Asian Journal of Andrology (2015) 17, 925–928

www.asiaandro.com; www.ajandrology.com

Open Access

INVITED RESEARCH HIGHLIGHT

Prostate Cancer

New therapies for relapsed castration-resistant prostate cancer based on peptide analogs of hypothalamic hormones

Andrew V Schally[1,2,3,4,5], Norman L Block[1,2,3], Ferenc G Rick[1,6]

Asian Journal of Andrology (2015) **17**, 925–928; doi: 10.4103/1008-682X.152819; published online: 26 June 2015

It is a pleasure to contribute our presentation at the International Prostate Forum of the Annual Meeting of the American Urological Association (AUA) to this special issue of the *Asian Journal of Andrology*.

We are gratified that the method developed in our laboratories[1] based on agonistic analogs of hypothalamic luteinizing hormone-releasing hormone (LHRH), also called gonadotropin-releasing hormone, which was discovered and characterized by one of us (AVS) in the 1970s,[2–6] has been used since the early 1980s for the treatment of hormone-dependent prostate cancer.[7–11] Since that time our laboratory has been trying to develop therapies for relapsed androgen-independent, castration-resistant prostate cancer (CRPC). These new therapies are also based on various analogs of hypothalamic hormones and are sorely needed when the patients with hormone-dependent prostate cancer undergo loss of effect of androgen deprivation therapy and relapse. New drugs such as abiraterone, enzalutamide (MDV 3100), cabazitaxel, sipuleucel-T, zoledronic acid, and radium-223[12–17] are welcome additions to the oncological-urological armamentarium but after an initial response, resistance to most of these agents appears to develop.

Consequently, in our view, synthetic antagonistic analogs of peptides such as growth hormone-releasing hormone (GHRH), LHRH, bombesin/gastrin releasing peptide (BN/GRP) that inhibit growth factors such as epidermal growth factor, vascular endothelial growth factor, insulin-like growth factor-I, II or analogs with cytotoxic moieties, which can target receptors on tumors, must be developed.[8–10,18–20]

As we know, therapies for metastatic hormone-sensitive prostate cancer, as introduced by Huggins and Hodges[21] and others include: orchiectomy, estrogens, adrenalectomy, corticosteroids, hypophysectomy, and anti-androgens. Each can induce palliation in advanced prostate cancer; however, each has its pros and cons, morbidity and mortality, and limited long-term efficacy. Our laboratory many years ago introduced LHRH agonists, which in chronic application downregulate pituitary LHRH receptors. In turn, this downregulation of LHRH receptors leads to an inhibition of the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), producing a suppression of androgen and estrogen levels in blood.[1,7–10,22] The treatment of patients with prostate cancer has been greatly facilitated by the introduction of sustained delivery systems[10,22] as the administration of microcapsules containing the agonist can be performed only every 3–6 months.

Following elucidation of the effects of LHRH agonists on prostate cancer and recognition of its associated effects, we turned our attention to LHRH antagonists. We and others synthesized antagonists of LHRH that block pituitary receptors for LHRH and produce an immediate cessation of secretion of LH, FSH and sex steroids.[8–10] Early LHRH antagonists had some in edematogenic activities due to histamine release. In the LHRH antagonist, cetrorelix, developed by us, these activities were greatly reduced, and it was possible to carry out clinical studies in patients with prostate cancer and benign prostatic hyperplasia.[8–10,23] Modern LHRH antagonist, degarelix, developed by Ferring, is the current drug of choice in this category.[24–27] Degarelix has no serious adverse effects and avoids the flare phenomenon of LHRH agonists. Degarelix provides fast and effective control of testosterone, prostate specific antigen, LH, and FSH levels. It produces lower FSH levels than leuprolide, eliminates microsurges of testosterone, and leads to fewer cardiovascular and metabolic consequences than the LHRH agonists.[24–27]

