

**SHORT COMMUNICATION**

# Clinical investigation of neuroendocrine differentiation in prostate cancer: A study focused on neuroendocrine tumor markers

**Tatsuya Shimomura<sup>1\*</sup> , Masaya Murakami<sup>1,2</sup>, Kanako Kasai<sup>1,2</sup>, Yu Imai<sup>1,2</sup>, Fumihiko Urabe<sup>1</sup>, Katsuki Muramoto<sup>1</sup>, Takafumi Yanagisawa<sup>1</sup>, Wataru Fukuokaya<sup>1</sup>, Keiichiro Mori<sup>1</sup>, Kojiro Tashiro<sup>1</sup>, Kota Katsumi<sup>1</sup>, Hidetsugu Takahashi<sup>1</sup>, Kentaro Yoshihara<sup>1</sup>, Keiichiro Miyajima<sup>1</sup>, Kosuke Iwatani<sup>1</sup>, Sotaro Kayano<sup>1</sup>, Taro Igarashi<sup>1</sup>, Akihiro Matsukawa<sup>1</sup>, Shunsuke Tsuzuki<sup>1</sup>, Hiroki Yamada<sup>1</sup>, Jun Miki<sup>1</sup>, and Takahiro Kimura<sup>1</sup> **

<sup>1</sup>Department of Urology, The Jikei University School of Medicine, Minato, Tokyo, Japan

<sup>2</sup>Department of Urology, Fuji City General Hospital, Fuji, Shizuoka, Japan

## Abstract

Compared to prostate adenocarcinoma, neuroendocrine prostate carcinoma is characterized by aggressive tumor biology and a poor prognosis. Several studies have focused on tumor markers to assess neuroendocrine differentiation and treatment outcomes. However, few studies have investigated changes in tumor markers throughout the clinical course. This observational study evaluated neuroendocrine differentiation, survival outcomes, and temporal changes in neuroendocrine marker positivity in patients with prostate cancer. A total of 193 prostate cancer cases were included in this study. We measured circulating neuroendocrine tumor markers, including neuron-specific enolase (NSE) and pro-gastrin-releasing peptide (pro-GRP), and calculated their positivity rates. In relation to castration-resistant prostate cancer (CRPC), we assessed the changes in the positive rates of neuroendocrine tumor markers over time. In the cross-sectional cohort, the positivity rate of circulating neuroendocrine markers was 19.8% in hormone-sensitive prostate cancer and 64.9% in CRPC (24/37). Temporal changes in marker positivity were evaluated in a separate longitudinal CRPC follow-up cohort ( $n = 39$ ) with serial NSE/pro-GRP measurements. In the context of CRPC, pro-GRP was significantly associated with prognosis. The median overall survival for patients with normal pro-GRP levels was 78 months, whereas that for patients with elevated pro-GRP levels was significantly shorter (36.5 months,  $p = 0.0216$ ). In the longitudinal CRPC follow-up cohort ( $n = 39$ ), the overall marker positivity rate increased from 61.5% at baseline to 92.3% at approximately four years. Neuroendocrine tumor markers were significantly elevated in CRPC and correlated with survival outcomes. The positivity rate of these tumor markers increased during CRPC treatment. This suggests that treatment-emergent neuroendocrine differentiation may occur more frequently than previously anticipated.

**Keywords:** Neuroendocrine differentiation; Prostate cancer; Neuroendocrine tumor markers; Neuron-specific enolase; Pro-gastrin-releasing peptide

### \*Corresponding author:

Tatsuya Shimomura  
(shimomura@jikei.ac.jp)

**Citation:** Shimomura T, Murakami M, Kasai K, *et al.* Clinical investigation of neuroendocrine differentiation in prostate cancer: A study focused on neuroendocrine tumor markers. *Tumor Discov.* 2026;5(3):025350086.  
doi: 10.36922/TD025350086

