized GnRH receptors: function and relative role. *Trends Endocrinol Metab* 2004;15:383-92
13. Shedgley KR, Finch AR, Caunt CJ, McArdle CA. Intracellular gonadotropin-releasing hormone receptors in breast cancer and gonadotrope lineage cells. *J Endocrinol* 2006;191:625-36
14. Millar RP, Lowe WN, Conklin D, et al. A novel mammalian receptor for the evolutionarily conserved type II GnRH. *Proc Natl Acad Sci USA* 2001;98:9636-41
15. Neill JD, Duck LW, Sellers JC, Musgrove LC. A gonadotropin-releasing hormone (GnRH) receptor specific for GnRH-II in primates. *Biochem Biophys Res Commun* 2001;282:1012-8
16. Caunt CJ, Finch AR, Sedgley KR, et al. Arrestin-mediated ERK activation by gonadotropin-releasing hormone receptors: receptor-specific activation mechanisms and compartmentalization. *J Biol Chem* 2006;281:2701-10
17. Gründker C, Günthert AR, Millar RP, Emons G. Expression of gonadotropin-releasing hormone II (GnRH-II) receptor in human endometrial and ovarian cancer cells and effects of GnRH-II on tumor cell proliferation. *J Clin Endocrinol Metab* 2002;87:1427-30
- Important data about the expression of GnRH-IIR on human cancer cells.

Acknowledgments

The authors thank S Bajusz for critically reading the manuscript and for his advice.

Declaration of interest

The manuscript represents original work and has not been published previously or simultaneously submitted elsewhere for publication. The manuscript has been read and approved by both authors. The authors state no conflict of interest and have received no payment in preparation of this manuscript.

