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INVITED RESEARCH HIGHLIGHT

Prostate Cancer

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

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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 laboratories¹ 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 Hodges²¹ 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, cetorelix, 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 microsurgical 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

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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 GRP³⁰ 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 CRPC 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.

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

	Chemical structure of MIA-602
MIA-602	[(PhAc-Ada) ⁹ -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

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