**Received:** August 27, 2025

**Revised:** November 12, 2025

**Accepted:** December 5, 2025

**Published online:** February 13, 2026

**Copyright:** © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

**Publisher's Note:** AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## 1. Introduction

Prostate cancer is the most common cancer among men in the United States and Europe, and it ranks as the second leading cause of cancer-related deaths.<sup>1</sup> Neuroendocrine prostate carcinoma (NEPC) is a rare tumor type; however, its incidence is on the rise.<sup>2</sup> Recent studies indicate that neuroendocrine characteristics are detected in nearly one-fifth of patients with castration-resistant prostate cancer (CRPC), a higher prevalence than previously expected.<sup>3</sup> NEPC can develop spontaneously, but most instances are a result of androgen deprivation therapy (ADT), with some cases associated with radiation therapy.<sup>4</sup> In comparison to typical prostate adenocarcinoma, NEPC demonstrates a more aggressive tumor behavior, including the absence of prostate-specific antigen (PSA) secretion, resistance to ADT, the emergence of visceral metastases, and a poor prognosis.<sup>5</sup> Recent advancements in the genomic and molecular classification of NEPC, along with the development of new biomarkers, could facilitate early diagnosis, identify potential therapeutic targets, and improve the selection of patients who are most likely to benefit from NEPC-targeted treatments.<sup>6</sup> Nevertheless, current treatment outcomes for neuroendocrine carcinoma remain suboptimal. Several studies have explored tumor markers to investigate neuroendocrine differentiation (NED), treatment results, and survival rates in prostate cancer, with the majority focusing on treatment outcomes in CRPC and only a few examining the changes in tumor markers over time.<sup>7–18</sup> This study aims to investigate NED, survival rates, and the temporal changes in tumor markers in prostate cancer.

## 2. Materials and methods

This study included a total of 193 cases of prostate cancer. Among these, 91 cases were hormone-sensitive prostate cancer (HSPC) treated with ADT or combined androgen blockade (CAB) therapy, which involves ADT along with bicalutamide or flutamide. There were 54 cases of recurrent prostate cancer after radiation therapy, with or without ADT, and 37 cases were classified as CRPC. Additionally, 11 cases showed NED, as indicated by positive neuroendocrine tumor markers detected via immunohistochemical (IHC) staining. This study comprised two analytic datasets: (i) a cross-sectional cohort of 193 prostate cancer cases (including 37 CRPC cases) used for comparisons of marker positivity across disease states and for correlation analyses (Figure 1, Tables 1 and 2, and Figure A1); and (ii) a longitudinal CRPC follow-up cohort ( $n = 39$ ) assembled based on availability of serial neuron-specific enolase (NSE)/pro-gastrin-releasing peptide (pro-GRP) assessments, used for analyses of temporal changes and survival (Table 3 and Figures 2–

4). The two CRPC datasets partially overlap; therefore, all analyses are reported with their applicable denominators. Circulating neuroendocrine markers, such as NSE and pro-GRP, were tested between February and April 2017. Serum chromogranin A (CgA) was not evaluated because it is not approved for clinical use in Japan. We analyzed the positive rates of these markers, excluding patients with creatinine levels over 1.5 mg/dL to minimize false-positive elevations of serum markers due to renal dysfunction. Accordingly, cross-sectional analyses ( $n = 37$  CRPC) were restricted to patients with evaluable baseline PSA/NSE/pro-GRP data meeting the creatinine criterion. Baseline PSA, NSE, pro-GRP, and treatment exposures were recorded. In the longitudinal CRPC follow-up cohort ( $n = 39$ ), patients receiving androgen receptor pathway inhibitors or docetaxel were assessed for circulating neuroendocrine tumor markers (NSE and pro-GRP) at three different time points: at baseline (first evaluation), one year later (second evaluation), and four years later (third evaluation). The goal was to evaluate changes in the positivity rates of circulating neuroendocrine tumor markers (NSE and/or pro-GRP) and their relationship with survival outcomes. Positivity for the markers was defined as results exceeding the normal ranges of 0–16.3 ng/mL for NSE and 0–80.9 pg/mL for pro-GRP. To assess the state of neuroendocrine transformation at the time of prostate cancer diagnosis, IHC staining for neuroendocrine tumor markers (CgA, CD56, synaptophysin) was conducted on cases with available prior biopsy specimens, with diagnoses confirmed by a pathologist. This study was approved by the Institutional Review Board of Fuji City General Hospital (Approval No. 282).