Luteinizing hormone-releasing hormone analogs provide effective palliative therapy for patients with advanced prostate carcinomas resulting in objective stable disease, partial remission and occasionally long-term complete remission.[8,9] However, all hormonal therapies aimed at androgen deprivation, including orchiectomy, anti-androgens, LHRH agonists or antagonists, with or without anti-androgens, usually provide remissions of only limited duration.[8,9] Most patients who live long enough with advanced prostatic carcinoma relapse. The prognosis of these patients with androgen-independent prostate cancer is very poor.[8,9] The latest therapeutic drugs for prostate cancer, like abiraterone, extend the survival only by a few months.[12,15,16]

Continuing our studies, it became clear that some effects of LHRH analogs are exerted directly, rather than indirectly, on prostate cancer cells.[8–10,28] We were able to

[1]Veterans Affairs Medical Center and South Florida Veterans Affairs Foundation for Research and Education, Miami, FL 33125, USA; [2]Department of Pathology, University of Miami Miller School of Medicine, Miami, FL 33136, USA; [3]Department of Medicine, Division of Hematology/Oncology, University of Miami Miller School of Medicine, Miami, FL 33136, USA; [4]Department of Medicine, Division of Endocrinology, University of Miami Miller School of Medicine, Miami, FL 33136, USA; [5]Department of Medicine, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL 33136, USA; [6]Department of Urology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL, 33174, USA.

Correspondence: Dr. AV Schally (andrew.schally@va.gov)

*This article is based on a presentation delivered on the International Prostate Forum at the Annual Meeting of the American Urological Association, Orlando, FI, USA, May 18, 2014.

demonstrate the presence of high levels of receptors for LHRH, BN/GRP, and somatostatin, as well as mRNAs for their receptor subtypes, in human prostate cancer specimens.[8–10,28] In experimental studies on human prostate cancer lines xenografted into nude mice, analogs of LHRH,[10] analogs of somatostatin,[29] and analogs of GRP[30] each inhibited prostate tumors growth and *in vitro* reduced their proliferation.[8–10,28] *In vitro* effects demonstrated a direct effect of these analogs on tumors.[10] Following the example with Estracyt (estramustine) consisting of nitrogen mustard coupled to carrier estrogen, we developed cytotoxic analogs of LHRH, somatostatin, and GRP which actively target respective receptors on prostate cancers.[18–20,31–37] Thus, we have synthesized a targeted cytotoxic LHRH analog, denoted AN-152 (commercially AEZS-108, zoptarelin-doxorubicin), which combines a D-Lys-6 LHRH agonist moiety (necessary for binding to LHRH receptors on cancer cell surfaces) with the cytotoxic doxorubicin.[19] This analog delivers doxorubicin to only those cells with LHRH receptors on the cell membrane, thus avoiding cytotoxic side effects in normal cells. We found that after binding to LHRH receptors, AN-152 (AEZS-108) is internalized into the tumor cells.[18,19,32,37]

AN-152 (AEZS-108) was extensively tested *in vitro* and also *in vivo* in nude mice with xenografted human cancer lines expressing LHRH receptors.[8,9,37] This drug inhibited the growth of a wide range of carcinomas (including kidney, prostate, urothelium) and sarcomas. These included DU-145 human androgen-independent prostate cancer as well as HT-1376, J82, RT-4 and HT-1197 bladder cancer lines.[8,9,37–39] The administration of AN-152 (AEZS-108) produced a powerful inhibition of tumor growth, greater than that induced by doxorubicin alone. The hybrid was found to be more efficacious and less toxic than doxorubicin.[37] These studies were followed by Phase I and II human trials in women with endometrial and ovarian cancer expressing LHRH receptors.[40,41]

Dose escalation studies established that the maximum tolerated dose of AEZS-108 is 267 mg m^{-2}. Dose-limiting leukopenia and neutropenia were observed at the highest dose.[40] Liu, Pinski *et al*. also subjected AN-152 (AEZS-108) to Phase II trials in patients with CPRC resistant to taxane.[42] AEZS-108 is currently in Phase III trials in USA and Europe in women with endometrial cancer. We have great expectations for it.