18. Engel JB, Schally AV. Drug Insight: clinical use of agonists and antagonists of luteinizing-hormone-releasing hormone. *Nat Clin Endocrinol Metab* 2007;3:157-67
19. Gründker C, Schlotawa L, Viereck V, et al. Antiproliferative effects of the GnRH antagonist cetrorelix and of GnRH-II on human endometrial and ovarian cancer cells are not mediated through the GnRH type I receptor. *Eur J Endocrinol* 2004;151:141-9
20. Fister S, Günther AR, Emons G, Gründker C. Gonadotropin-releasing hormone type II antagonists induce apoptotic cell death in human endometrial and ovarian cancer cells in vitro and in vivo. *Cancer Res* 2007;67:1750-6
21. Nagy A, Schally AV. Targeting of cytotoxic luteinizing hormone-releasing hormone analogs to breast, ovarian, endometrial, and prostate cancers. *Biol Reprod* 2005;73:851-9
- **A comprehensive overview of antitumor effect of doxorubicin-GnRH conjugates on different tumor types.**
22. Keller G, Schally AV, Gaiser T, et al. Human malignant melanomas express receptors for luteinizing hormone releasing hormone allowing targeted therapy with cytotoxic luteinizing hormone releasing hormone analogue. *Cancer Res* 2005;65:5857-63
23. Keller G, Schally AV, Gaiser T, et al. Receptors for luteinizing hormone releasing hormone (LHRH) expressed in human non-Hodgkin's lymphomas can be targeted for therapy with cytotoxic LHRH analogue AN-207. *Eur J Cancer* 2005;41:2196-202
24. Nagy A, Schally AV. Cytotoxic analogs of luteinizing hormone-releasing hormone (LHRH): a new approach to targeted chemotherapy. *Drugs Future* 2002;27:359-70
25. Mező G, Manea M, Szabó I, et al. New derivatives of GnRH as potential anticancer therapeutic agents. *Curr Med Chem* 2008;15:2366-79
26. Nagy A, Schally AV, Armatis P, et al. Cytotoxic analog luteinizing hormone-releasing hormone containing doxorubicin or 2-pyrrolinodoxorubicin, a derivative 500-1000 times more potent. *Proc Natl Acad Sci USA* 1996;93:7269-73
- **First indication of the increased antitumor effect of 2-pyrrolinodoxorubicin and AN-207 conjugate (compared with doxorubicin and AN-152 conjugate).**
27. Chatzistamou I, Schally AV, Nagy A, et al. Effective treatment of metastatic MDA-MB-435 human estrogen-independent breast carcinomas with a targeted cytotoxic analogue of luteinizing hormone-releasing hormone AN-207. *Clin Cancer Res* 2000;6:4158-65
28. Schally AV. New approaches to the therapy of various tumors based on peptide analogues. *Horm Metab Res* 2008;40:315-22
29. Kovács M, Szepesházi K, Schally AV. Endocrine and antineoplastic effects of antagonistic and cytotoxic analogs of luteinizing hormone-releasing hormone. In: Kovács M, Merchenhalter I, editors. *Neuropeptides and peptide analogs*. Research Signpost, Kerala, India; 2009. p. 33-57
30. Keller G, Schally AV, Gaiser T, et al. Receptors for luteinizing hormone releasing hormone expressed on human renal cell carcinomas can be used for targeted chemotherapy with cytotoxic luteinizing hormone releasing hormone analogues. *Clin Cancer Res* 2005;11:5549-57
31. Szepesházi K, Schally AV, Halmos G. LH-RH receptors in human colorectal cancers: unexpected molecular targets for experimental therapy. *Int J Oncol* 2007;30:1485-92
32. Szepesházi K, Schally AV, Treszl A, et al. Therapy of experimental hepatic cancers with cytotoxic peptide analogs targeted to receptors for luteinizing hormone-releasing hormone, somatostatin or bombesin. *Anticancer Drugs* 2008;19:349-58
- **First report on the possible benefits of combination therapy using different hormones as targeting moieties.**
33. Günther A, Gründker C, et al. Internalization of cytotoxic analog AN-152 of luteinizing hormone-releasing hormone induces apoptosis in human endometrial and ovarian cancer cell lines independent of multidrug resistance-1 (MDR-1) system. *Am J Obstet Gynecol* 2004;191:1164-72
34. Emons G, Sindermann H, Engel J, et al. Luteinizing hormone-releasing hormone receptor-targeted chemotherapy using AN-152. *Neuroendocrinol* 2009;90:15-8
35. Nagy A, Plonowski A, Schally AV. Stability of cytotoxic luteinizing hormone-releasing hormone conjugate (AN-152) containing doxorubicin-14-O-hemiglutarate in mouse and human serum in vitro: implications for the preclinical studies. *Proc Natl Acad Sci USA* 2000;97:829-34
36. Kovács M, Schally AV, Nagy A, et al. Recovery of pituitary function after treatment with a targeted cytotoxic analog of luteinizing hormone-releasing hormone. *Proc Natl Acad Sci USA* 1997;94:1420-5
37. Schally AV, Halmos G, Rékasi Z, Arencibia-Jimenez JM. The actions of LH-RH agonists and antagonists and cytotoxic analogs on the LH-RH receptors on the pituitary and tumors. In: P Devroey, editor. *Infertility and reproductive medicine clinica of North America*. Philadelphia: Saunders. 2001;12:17-44
38. Sower SA, Chiang Y-C, Lovas S, Conlon JM. Primary structure and biological activity of a third gonadotropin-releasing hormone from lamprey brain. *Endocrinol* 1993;132:1125-31
39. Lovas S, Pályi I, Vincze B, et al. Direct anticancer activity of gonadotropin-releasing hormone-III. *J Pept Res* 1998;52:384-9
40. Kovács M, Seprődi J, Koppán M, et al. Lamprey gonadotropin hormone-releasing hormone-III has no selective follicle-stimulating hormone-releasing effect in rats. *J Neuroendocrinol* 2002;14:1-14
41. Szabó I, Manea M, Orbán E, et al. Development of an oxime bond containing daunorubicin-gonadotropin-releasing hormone-III conjugate as a potential anticancer drug. *Bioconj Chem* 2009;20:656-65
- **First data showing the *in vivo* antitumor activity of an oxime bond-linked daunorubicin-GnRH-III conjugate.**
42. Kovács M, Vincze B, Horváth JE, Seprődi J. Structure-activity study on the LH- and FSH-releasing and anticancer effects of gonadotropin-releasing hormone (GnRH)-III analogs. *Peptides* 2007;28:821-9
- **Important data regarding the selective antitumor activity of GnRH-III.**
43. Sibrian-Vazquez M, Jensen TJ, Vicente MG. Synthesis, characterization,