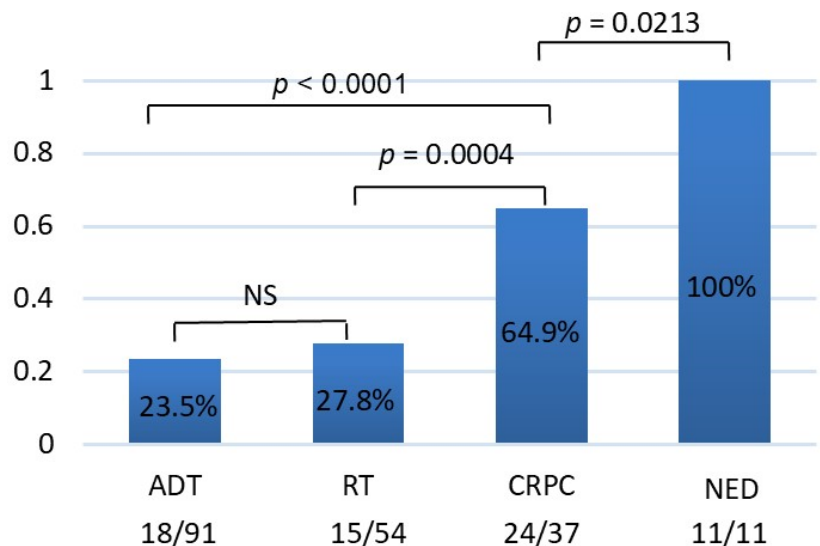
### 2.1. Statistical analysis

We performed the Mann–Whitney  $U$  test,  $t$ -test, and Fisher's exact test to assess the differences among the groups. For multiple comparisons,  $p$ -values were adjusted using the Holm (Bonferroni) method. Survival rates were calculated using the Kaplan–Meier method, and we compared survival distributions with the log-rank test. A  $p$ -value below 0.05 was used as the threshold for statistical significance. All statistical analyses were conducted using EZR (Version 1.65, Jichi Medical University, Japan).<sup>19</sup>