Another class of new and important antitumor peptides that could inhibit CRPC consists of antagonists of GHRH.[43–46] The story behind these compounds is most interesting. One of us (AVS) was among the investigators who discovered GHRH in hypothalami of animals in the 1960s.[43] Because only extremely small quantities of GHRH are present in the hypothalamus, it was not possible to characterize GHRH structurally until the early 1980s.[43] At that time, the clinicians became aware of cases of paraneoplastic acromegaly in some patients with pancreatic carcinoma. These tumors were actually producing GHRH, which then induced GH release from the pituitary. Thus, GHRH can be produced by tumors themselves, and it thus functions in an autocrine/paracrine fashion as a growth factor. Samples of these tumors were used for the isolation and structural elucidation of GHRH, which was then synthesized.[43–46] Over the past 30 years, we have been investigating the role of GHRH in tumor growth and found that many tumors produced GHRH and had GHRH receptors.[43–46] We determined that human prostate cancer specimens and human prostatic cell lines express GHRH and GHRH receptors.[47] We also demonstrated the expression of splice variants of these receptors.[47–49] This presence of GHRH receptors provides the basis for a new approach to the treatment of CRPC based on antagonists of GHRH.[8,9,43] Thus, over the past 20 years we produced nearly 2000 synthetic antagonistic analogs of GHRH, with each step improving their potency and half-life.[43–46,50] We substituted some of the natural (coded) L-amino acids in the N-terminal 29 amino acid sequence of GHRH that has all the biological activity either with their "D" isomers or with totally synthetic amino acids.[43–46,50] The structure of one of our GHRH antagonists, MIA-602, the one we have chosen for clinical development, is shown in **Table 1**. We have found that these GHRH antagonists can block the growth of over 20 different human tumor types, as exemplified by over 60 human cancer cell lines xenografted into nude mice.[43–46,51–69] We showed that MIA-602 and our other GHRH antagonists inhibited growth of PC-3 and 22Rv1 human androgen-independent prostate cancer cell lines and also hormone-dependent prostate cancer lines.[58–62] Our GHRH antagonists also suppressed prostate, kidney, urothelial, breast, triple-negative breast, ovary, astrocytoma, melanoma, ENT tumors, esophagus, stomach, colon, lung, adrenal cortical, pheochromocytoma, uterus, osteosarcomas and multiple lymphoma types. In addition to their inherent effects on cancer, we found that the GHRH antagonists also potentiated the effects of cytotoxic chemotherapy without enhancing the toxicity![70]

The side effect/toxicity profile of GHRH analogs is minimal. Similarly, LHRH agonists and antagonists also have little or no toxicity, and it is really their anti-androgenic effect, not the drugs themselves that cause some adverse effects.[71,72]

PERSPECTIVES FOR THE IMPROVEMENT OF THERAPY FOR CASTRATION-RESISTANT PROSTATE CANCER

Novel drugs are required for the treatment of CRPC. The best option may not be the agents that target androgen receptors or compounds which inhibit enzymes involved in androgen biosynthesis. This is because the androgen deprivation created by these compounds can be overcome by mutations in androgen receptors, the appearance of splice variants of these receptors or alternate biochemical pathways.

The use of currently available cytotoxic analogs of LHRH, or somatostatin that can be targeted to prostate cancers may lead to an improvement in the treatment of CRPC and an increase in the survival rate. A new modality based on GHRH antagonists also appears to be useful for the treatment of metastatic CRPC.

