# GnRH Receptor and Androgen Receptor Status and Outcome of Advanced Prostate Carcinomas

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**Abstract.** *Background: The effect of gonadotropin-releasing hormone (GnRH) analogs on prostate carcinoma is partly a result of breaking the pituitary–gonadal axis, and partly the direct action on tumor cells expressing the GnRH receptor (GnRHR). The aim of this study was to detect the extent of correlation between the expression of GnRHR and also the androgen receptor (AR) and the efficacy of total androgen blockade (TAB). Patients and Methods: Needle biopsy samples of twenty advanced prostate carcinoma patients were investigated histologically regarding Gleason score, AR and GnRHR status of the tumor cells. An affinity purified polyclonal antibody reacting with GnRHR and a monoclonal antibody for AR were applied for immunoperoxidase reactions. TAB was started in each case. Pathological, radiological and laboratory-prostate-specific antigen (PSA) data obtained before the start of TAB and survival, PSA values, and radiological findings after three years of TAB were releated to AR and GnRHR. Results: Regarding the clinical, radiological and laboratory findings before and after three years of TAB, 13 patients (group A) were considered to show a 'favourable' and 7 (group B) to show a 'poor' outcome. Twelve patients out of 14 with AR-positive tumor cells and all nine patients with GnRHR-positive tumor cells, as well as all eight patients with both AR- and GnRHR-positive tumor cells belonged to group A. The majority of AR-negative, GnRHR-negative or both AR- and GnRHR-negative cases belonged to group B. Conclusion: The presence of GnRHR and AR or both of these receptors indicates a more favourable outcome of advanced prostate carcinoma when treated with TAB, compared to GnRHR- and AR-negative cases. GnRHR and AR negativity may indicate a need for supplementary chemo- or radiotherapy.*

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The theoretical basis of hormonal therapy of hormone-sensitive tumors is the presence of hormone receptors in the tumor cells (1). Anti-androgen treatment presumes androgen receptor (AR) positivity. The well-known effect of gonadotropin-releasing hormone (GnRH) analogues is exerted on the pituitary–gonadal axis (2), but a number of studies carried out by Schally *et al.* (3) and others (4-8) have pointed to a direct effect of these compounds on tumor cells. The direct effect is necessarily bound to the presence of GnRH receptors (GnRHR). Such receptors have been found in prostate carcinoma cells by various molecular biological methods (9-11). The first immunohistochemical demonstration of GnRHRs in biopsy samples of human prostate carcinoma has been reported by our group, using affinity purified polyclonal antibodies reacting with GnRHR in frozen sections (12). In order to facilitate further serial studies, the immunoperoxidase method has been adapted by our group to formalin-fixed, paraffin embedded material. The aim of the present study was to start a systematic investigation of the association between GnRHR and efficacy of GnRH analog therapy. As a first step, the GnRHR and also the androgen receptor (AR) status of twenty newly diagnosed patients (including those mentioned in reference 12) with advanced prostate carcinoma was determined. The AR and GnRHR status was also related to the TNM stage, Gleason score, and serum prostate-specific antigen (PSA) values at the start of total androgen blockade (TAB), as well as to the survival, serum PSA values, and the results of repeated bone scintigraphy and repeated CT for lymph node metastases after three years of TAB.

## Patients and Methods

Twenty consecutive patients including ten of our previous cases (12) with suspicious digital rectal findings and elevated PSA levels measured using MEIA (microparticular enzyme immune assay) (Abbot Laboratory, Chicago, MI, USA) were enrolled into this study. Informed consent of the patients was obtained before the start of any diagnostic and therapeutic activity, according to the regulations of the Institutional Ethics Commission. Transrectal, ultrasound directed fifteen needle biopsies were performed and the formalin-fixed samples