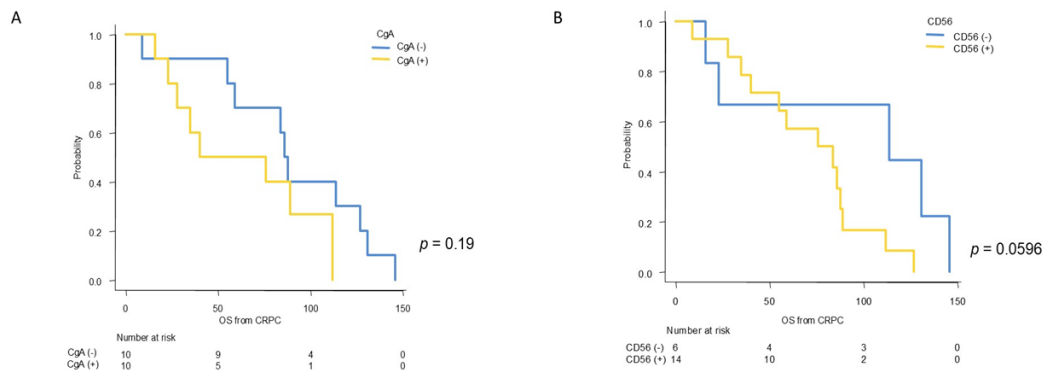
## 3. Results

### 3.1. Positive rates of circulating neuroendocrine tumor markers

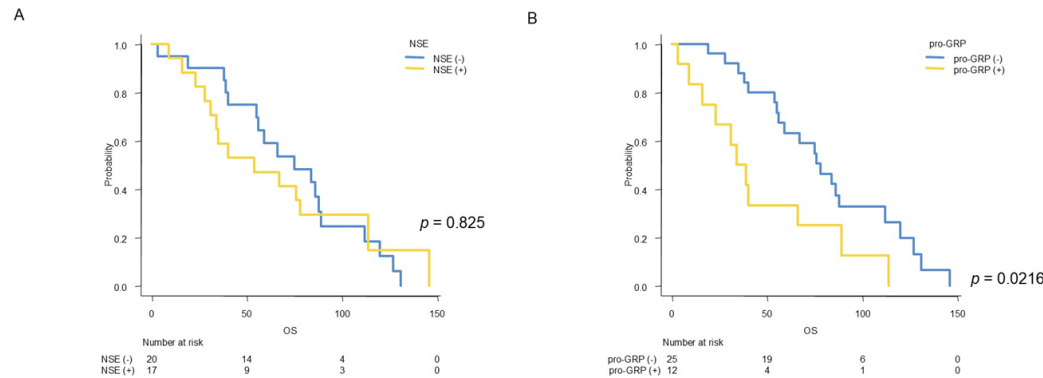
In the cross-sectional dataset (CRPC  $n = 37$ ), the positivity rates of circulating neuroendocrine markers (NSE and/or pro-GRP; defined as values above the normal thresholds) were 19.8% in HSPC treated with ADT or CAB (18 out of 91 cases), 27.8% in prostate cancer after radiation



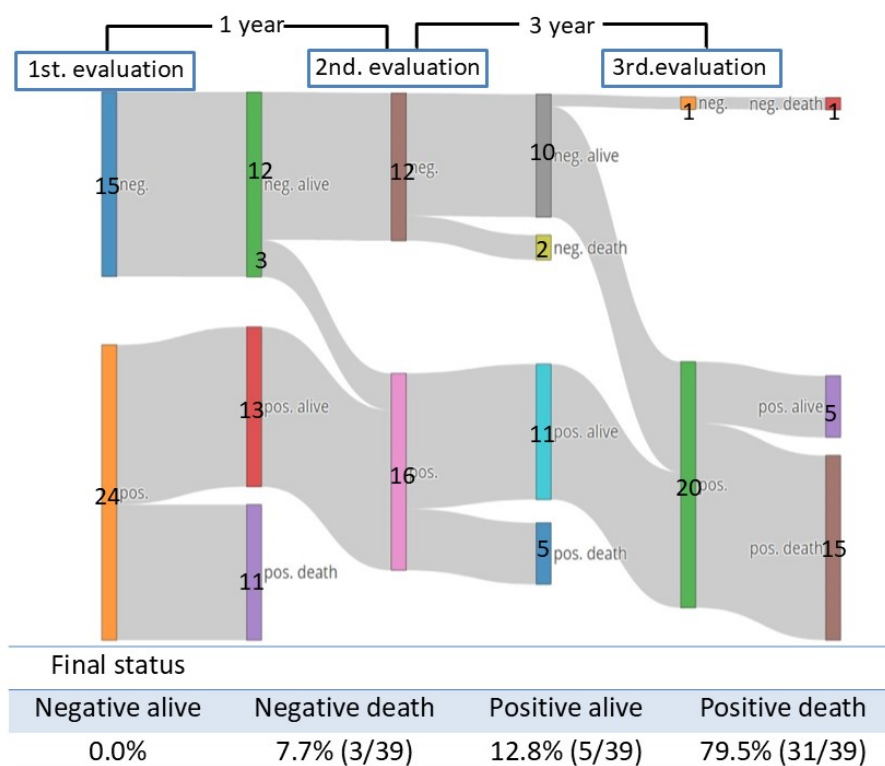
**Figure 1.** Positive rate of serum neuroendocrine tumor markers by prostate cancer status (cross-sectional cohort; hormone-sensitive prostate cancer [HSPC]  $n = 91$ , post-radiation therapy  $n = 54$ , castration-resistant prostate cancer [CRPC]  $n = 37$ , neuroendocrine differentiation [NED]  $n = 11$ ). Abbreviation: NS: Not significant.