ACKNOWLEDGMENTS

This material is based upon work supported in part by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development of the Miami VA Healthcare System; by the Departments of Pathology and Medicine, Sylvester Comprehensive Cancer Center; and Division of Hematology/Oncology of the Miller Medical School, Department of the Miller School of Medicine, University of Miami; by the South Florida Veterans Affairs Foundation for Research and Education (all to AVS); and by the L. Austin Weeks Endowment for Urologic Research (NLB).

Table 1: Structure of growth hormone-releasing hormone antagonist, MIA-602

	Chemical structure of MIA-602
MIA-602	[(PhAc-Ada)^{0}-Tyr^{1}, D-Arg^{2}, Phe (F)$_{5}$^{6}, Ala^{8}, Har^{9}, Tyr (Me)^{10}, His^{11}, Orn^{12}, Abu^{15}, His^{20}, Orn^{21}, Nle^{27}, D-Arg^{28}, Har^{29}] hGH-RH (1-29) NH_2

Abu: alpha-aminobutyric acid; Ada: 12-aminododecanoyl; Har: homoarginine; Nle: norleucine; Orn: ornithine; PhAc: phenylacetyl; Tyr (Me): O-methyltyrosine; Ac: acetyl; Agm: agmatine; Amc: 8-aminocaprylyl; Amp: para-amidino-phenylalanine; Oct: octyl; Tyr (Et): O-ethyltyrosine; Ibu: isobutyryl

FGR received support from the Urology Care Foundation Research Scholars Program and the AUA Southeastern Section. The contents of this work do not represent the views of the Department of Veterans Affairs or the United States Government.