Table I. *Clinicopathological characteristics before the start of TAB and survival, serum PSA, of bone scintigraphy and CT after three years of TAB in 20 advanced prostate carcinoma patients.*

| Age (years) | TNM stage | PSA (ng/ml) | Gn-RHR | Androgen receptor | Gleason score | PSA* | Alive + Died – | Bone Scintigraphy† | CT†† |
|---|---|---|---|---|---|---|---|---|---|
| 59 | T2cNoMo | 12.5 | Neg | Neg | 7 | 42.40 | + | + | + |
| 81 | T2cNoMo | 13.9 | ++ | Neg | 7 | 3.40 | + | – | – |
| 75 | T3cN2M1b | 400.0 | Neg | Pos | 8 | 180.00 | + | + | + |
| 52 | T2cN1M1b | 1500.0 | Neg | Neg | 8 | ND | –# | + | + |
| 79 | T3bN0M0 | 80.6 | ++ | Pos | 7 | 1.20 | + | – | – |
| 72 | T2cNoMo | 25.0 | Neg | Pos | 8 | 0.00 | + | – | – |
| 71 | T2bNoMo | 21.4 | ++ | Pos | 7 | 0.00 | + | – | – |
| 77 | T3cNoMo | 20.0 | ++ | Pos | 7 | 0.50 | + | – | – |
| 75 | T2bN0M0 | 5.89 | + | Pos | 7 | 0.00 | + | – | – |
| 61 | T3N0M0 | 0.5 | Neg | Pos | 8 | 0.00 | + | – | – |
| 72 | T3aNoM1 | 892.0 | Neg | Neg | 7 | 0.00 | + | + | – |
| 70 | T2aNoMo | 42.9 | + | Pos | 7 | 0.00 | + | – | – |
| 87 | T2cNoMo | 56.0 | Neg | Neg | 8 | 0.13 | + | – | – |
| 67 | T3aNoMo | 21.8 | + | Pos | 9 | 2.73 | + | – | – |
| 80 | T3aNoM1 | 850.0 | Neg | Neg | 7 | 7.70 | + | + | – |
| 75 | T3cNoM1 | 80.0 | Neg | Pos | 9 | 270.80 | –## | + | + |
| 59 | T2bNoMo | 35.9 | ++ | Pos | 7 | 0.08 | + | – | – |
| 77 | T2cNoMo | 79.0 | Neg | Pos | 7 | 0.00 | + | – | – |
| 63 | T1cNoMo | 99.4 | Neg | Pos | 8 | 0.03 | + | + | – |
| 58 | T2bNoMo | 17.5 | + | Pos | 7 | 13.16 | + | – | – |

*Repeated after 3 years of TAB (total androgen blockade); GnRHR: gonadotropin-releasing hormone receptor; †: +, bone metastases; – no signs of metastasis; ††: +, lymph node metastases; –, no signs of metastases; PSA: prostate-specific antigen; #6 months' survival; ##13 months' survival.

were routinely 8% neutral formalin fixed and paraffin-embedded. The 8 μm thin sections were stained with hematoxylin and eosin (H and E) in order to establish the diagnosis of prostate carcinoma and to determine the Gleason score. Duplicate samples of the sections, each containing at least 100 tumor cells were processed for immunohistochemical stained presentation of androgen receptor (AR) and (GnRHR). H and E as well as immunohistochemical stained samples were investigated and scored by two independent pathologists in a blinded fashion.

The immunoperoxidase reaction was performed on the paraffin sections using anti-androgen receptor antibody (Dako, Glostrup, Denmark) and the polyclonal anti-GnRHR (C-18) Sc 8681 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). The dilution of the AR antibody was 1:100 and that of the anti-GnRHR was 1:20.

The antibodies were applied overnight at 37˚C. The sections were treated with proteinase K and, to inactivate the endogenous peroxidase, $H_2O_2$; the secondary antibodies (anti-mouse IgG and anti-goat IgG, respectively) (Dako) were used at a dilution of 1:100 the next day. Diaminobenzidine served as chromogen and methyl green as counterstain. The sections were viewed using an Axiophot microscope (Zeiss, Jena, Germany).

Nuclear positivity for AR was recorded if more than 60% of the tumor cells showed positive reaction. For GnRHR, positivity was recorded as + if 40-60% of the tumor cells, showed a cytoplasmic positive reaction ++ if more than 60% of the tumor cells did.

All the patients were treated with implants of Zoladex depot LA (long activity) (10.8 mg) (Astra Zeneca, Macclesfield, UK) every twelfth week and orally with a daily dose of 50 mg Casodex (Astra-Zeneca).

The clinical, radiological (bone scintigraphy, CT), laboratory (PSA) and pathological data, including GnRHR and AR status obtained before the start of TAB and the clinical, laboratory and radiological data after three years of treatment were compared.