**Figure 2.** Kaplan-Meier curve of overall survival (OS) from castration-resistant prostate cancer (CRPC) diagnosis by immunohistochemical staining at prostate biopsy (longitudinal CRPC cohort  $n = 39$ ). (A) Kaplan-Meier curve of OS from CRPC diagnosis via expression of chromogranin A (CgA). (B) Kaplan-Meier curve of OS from CRPC diagnosis via CD56 expression.



**Figure 3.** Kaplan-Meier curve of overall survival (OS) from castration-resistant prostate cancer (CRPC) diagnosis by serum neuroendocrine tumor markers (longitudinal CRPC cohort  $n = 39$ ). (A) Kaplan-Meier curve of OS from CRPC diagnosis comparing neuron-specific enolase (NSE) (+) and NSE (-). (B) Kaplan-Meier curve of OS from CRPC diagnosis comparing pro-gastrin-releasing peptide (pro-GRP) (+) and pro-GRP (-).



**Figure 4.** Observation of the positive rate of neuroendocrine markers, changes during the follow-up period, and survival outcome (longitudinal castration-resistant prostate cancer cohort,  $n = 39$ )

Abbreviations: neg.: Negative; pos.: Positive.

**Table 1. Positive rate of NSE, pro-GRP in CRPC (cross-sectional cohort,  $n = 37$ )**

| (cross-sectional cohort, $n = 37$ ) |               |               |                |                 |
|-------------------------------------|---------------|---------------|----------------|-----------------|
| Markers                             | NSE           | pro-GRP       | NSE or pro-GRP | NSE and pro-GRP |
| Positive rate (%)                   | 17/37 (45.9%) | 12/37 (32.4%) | 24/37 (64.9%)  | 7/37 (18.9%)    |

Abbreviations: CRPC: Castration-resistant prostate cancer; NSE: Neuron-specific enolase; pro-GRP: pro-gastrin-releasing peptide.

**Table 2. Correlation between PSA, NSE, and pro-GRP in CRPC (cross-sectional cohort,  $n = 37$ )**

| pro GRP in CRPC (cross-sectional cohort, $n = 37$ ) |         |                |            |
|-----------------------------------------------------|---------|----------------|------------|
| Groups                                              | $r$     | 95% CI         | $p$ -value |
| PSA vs. NSE                                         | -0.0242 | -0.346, 0.302  | 0.887      |
| PSA vs. pro-GRP                                     | 0.903   | 0.819, 0.95    | < 0.0001   |
| NSE vs. pro-GRP                                     | 0.235   | -0.0966, 0.519 | 0.162      |

Abbreviations: CI: Confidence interval; CRPC: Castration-resistant prostate cancer; NSE: Neuron-specific enolase; pro-GRP: pro-gastrin-releasing peptide; PSA: Prostate-specific antigen.

therapy with or without ADT (RT; 15 out of 54 cases), 64.9% in CRPC (24 out of 37 cases), and 100% in cases with NED (11 out of 11 cases). There was no significant difference between HSPC and RT. However, significant differences were observed between HSPC and CRPC, as well as between RT and CRPC ( $p < 0.0001$  and  $p < 0.01$ ,

respectively), and between NED and CRPC ( $p = 0.0457$ ) (Figure 1).