REFERENCES

1 Redding TW, Schally AV. Inhibition of prostate tumor growth in two rat models by chronic administration of D-Trp6 analogue of luteinizing hormone-releasing hormone. *Proc Natl Acad Sci U S A* 1981; 78: 6509–12.
2 Schally AV, Arimura A, Baba Y, Nair RM, Matsuo H, *et al*. Isolation and properties of the FSH and LH-releasing hormone. *Biochem Biophys Res Commun* 1971; 43: 393–9.
3 Matsuo H, Baba Y, Nair RM, Arimura A, Schally AV. Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. *Biochem Biophys Res Commun* 1971; 43: 1334–9.
4 Matsuo H, Arimura A, Nair RM, Schally AV. Synthesis of the porcine LH- and FSH-releasing hormone by the solid-phase method. *Biochem Biophys Res Commun* 1971; 45: 822–7.
5 Schally AV, Arimura A, Kastin AJ, Matsuo H, Baba Y, *et al*. Gonadotropin-releasing hormone: one polypeptide regulates secretion of luteinizing and follicle-stimulating hormones. *Science* 1971; 173: 1036–8.
6 Schally AV, Kastin AJ, Arimura A. Hypothalamic follicle-stimulating hormone (FSH) and luteinizing hormone (LH)-regulating hormone: structure, physiology, and clinical studies. *Fertil Steril* 1971; 22: 703–21.
7 Tolis G, Ackman D, Stellos A, Mehta A, Labrie F, *et al*. Tumor growth inhibition in patients with prostatic carcinoma treated with luteinizing hormone-releasing hormone agonists. *Proc Natl Acad Sci U S A* 1982; 79: 1658–62.
8 Schally AV, Comaru-Schally AM, Nagy A, Kovacs M, Szepeshazi K, *et al*. Hypothalamic hormones and cancer. *Front Neuroendocrinol* 2001; 22: 248–91.
9 Schally AV, Comaru-Schally AM. Hypothalamic and other peptide hormones. In: Kufe DW, Pollock RE, Weichselbaum RR, Bast RC Jr, Gasler TS, *et al*., editors. Cancer Medicine. 7th ed. Hamilton, Ontario: B. C. Dekker Publishers; 2006. p. 802–16.
10 Schally AV. Luteinizing hormone-releasing hormone analogs: their impact on the control of tumorigenesis. *Peptides* 1999; 20: 1247–62.
11 Rick FG, Block NL, Schally AV. Agonists of luteinizing hormone-releasing hormone in prostate cancer. *Expert Opin Pharmacother* 2013; 14: 2237–47.
12 Shore N, Mason M, de Reijke TM. New developments in castrate-resistant prostate cancer. *BJU Int* 2012; 109 Suppl 6: 22–32.
13 Kawalec P, Paszulewicz A, Holko P, Pilc A. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. A systematic review and meta-analysis. *Arch Med Sci* 2012; 8: 767–75.
14 Schellhammer PF, Chodak G, Whitmore JB, Sims R, Frohlich MW, *et al*. Lower baseline prostate-specific antigen is associated with a greater overall survival benefit from sipuleucel-T in the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT) trial. *Urology* 2013; 81: 1297–302.
15 de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, *et al*. Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med* 2011; 364: 1995–2005.
16 Ryan CJ, Smith MR, Fizazi K, Saad F, Mulders PF, *et al*. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. *Lancet Oncol*. 2015;16: 152–60.
17 Mulcahy N. Enzalutamide Before Chemo Slows Metastatic Prostate Cancer. In: Medscape Medical News; 2014.
18 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.
19 Nagy A, Schally AV, Armatis P, Szepeshazi K, Halmos G, *et al*. Cytotoxic analogs of luteinizing hormone-releasing hormone containing doxorubicin or 2-pyrrolinodoxorubicin, a derivative 500-1000 times more potent. *Proc Natl Acad Sci U S A* 1996; 93: 7269–73.