## Results

Table I shows the clinicopathological data of the patients. Before the start of TAB, the PSA values were moderately or highly elevated in all cases. The T stage ranged between T1 c and T3 c and the Gleason score between 7 and 9. Figures 1 and 2 show cytoplasmic GnRHR positivity in the prostate carcinoma cells.

The patients could be devided into two groups based on survival, radiological (bone scintigraphy, CT) and laboratory (PSA) examination. Group A (favourable outcome): thirteen patients were free of radiological signs of bone and lymph node metastasis both at the start of TAB and after three years of treatment. Group B (poor outcome): five patients showed bone and two patients lymph node metastases even at the start of TAB and two further patients developed bone metastases during the course of the three year period of TAB. Two patients out of this group died as a consequence of tumor progression. Four out of the seven patients with metastases had PSA values 400 ng/ml or higher before the start of TAB. In two other cases, the PSA values increased after three years of TAB.

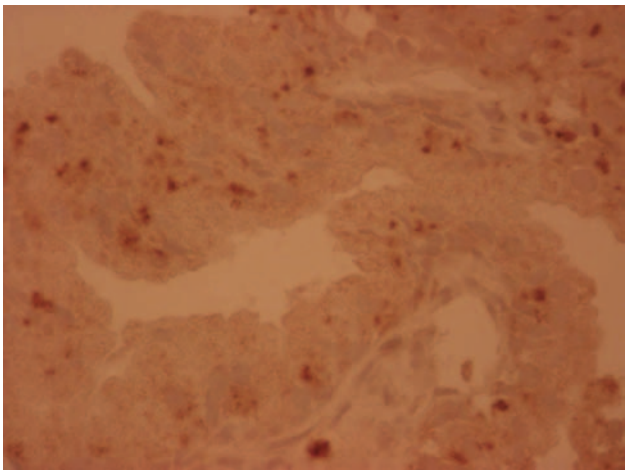

Figure 1. *GnRHRs in cells of a prostate carcinoma (case 4). Fine granular positivity in the cytoplasm of the tumor cells. GnRHR immunoperoxidase, ×600.*

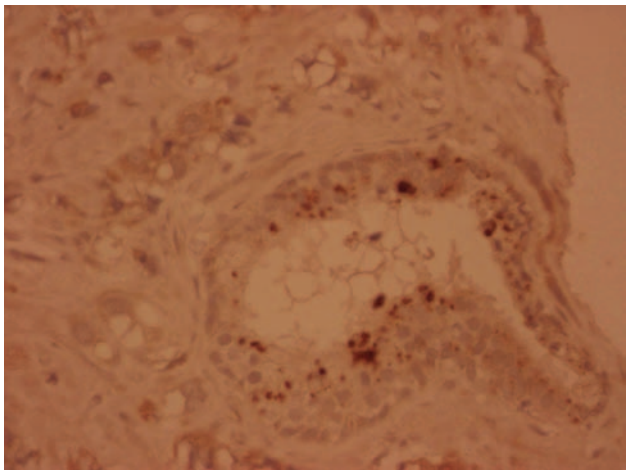

Figure 2. *GnRHRs in cells of a prostate carcinoma (case 6). Positivity is presented as fine granules and larger droplets in the cytoplasm of the tumor cells. GnRHR immunoperoxidase, ×600.*

Table II. *Association between AR and GnRHR status and favourable (A) or poor (B) clinical behaviour of 20 advanced prostate carcinoma cases*

| Receptor status | Favourable outcome (A) | Poor outcome (B) | Total |
|---|---|---|---|
| AR-positive | 12 | 2 | 14 |
| AR-negative | 2 | 4 | 6 |
| GnRHR-positive | 9 | - | 9 |
| GnRHR-negative | 4 | 7 | 11 |
| AR- and GnRHR-positive | 7 | - | 7 |
| AR- and GnRHR-negative | 1 | 4 | 5 |
| One of AR- or GnRHR-negative | 4 | 3 | 7 |

AR: Androgen receptor; GnRHR: gonadotropin-releasing hormone receptor.