### 3.2. Positive rate of neuroendocrine serum tumor markers in castration-resistant prostate cancer

Among CRPC patients in the cross-sectional cohort ( $n =$

Table 3. Characteristics of the longitudinal CRPC follow-up cohort ( $n = 39$ )

| the longitudinal CRPC follow- up cohort ( $n = 39$ ) |                 | Value                |
|------------------------------------------------------|-----------------|----------------------|
| iPSA                                                 |                 | 156.141 (7.55–9540)  |
| GS                                                   | $\leq 7$        | 8 (24.2%)            |
|                                                      | $\geq 8$        | 25 (75.8%)           |
|                                                      | NA              | 6                    |
| Metastasis (–)                                       |                 | 12 (32.4%)           |
|                                                      | (+)             | 25 (67.6%)           |
|                                                      | NA              | 2                    |
| CgA                                                  | (–)             | 10 (50%)             |
|                                                      | (+)             | 10 (50%)             |
| Synaptophysin (–)                                    |                 | 1 (5%)               |
|                                                      | (+)             | 19 (95%)             |
| CD 56                                                | (–)             | 6 (30%)              |
|                                                      | (+)             | 14 (70%)             |
| Ki 67                                                |                 | 15.00 [1.00, 95.00]  |
| ttCRPC                                               |                 | 20.5 (4–76)          |
| PSA                                                  |                 | 32.251 [0.032, 7490] |
| NSE                                                  | (median, range) | 14.6 [7.3, 450.6]    |
| NSE                                                  | (normal)        | 21 (53.8%)           |
|                                                      | (positive)      | 18 (46.2%)           |
| pro-GRP (median, range)                              |                 | 56.7 [26.1, 520.2]   |
| pro-GRP (normal)                                     |                 | 25 (64.1%)           |
|                                                      | (positive)      | 14 (35.9%)           |
| Tx                                                   | ABI             | 15 (38.5%)           |
|                                                      | ENZ             | 16 (41.0%)           |
|                                                      | DOC             | 8 (20.5%)            |

Abbreviations: ABI: Abiraterone acetate; CgA: Chromogranin A; CRPC: Castration-resistant prostate cancer; DOC: Docetaxel; ENZ: Enzalutamide; iPSA: Initial prostate-specific antigen; NA: Not available; NSE: Neuron-specific enolase; pro-GRP: pro-gastrin-releasing peptide; ttCRPC: Time to castration-resistant prostate cancer; Tx: Treatment.

37), positivity rates for NSE and pro-GRP were as follows (Table 1): 45.9% (17 out of 37 cases) and 32.4% (12 out of 37 cases), respectively. Additionally, 64.9% (24 of 37 cases) had at least one marker test positive, and 18.9% (7 of 37 cases) tested positive on both markers.

### 3.3. Correlation between neuron-specific enolase, pro-gastrin-releasing peptide, and prostate-specific antigen

We examined the relationship between circulating tumor markers (NSE, pro-GRP, and PSA) in CRPC patients in the cross-sectional cohort ( $n = 37$ ). As indicated in Table 2 and Figure A1A–A1C, a significant correlation was found only between PSA and pro-GRP ( $r = 0.903$ ,  $p < 0.0001$ ). There were no significant associations between PSA and NSE,

or between NSE and pro-GRP ( $p = 0.887$  and  $p = 0.162$ , respectively).

### 3.4. Baseline characteristics of the longitudinal castration-resistant prostate cancer follow-up cohort ( $n = 39$ )

The characteristics of the CRPC follow-up are detailed in Table 3. The median initial PSA level was 156.141 ng/mL, with a range of 7.55 to 9,540 ng/mL. Among the patients, 24.2% had a Gleason score (GS) of 7 or lower, while 75.8% had a GS of 8 or higher. The median time to CRPC was 20.5 months, ranging from 4 to 76 months. The positivity rates for histological neuroendocrine markers at the initial prostate biopsy (immunostaining was conducted in 20 out of 39 cases) were as follows: CgA at 50%, synaptophysin

at 95%, and CD56 at 70%. At the start of this study, the median PSA level (first evaluation) was 32.251 ng/mL, with a range of 0.032 to 7,490 ng/mL. The median levels for NSE and pro-GRP were 14.6 ng/mL (range: 7.3–450.6) and 56.7 pg/mL (range: 26.1–520.2), respectively. At the beginning of the study, the treatment agents introduced were abiraterone for 15 patients (38.5%), enzalutamide (ENZ) for 16 patients (41.0%), and docetaxel (DOC) for 8 patients (20.5%).