20 Nagy A, Schally AV, Halmos G, Armatis P, Cai RZ, *et al*. Synthesis and biological evaluation of cytotoxic analogs of somatostatin containing doxorubicin or its intensely potent derivative, 2-pyrrolinodoxorubicin. *Proc Natl Acad Sci U S A* 1998; 95: 1794–9.
21 Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res* 1941; 1: 293–7.
22 Redding TW, Schally AV, Tice TR, Meyers WE. Long-acting delivery systems for peptides: inhibition of rat prostate tumors by controlled release of [D-Trp6] luteinizing hormone-releasing hormone from injectable microcapsules. *Proc Natl Acad Sci U S A* 1984; 81: 5845–8.
23 Rick FG, Schally AV, Block NL, Halmos G, Perez R, *et al*. LHRH antagonist Cetrorelix reduces prostate size and gene expression of proinflammatory cytokines and growth factors in a rat model of benign prostatic hyperplasia. *Prostate* 2011; 71: 736–47.
24 Crawford ED, Hou AH. The role of LHRH antagonists in the treatment of prostate cancer. *Oncology (Williston Park)* 2009; 23: 626–30.
25 Klotz L, Miller K, Crawford ED, Shore N, Tombal B, *et al*. Disease control outcomes from analysis of pooled individual patient data from five comparative randomised clinical trials of degarelix versus luteinising hormone-releasing hormone agonists. *Eur Urol* 2014; 66: 1101–8.
26 Crawford ED, Tombal B, Miller K, Boccon-Gibod L, Schröder F, *et al*. A phase III extension trial with a 1-arm crossover from leuprolide to degarelix: comparison of gonadotropin-releasing hormone agonist and antagonist effect on prostate cancer. *J Urol* 2011; 186: 889–97.
27 Rick FG, Block NL, Schally AV. An update on the use of degarelix in the treatment of advanced hormone-dependent prostate cancer. *Onco Targets Ther* 2013; 6: 391–402.
28 Halmos G, Arencibia JM, Schally AV, Davis R, Bostwick DG. High incidence of receptors for luteinizing hormone-releasing hormone (LHRH) and LHRH receptor gene expression in human prostate cancers. *J Urol* 2000; 163: 623–9.
29 Cai RZ, Szoke B, Lu R, Fu D, Redding TW, *et al*. Synthesis and biological activity of highly potent octapeptide analogs of somatostatin. *Proc Natl Acad Sci U S A* 1986; 83: 1896–900.
30 Cai RZ, Reile H, Armatis P, Schally AV. Potent bombesin antagonists with C-terminal Leu-psi (CH2-N)-Tac-NH2 or its derivatives. *Proc Natl Acad Sci U S A* 1994; 91: 12664–8.
31 Nagy A, Armatis P, Cai RZ, Szepeshazi K, Halmos G, *et al*. Design, synthesis, and *in vitro* evaluation of cytotoxic analogs of bombesin-like peptides containing doxorubicin or its intensely potent derivative, 2-pyrrolinodoxorubicin. *Proc Natl Acad Sci U S A* 1997; 94: 652–6.
32 Letsch M, Schally AV, Szepeshazi K, Halmos G, Nagy A. Preclinical evaluation of targeted cytotoxic luteinizing hormone-releasing hormone analogue AN-152 in androgen-sensitive and insensitive prostate cancers. *Clin Cancer Res* 2003; 9: 4505–13.
33 Plonowski A, Schally AV, Nagy A, Groot K, Krupa M, *et al*. Inhibition of *in vivo* proliferation of MDA-PCa-2b human prostate cancer by a targeted cytotoxic analog of luteinizing hormone-releasing hormone AN-207. *Cancer Lett* 2002; 176: 57–63.