- and metabolic stability of porphyrin-peptide conjugates bearing bifunctional signaling sequences
J Med Chem 2008;51:2915-23
44. Bai KB, Láng O, Orbán E, et al. Design, synthesis, and in vitro activity of novel drug delivery systems containing tuftsin derivatives and methotrexate. *Bioconjug Chem* 2008;19:2260-9
 45. Rahimpour S, Ben-Aroya N, Ziv K, et al. Receptor-mediated targeting of a photosensitizer by its conjugation to gonadotropin-releasing hormone analogues. *J Med Chem* 2003;46:3965-74
 46. Dharap SS, Qui B, Williams GC, et al. Molecular targeting of drug delivery systems to ovarian cancer by BH3 and LHRH peptides. *J Control Release* 2003;91:61-73
 47. Dharap SS, Wang Y, Chandna P, et al. Tumor-specific targeting of an anticancer drug delivery system by LHRH peptide. *Proc Natl Acad Sci USA* 2005;102:12962-7
 48. Khandare JJ, Chandna P, Wang Y, et al. Novel polymeric prodrug with multivalent components for cancer therapy. *J Pharm Exp Ther* 2006;317:929-37
 49. Chandna P, Saad M, Wang Y, et al. Targeted proapoptotic anticancer drug delivery system. *Mol Pharm* 2007;4:668-78
 - **First indication of a four-component drug delivery system containing GnRH as a targeting moiety.**
 50. Dharap SS, Minko T. Targeted proapoptotic LHRH-BH3 peptide. *Pharm Res* 2003;20:889-96
 51. Barda Y, Cohen N, Lev V, et al. Backbone metal cyclization: novel ^{99m}Tc labeled GnRH analog as potential SPECT molecular imaging agent in cancer. *Nucl Med Biol* 2004;31:921-33
 52. Schottelius M, Berger S, Poethko T, et al. Development of novel ⁶⁸Ga- and ¹⁸F-labeled GnRH-I analogues with high GnRH-targeting efficiency. *Bioconjug Chem* 2008;19:1256-68
 53. Pawson AJ, Faccenda E, Maudsley S, et al. Mammalian type I Gonadotropin-releasing hormone receptors undergo slow, constitutive, agonist-independent internalization. *Endocrinology* 2008;149:1415-22
 54. Montagnani-Marelli M, Moretti RM, Januszkiewicz-Caulier J, et al. Gonadotropin-releasing hormone (GnRH) receptor sin tumors: a new rationale for the therapeutical application of GnRH analogs in cancer patients? *Curr Cancer Drug Targets* 2006;6:257-69
 55. Bauer W, Briner U, Doepfner W, et al. SMS-201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci* 1982;31:1133-40
 56. Cai R-Z, Szőke B, Lu R, et al. Synthesis and biological activity of highly potent octapeptide analogs of somatostatin. *Proc Natl Acad Sci USA* 1986;83:1896-900
 57. Nagy A, Schally AV, Halmos G, et al. Synthesis and biological evaluation of cytotoxic analogs of somatostatin containing doxorubicin or its intensely potent derivative 2-pyrrolinodoxorubicin. *Proc Natl Acad Sci USA* 1998;95:1794-99
 58. Schally AV, Comaru-Schally M, Nagy A, et al. Hypothalamic hormones and cancer. *Front Neuroendocrinol* 2001;22:248-91
 59. Schally AV, Nagy A. New approaches to treatment of various cancers based on cytotoxic analogs of LHRH, somatostatin and bombesin. *Life Sci* 2003;72:2305-20