### 3.5. Survival outcomes in the longitudinal castration-resistant prostate cancer follow-up cohort ( $n = 39$ )

Figure 2A shows the Kaplan–Meier curve for overall survival (OS) after CRPC diagnosis, categorized by CgA positivity based on immunohistochemistry (IHC). Median OS was 87 months (95% confidence interval [CI]: 9–127) in CgA-negative patients and 58 months (95% CI: 16–NA) in CgA-positive patients ( $p = 0.19$ ). Figure 2B presents the Kaplan–Meier curve for OS from CRPC diagnosis based on CD56 IHC positivity. The median OS for CD56-negative patients was 114 months (95% CI: 16–NA), compared to 80 months (95% CI: 35–88) for CD56-positive patients, with a  $p$ -value of 0.0596. For synaptophysin, a comparison between positive and negative cases was not performed because there was only one negative case.

Figure 3A shows the Kaplan–Meier curve for OS after CRPC diagnosis, categorized by NSE positivity. Patients with normal NSE levels had a median OS of 75 months (95% confidence interval [CI]: 40–89), whereas those with elevated NSE levels had a median OS of 54 months (95% CI: 28–114), with a  $p$ -value of 0.825. Additionally, Figure 3B presents the Kaplan–Meier curve for OS from CRPC diagnosis based on pro-GRP positivity. The median OS for patients with normal pro-GRP levels was 78 months (95% CI: 55–112), compared to 36.5 months (95% CI: 9–89) for those with positive pro-GRP levels ( $p$ -value = 0.0216).

### 3.6. Changes in serum tumor markers over time and survival

In the longitudinal CRPC follow-up cohort ( $n = 39$ ), marker positivity was assessed at baseline, at approximately 1 year, and at approximately 4 years. At the start of this study (first evaluation), 24 cases (61.5%) had positive tumor markers (NSE and/or pro-GRP), while 15 cases (38.5%) were negative, reporting normal levels for both markers. Between the first and second evaluations, 11 cases (45.8%) in the marker-positive group died. During the second follow-up, 16 cases showed positive tumor markers, including 3 cases (20%) that converted from negative to positive. Between the second and third evaluations, 5 cases (31.3%) in the marker-positive group died. At the third

follow-up, 9 cases (75%) from the negative group turned positive (Figure 4). Analyzing the changes in serum tumor markers, the overall positive rate (defined as at least one of NSE or pro-GRP being positive) at the beginning of the study was 61.5%. However, by the third evaluation (approximately four years later), this rate increased to 92.3%. Among the 15 cases that were negative at the start, 12 cases (80%) became positive during follow-up. At the end of the follow-up period, 31 of 39 cases (79.5%) died; most deaths occurred among patients with positive serum neuroendocrine markers (above the normal limits for NSE or pro-GRP). Only 3 out of 39 cases (7.8%) maintained negative serum neuroendocrine markers, with both NSE and pro-GRP levels remaining normal.