34 Stangelberger A, Schally AV, Nagy A, Szepeshazi K, Kanashiro CA, *et al*. Inhibition of human experimental prostate cancers by a targeted cytotoxic luteinizing hormone-releasing hormone analog AN-207. *Prostate* 2006; 66: 200–10.
35 Plonowski A, Schally AV, Nagy A, Sun B, Szepeshazi K. Inhibition of PC-3 human androgen-independent prostate cancer and its metastases by cytotoxic somatostatin analogue AN-238. *Cancer Res* 1999; 59: 1947–53.
36 Letsch M, Schally AV, Szepeshazi K, Halmos G, Nagy A. Effective treatment of experimental androgen sensitive and androgen independent intraosseous prostate cancer with targeted cytotoxic somatostatin analogue AN-238. *J Urol* 2004; 171: 911–5.
37 Schally AV, Nagy A. Chemotherapy targeted to cancers through tumoral hormone receptors. *Trends Endocrinol Metab* 2004; 15: 300–10.
38 Popovics P, Schally AV, Szalontay L, Block NL, Rick FG. Targeted cytotoxic analog of luteinizing hormone-releasing hormone (LHRH), AEZS-108 (AN-152), inhibits the growth of DU-145 human castration-resistant prostate cancer *in vivo* and *in vitro* through elevating p21 and ROS levels. *Oncotarget* 2014; 5: 4567–78.
39 Szepeshazi K, Schally AV, Keller G, Block NL, Benten D, *et al*. Receptor-targeted therapy of human experimental urinary bladder cancers with cytotoxic LH-RH analog AN-152 [AEZS- 108]. *Oncotarget* 2012; 3: 686–99.
40 Emons G, Sindermann H, Engel J, Schally AV, Gründker C. Luteinizing hormone-releasing hormone receptor-targeted chemotherapy using AN-152. *Neuroendocrinology* 2009; 90: 15–8.
41 Emons G, Kaufmann M, Gorchev G, Tsekova V, Gründker C, *et al*. Dose escalation and pharmacokinetic study of AEZS-108 (AN-152), an LHRH agonist linked to doxorubicin, in women with LHRH receptor-positive tumors. *Gynecol Oncol* 2010; 119: 457–61.
42 Liu SV, Tsao-Wei DD, Xiong S, Groshen S, Dorff TB, *et al*. Phase I, dose-escalation study of the targeted cytotoxic LHRH analog AEZS-108 in patients with castration- and taxane-resistant prostate cancer. *Clin Cancer Res*. 2014;20: 6277–83.
43 Schally AV, Varga JL, Engel JB. Antagonists of growth-hormone-releasing hormone: an emerging new therapy for cancer. *Nat Clin Pract Endocrinol Metab* 2008; 4: 33–43.
44 Schally AV, Varga JL. Antagonists of growth hormone-releasing hormone in oncology. *Comb Chem High Throughput Screen* 2006; 9: 163–70.
45 Varga J, Schally AV. Analogues of growth hormone-releasing hormone (GH-RH) in cancer. In: Kastin AJ, editor. Handbook of Peptides. London: Elsevier/Academic Press; 2006. p. 483–9.
46 Schally AV, Varga JL. Antagonistic analogs of growth hormone-releasing hormone: new potential antitumor agents. *Trends Endocrinol Metab* 1999; 10: 383–91.
47 Halmos G, Schally AV, Czompoly T, Krupa M, Varga JL, *et al*. Expression of growth hormone-releasing hormone and its receptor splice variants in human prostate cancer. *J Clin Endocrinol Metab* 2002; 87: 4707–14.
48 Rekasi Z, Czompoly T, Schally AV, Halmos G. Isolation and sequencing of cDNAs for splice variants of growth hormone-releasing hormone receptors from human cancers. *Proc Natl Acad Sci U S A* 2000; 97: 10561–6.
49 Havt A, Schally AV, Halmos G, Varga JL, Toller GL, *et al*. The expression of the pituitary growth hormone-releasing hormone receptor and its splice