Regarding the AR and GnRHR status, the following associations were observed (Table II). Twelve out of the 14 AR-positive cases belonged to group A, while 4 out of the 6 AR-negative cases belonged to group B. All 9 patients with GnRHR positivity were members of group A, while 7 of the 11 GnRHR-negative cases belonged to group B. All 7 AR- and GnRHR-positive patients were members of group A. Four out of the 5 patients with both AR and GnRHR negativity belonged to group B, while only 3 patients of the remaining 15 patients were members of group B. Seven patients showed either AR or GnRHR negativity: 4 in group A and 3 in group B.

The distribution of GnRHR- and AR-positive cases between group A and group B is shown in Figure 3a and b.

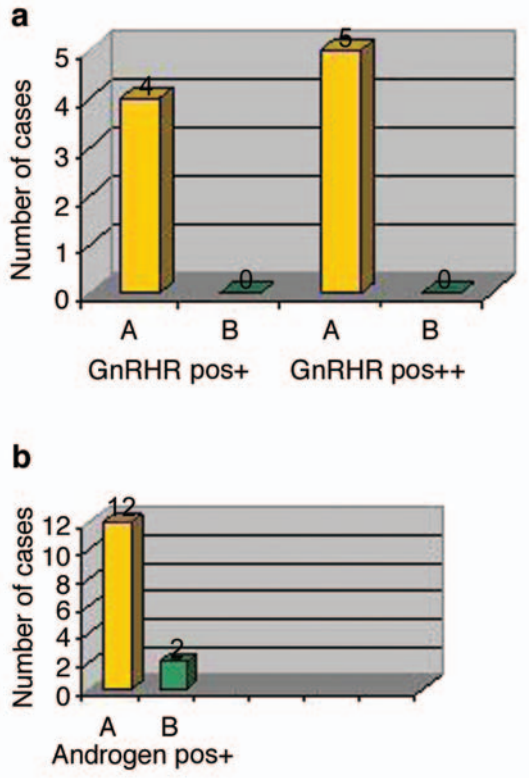


Figure 3. *a. Distribution of GnRHR-positive cases between group A and group B. b. Distribution of androgen-receptor positive cases between group A and group B.*

## Discussion

The administration of GnRH analogs can be used as monotherapy or can be a part of hormonal therapy, *i.e.* total androgen blockade of advanced prostate carcinoma (13).

The most probable mode of action of GnRH analogs is activation of a phosphotyrosine phosphatase which counteracts the effects of the receptor-associated tyrosine kinase (14). The efficacy of GnRH analog therapy is in close association with the expression of GnRHR and also AR as shown in our recent

study. Mutations and modulations of these receptors may influence the effect of TAB. The androgen receptor superfamily interacts with various coactivators or corepressors to modulate transcription of androgen target genes (15). Several attempts have been made to find predictive and prognostic factors regarding prostate carcinoma. Karakiewitz and Hutterer (16) found altogether 39 models for prediction in their review of English literature. Recently the molecular basis of poor outcome of prostate cancer related to androgen-independent stage has been studied. Jin *et al*. (17) pointed to the importance of the nuclear factor kappa B (NF kappa B) pathway in the progression of prostate cancer to androgen-independent growth.

The present results confirmed the presence of GnRHRs in nearly half of the untreated prostate carcinomas. It was also evident that formalin-fixed, paraffin-embedded blocks could be successfully used for the immunohistochemical demonstration of GnRHR, when the affinity purified polyclonal antibody was applied. This may facilitate further serial studies on the expression of this receptor in various human tumors, first of all in prostate and breast carcinoma. The method for improving the accuracy of prognosis of prostate cancer recommended by our group, *i.e*. the parallel assessment of androgen receptor and gonadotropin-relelasing hormone receptor by immunohistochemical reactions in addition to Gleason score, TNM stage and serum PSA values, has several advances. These include rapid performance of the reactions on paraffin-embedded material, high specificity and relatively low costs.

Our data on the AR and GnRHR suggested that immunohistochemical detection of both these receptors may provide a better approach to the estimation of the admission status and further outcome of advanced prostate carcinoma patients. In particular GnRHR positivity and GnRHR plus AR positivity pointed to a more favourable clinical status and outcome of the disease. Negativity of both AR and GnRHR may indicate the need for supplementary therapeutic tools such as chemo- or radiotherapy. (1)

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