 60. Engel JB, Schally AV, Halmos G, et al. Targeted therapy with a cytotoxic somatostatin analog, AN-238, inhibits growth of human experimental endometrial carcinomas expressing multidrug resistance protein MDR-I. *Cancer* 2005;104:1312-21
 61. O'Byrne KJ, Schally AV, Thomas A, et al. Somatostatin, its receptors and analogs, in lung cancer. *Chemotherapy* 2001;47(S):78-108
 62. Menda Y, Kahn D. Somatostatin receptor imaging of non-small cell lung cancer with ^{99m}Tc depreotide. *Semin Nucl Med* 2002;32:92-6
 63. Kiaris H, Schally AV, Nagy A, et al. A targeted cytotoxic somatostatin (SST) analog, AN-238, inhibits the growth of H-69 small-cell lung carcinoma (SCLC) and H-157 non-SCLC in nude mice. *Eur J Cancer* 2001;37:620-8
 64. Szereday Z, Schally AV, Szepesházi K, et al. Effective treatment of H838 human non-small cell lung carcinoma with a targeted cytotoxic somatostatin analog, AN-238. *Int J Oncol* 2003;22:1141-6
 65. Treszl A, Schally AV, Seitz S, et al. Inhibition of human non-small cell lung cancers with a targeted cytotoxic somatostatin analog, AN-162. *Peptides* 2009;30:1643-50
 66. Seitz S, Schally AV, Treszl A, et al. Preclinical evaluation of properties of a new targeted cytotoxic somatostatin analog, AN-162 (AEZS-124), and its effects on tumor growth inhibition. *Anticancer Drugs* 2009;20:553-8
 - **First preclinical evaluation data of AN-162 conjugate on human breast cancer cells.**
 67. Huang C-M, Wu Y-T, Chen S-T. Targeting delivery of paclitaxel into tumor cells via somatostatin receptor endocytosis. *Chem Biol* 2000;7:453-61
 68. Nicolau KC, Dai WM, Guy RK. Chemistry and biology of taxol. *Angew Chem Int Ed Engl* 1994;33:15-44
 69. Sun ML, Wei JM, Wang XW, et al. Paclitaxel-octreotide conjugates inhibit growth of human non-small lung cancer cells in vitro. *Exp Oncol* 2007;29:186-91
 70. Shen H, Hu D, Du J, et al. Paclitaxel-octreotide conjugates in tumor growth inhibition of A549 human non-small cell lung cancer xenografted into nude mice. *Eur J Pharmacol* 2008;601:23-9
 71. Fuselier JA, Sun L, Woltering SN, et al. An adjustable release rate linking strategy for cytotoxin-peptide conjugates. *Bioorg Med Chem Lett* 2003;13:799-803
 72. Patterson LH, McKeown SR, Robson T, et al. Antitumour prodrug development using cytochrome P450 (CYP) mediated activation. *Anticancer Drug Des* 1999;14:473-86
 73. Cha SW, Gu HK, Lee KP, et al. Immunotoxicity of ethyl carbamate in female BALB/c mice: role of esterase and cytochrome P450. *Toxicology Lett* 2000;115:173-81
 74. Moody TW, Fuselier J, Coy DH, et al. Camptothecin-somatostatin conjugates inhibit the growth of small cell lung cancer cells. *Peptides* 2005;26:1560-6
 75. Sun L-C, Fuselier JA, Coy DH. Effects of camptothecin conjugated to a somatostatin analog vector on growth of tumor cell lines in culture and related tumor sin rodents. *Drug Deliv* 2004;11:231-8
 - **Application of a carbamate linker for the preparation of a somatostatin derivative-camptothecin conjugate.**