variants in normal and neoplastic human tissues. *Proc Natl Acad Sci U S A* 2005; 102: 17424–9.
50 Zarandi M, Varga JL, Schally AV, Horvath JE, Toller GL, *et al.* Lipopeptide antagonists of growth hormone-releasing hormone with improved antitumor activities. *Proc Natl Acad Sci U S A* 2006; 103: 4610–5.
51 Kahán Z, Varga JL, Schally AV, Rékási Z, Armatis P, *et al.* Antagonists of growth hormone-releasing hormone arrest the growth of MDA-MB-468 estrogen-independent human breast cancers in nude mice. *Breast Cancer Res Treat* 2000; 60: 71–9.
52 Kiaris H, Schally AV, Varga JL, Groot K, Armatis P. Growth hormone-releasing hormone: an autocrine growth factor for small cell lung carcinoma. *Proc Natl Acad Sci U S A* 1999; 96: 14894–8.
53 Engel JB, Keller G, Schally AV, Toller GL, Groot K, *et al.* Inhibition of growth of experimental human endometrial cancer by an antagonist of growth hormone-releasing hormone. *J Clin Endocrinol Metab* 2005; 90: 3614–21.
54 Szepeshazi K, Schally AV, Groot K, Armatis P, Hebert F, *et al*. Antagonists of growth hormone-releasing hormone (GH-RH) inhibit *in vivo* proliferation of experimental pancreatic cancers and decrease IGF-II levels in tumours. *Eur J Cancer* 2000; 36: 128–36.
55 Szepeshazi K, Schally AV, Groot K, Armatis P, Halmos G, *et al*. Antagonists of growth hormone-releasing hormone (GH-RH) inhibit IGF-II production and growth of HT-29 human colon cancers. *Br J Cancer* 2000; 82: 1724–31.
56 Kiaris H, Schally AV, Varga JL. Antagonists of growth hormone-releasing hormone inhibit the growth of U-87MG human glioblastoma in nude mice. *Neoplasia* 2000; 2: 242–50.
57 Keller G, Schally AV, Groot K, Toller GL, Havt A, *et al.* Effective treatment of experimental human non-Hodgkin's lymphomas with antagonists of growth hormone-releasing hormone. *Proc Natl Acad Sci U S A* 2005; 102: 10628–33.
58 Plonowski A, Schally AV, Letsch M, Krupa M, Hebert F, *et al.* Inhibition of proliferation of PC-3 human prostate cancer by antagonists of growth hormone-releasing hormone: lack of correlation with the levels of serum IGF-I and expression of tumoral IGF-II and vascular endothelial growth factor. *Prostate* 2002; 52: 173–82.
59 Stangelberger A, Schally AV, Rick FG, Varga JL, Baker B, *et al.* Inhibitory effects of antagonists of growth hormone releasing hormone on experimental prostate cancers are associated with upregulation of wild-type p53 and decrease in p21 and mutant p53 proteins. *Prostate* 2012; 72: 555–65.
60 Rick FG, Schally AV, Szalontay L, Block NL, Szepeshazi K, *et al*. Antagonists of growth hormone-releasing hormone inhibit growth of androgen-independent prostate cancer through inactivation of ERK and Akt kinases. *Proc Natl Acad Sci U S A* 2012; 109: 1655–60.
61 Fahrenholtz CD, Rick FG, Garcia MI, Zarandi M, Cai RZ, *et al.* Preclinical efficacy of growth hormone-releasing hormone antagonists for androgen-dependent and castration-resistant human prostate cancer. *Proc Natl Acad Sci U S A* 2014; 111: 1084–9.
62 Pinski J, Schally AV, Groot K, Halmos G, Szepeshazi K, *et al.* Inhibition of growth of human osteosarcomas by antagonists of growth hormone-releasing hormone. *J Natl Cancer Inst* 1995; 87: 1787–94.
63 Kovács M, Schally AV, Hohla F, Rick FG, Pozsgai E, *et al*. A correlation of endocrine and anticancer effects of some antagonists of GHRH. *Peptides* 2010; 31: 1839–46.
64 Pozsgai E, Schally AV, Hocsak E, Zarandi M, Rick F, *et al*. The effect of a novel antagonist of growth hormone releasing hormone on cell proliferation and on the key cell signaling pathways in nine different breast cancer cell lines. *Int J Oncol* 2011; 39: 1025–32.
65 Perez R, Schally AV, Vidaurre I, Rincon R, Block NL, *et al*. Antagonists of growth hormone-releasing hormone suppress *in vivo* tumor growth and gene expression in triple negative breast cancers. *Oncotarget* 2012; 3: 988–97.
66 Jaszberenyi M, Schally AV, Block NL, Zarandi M, Cai RZ, *et al*. Suppression of the proliferation of human U-87 MG glioblastoma cells by new antagonists of growth hormone-releasing hormone *in vivo* and *in vitro*. *Target Oncol* 2013; 8: 281–90.
67 Seitz S, Rick FG, Schally AV, Treszl A, Hohla F, *et al.* Combination of GHRH antagonists and docetaxel shows experimental effectiveness for the treatment of triple-negative breast cancers. *Oncol Rep* 2013; 30: 413–8.
68 Szalontay L, Schally AV, Popovics P, Vidaurre I, Krishan A, *et al.* Novel GHRH antagonists suppress the growth of human malignant melanoma by restoring nuclear p27 function. *Cell Cycle* 2014; 13: 2790–7.
69 Hohla F, Winder T, Greil R, Rick FG, Block NL, *et al*. Targeted therapy in advanced metastatic colorectal cancer: current concepts and perspectives. *World J Gastroenterol* 2014; 20: 6102–12.
70 Rick FG, Seitz S, Schally AV, Szalontay L, Krishan A, *et al.* GHRH antagonist when combined with cytotoxic agents induces S-phase arrest and additive growth inhibition of human colon cancer. *Cell Cycle* 2012; 11: 4203–10.
71 Saylor PJ, Smith MR. Metabolic complications of androgen deprivation therapy for prostate cancer. *J Urol* 2009; 181: 1998–2006.
72 Albertsen PC, Klotz L, Tombal B, Grady J, Olesen TK, *et al*. Cardiovascular morbidity associated with gonadotropin releasing hormone agonists and an antagonist. *Eur Urol* 2014; 65: 565–73.