76. Bezerra Y, Fuselier JA, Peyma GA, et al. Study of inhibition effects of antiangiogenic somatostatin camptothecin conjugate on laser-induced choroidal neovascularization in rats. *Retina* 2005;25:345-54
77. Sun L-C, Luo J, Mackey LV, et al. A conjugate of camptothecin and a somatostatin analog against prostate cancer cell invasion via a possible signaling pathway involving PI3K/Akt, alphaVbeta3/alphaVbeta5 and MMP-2/-9. *Cancer Lett* 2007;246:157-66
78. John A, Tuszyński G. The role of matrix metalloproteinases in tumor angiogenesis and tumor metastasis. *Pathol Oncol Res* 2001;7:14-23
79. Mier W, Gabel D, Haberkorn U, Eisenhut M. Conjugation of the closo-borane mercaptoundecahydrododecaborate (BSH) to a tumour selective peptide. *Z Anorg Chem* 2004;630:1258-62
80. Betzel T, Hess T, Waser B, et al. closo-Borane conjugated regulatory peptides retain high biological affinity: Synthesis of closo-borane conjugated Tyr3-octreotate derivatives for BNCT. *Bioconjug Chem* 2008;19:1796-802
- **First indication of a hormone-borane conjugate as a potential therapeutic agent for BNCT.**
81. de Jong M, Breeman WA, Bakker WH, et al. Comparison of (111)In-labeled somatostatin analogues for tumor scintigraphy and radionuclide therapy. *Cancer Res* 1998;58:437-41
82. Raderer M, Kurtaran A, Leimer M, et al. Value of peptide receptor scintigraphy using 123I-vasoactive intestinal peptide and 111In-DTPA-D-Phe1-octreotide in 194 carcinoid patients: Vienna university experience, 1993-1998. *J Clin Oncol* 2000;18:1331-6
83. Rufini V, Calcagini ML, Baum RP. Imaging of neuroendocrine tumors. *Semin Nucl Med* 2006;36:228-47
84. Kwekkeboom D, Krenning EP, de Jong M. Peptide receptor imaging and therapy. *J Nucl Med* 2000;41:1704-13
85. Bodei L, Cremonesi M, Zololi S, et al. Receptor-mediated radionuclide therapy with 90Y-DOTATOC in association with amino acid infusion; a phase 1 study. *Eur J Nucl Med* 2003;30:207-16
86. Reubi JC, Schaer JC, Waser B, et al. Affinity profiles for human somatostatin receptor sst1-sst5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. *Eur J Nucl Med* 2000;27:273-82
87. Henze M, Schumacher J, Hipp P, et al. PET imaging of somatostatin receptors using [68Ga] DOTA-D-Phe1-Tyr3-octreotide: first results in patients with meningiomas. *J Nucl Med* 2001;42:1053-6
88. Koukouraki S, Strauss LG, Georgoulas V, et al. Evaluation of the pharmacokinetics of 68Ga-DOTATOC in patients with metastatic neuroendocrine tumors scheduled for 90Y-DOTATOC therapy. *Eur J Nucl Med Mol Imaging* 2006;33:460-6
89. Storch D, Béhé M, Walter MA, et al. Evaluation of [99mTc/EDDA/HYNIC0] octreotide derivatives compared with [111In-DOTA0,Tyr3,Thr8]octreotide and [111In-DTPA0]octreotide: does tumor or pancreas uptake correlate with the rate of internalization? *J Nucl Med* 2005;46:1561-9
90. Gambini JP, López Lerena JJ, Quagliata A, et al. 99mTc-HYNIC octreotide in neuroblastoma. *Ann Nucl Med* 2008;22:817-9
91. Chen L, Li F, Zhuang H, et al. 99mTc-HYNIC-TOC scintigraphy is superior to 131I-MIBG imaging in the evaluation of extraadrenal pheochromocytoma. *J Nucl Med* 2009;50:397-400
92. Kwekkeboom DJ, Mueller-Brand J, Paganelli G, et al. Overview of results of peptide receptor radionuclide therapy with 3 radiolabeled somatostatin analogs. *J Nucl Med* 2005;46S:62-6
93. De Jong M, Valkema R, Jamar F, et al. Somatostatin receptor-targeted radionuclide therapy of tumors: preclinical and clinical findings. *Semin Nucl Med* 2002;32:133-40
94. Mier W, Graham KAN, Wang Q, et al. Synthesis of peptide conjugated chelator oligomers for endoradiotherapy and MRT imaging. *Tetrahedron Lett* 2004;45:5453-5
95. Nayak TK, Atcher RW, Prossnitz ER, Norenberg JP. Enhancement of somatostatin-receptor-targeted 177Lu-[DOTA0-Tyr3]-octreotide therapy by gemcitabine pretreatment-mediated receptor uptake, up-regulation and cell cycle modulation. *Nucl Med Biol* 2008;35:673-8
96. Nayak TK, Norenberg JP, Anderson TL, et al. Somatostatin-receptor-targeted alpha-emitting 213Bi is therapeutically more effective than beta-emitting 177Lu in human pancreatic adenocarcinoma cells. *Nucl Med Biol* 2007;34:185-93
97. Ginj M, Zhang H, Waser B, et al. Radiolabeled somatostatin receptor antagonists are preferable to agonists for in vivo peptide receptor targeting of tumors. *Proc Natl Acad Sci USA* 2006;103:16436-41
98. Cescato R, Schulz S, Waser B, et al. Internalization of sst2, sst3, and sst5 receptors: effects of somatostatin agonists and antagonists. *J Nucl Med* 2006;47:502-11
- **Important data on somatostatin antagonist derivatives and their potential tumor-targeting applications.**
99. Cescato R, Erchegyi J, Waser B, et al. Design and in vitro characterization of highly sst2-selective somatostatin antagonists suitable for radiotargeting. *J Med Chem* 2008;51:4030-7

Affiliation

Gábor Mező^{†1} & Marilena Manea^{2,3}

[†]Author for correspondence

¹Eötvös Loránd University, Research Group of Peptide Chemistry, Hungarian Academy of Sciences, 1117 Budapest, Pázmány P. stny. 1/A, Hungary
Tel: +36 1 372 2500/1433; Fax: +36 1 372 2620; E-mail: gmezo@elte.hu

²University of Konstanz, Department of Chemistry, Laboratory of Analytical Chemistry and Biopolymer Structure Analysis, 78457 Konstanz, Germany

³University of Konstanz, Zukunftskolleg, 78457 Konstanz, Germany