## 4. Discussion

Neuroendocrine prostate carcinoma is a rare tumor type, yet its incidence is on the rise. There are two forms of NEPC: *de novo* NEPC, which arises independently, and treatment-emergent NEPC, which develops as a consequence of ADT or radiation treatment. While *de novo* NEPC is infrequent, recent findings indicate that the prevalence of treatment-emergent NEPC has increased compared to previous studies. A study conducted by Aggarwal *et al.*<sup>3</sup> involved a prospective biopsy program at metastatic sites in patients with CRPC, focusing on the pathology and genomics of treatment-emergent small cell prostate cancer (t-SCNC). They reported that the incidence of t-SCNC in CRPC was approximately 17%, and that androgen receptors were present in 75% of cases. In our study, we examined circulating neuroendocrine tumor markers (NSE and pro-GRP) to assess the prevalence of elevated neuroendocrine tumor markers as a surrogate of neuroendocrine differentiation. The positive rate for NSE was 45.9% (17 of 37 cases), whereas for pro-GRP, it was 32.4% (12 of 37 cases) in the CRPC group. The combined positive rate for NSE and pro-GRP was 64.9% (24 of 37 cases) in CRPC. Although we did not evaluate pathological findings as in the aforementioned prospective study, these results suggest a potentially high incidence of NEPC in CRPC. Additionally, Heck *et al.*<sup>11</sup> reported an 80% positive rate for tumor markers (NSE and chromogranin A) in CRPC. Hvamstada *et al.*<sup>20</sup> reported that NSE was positive in 33% and chromogranin A in 58% of hormone-resistant prostate cancer cases. In our study, we assessed HSPC, in which the rate of positive tumor markers (NSE or pro-GRP) among HSPC patients undergoing ADT or CAB was 23.5%. Berruti *et al.*<sup>9</sup> noted that elevated NSE levels were present in 16.0% (4 of 25 cases) and elevated CgA levels in 36.0% (9 of 25 cases) within their HSPC cohort. Because serum CgA testing is not authorized in our country, we did not include it in our study. Positive neuroendocrine

tumor markers may be detected in approximately 20–30% of HSPC patients. We also investigated the relationship between PSA, NSE, and pro-GRP in CRPC cases. A significant correlation was identified between PSA and pro-GRP ( $p < 0.0001$ ), but no correlation was found between PSA and NSE, or between NSE and pro-GRP ( $p = 0.887$  and  $p = 0.162$ , respectively). Kamiya *et al.*<sup>7</sup> also reported no correlation between NSE and PSA, or between CgA and NSE ( $p = 0.8434$  and  $p = 0.418$ , respectively). Since circulating neuroendocrine markers do not correlate with one another (NSE and CgA, NSE and pro-GRP), it is important to measure each marker individually. We assessed neuroendocrine tumor markers and survival outcomes by analyzing pathological markers (CgA, CD56, and synaptophysin) from prostate biopsies, along with circulating markers (NSE and pro-GRP) at the beginning of this observational study. Although the log-rank test did not reveal significant differences in histological markers, cases that were positive for these markers exhibited a lower survival rate. Huang *et al.*<sup>8</sup> found a link between positive CgA staining and survival outcomes, indicating that patients with positive CgA staining had significantly worse OS compared to those with negative staining. However, there are no existing reports on the relationship between CD56 or synaptophysin staining and survival outcomes in prostate cancer. For circulating neuroendocrine tumor markers, patients who tested positive for pro-GRP had a significantly lower survival rate from the time of CRPC diagnosis in this study. Conversely, although NSE-positive patients also had a lower survival rate, the difference was not statistically significant. Several studies have explored the connection between circulating markers and survival in CRPC. Both CgA and NSE are associated with progression-free survival and OS, with elevated levels of these markers indicating a significantly worse prognosis.<sup>10–18</sup> Regarding pro-GRP, Yashi *et al.*<sup>21</sup> recently reported that high pro-GRP levels were associated with worse OS in multivariate analysis in their CRPC cohort. Their report, along with our study, suggests that pro-GRP could serve as a prognostic indicator for CRPC. Moving forward, it will be essential to evaluate not only CgA and NSE but also pro-GRP.

Szarvas *et al.*<sup>18</sup> noted changes in CRPC-related markers, but they did not observe a significant increase in CgA or NSE during the evaluation conducted three months after treatment began. In our study, we investigated whether the markers became positive (exceeding the normal limits of NSE or pro-GRP) during the observation period for each case. Remarkably, 80% of initially negative cases became positive during the study, suggesting that circulating neuroendocrine tumor markers often increase during CRPC treatment. While this observation alone does not confirm neuroendocrine transformation, it highlights the

importance of monitoring tumor markers during CRPC treatment. This study has several limitations: (i) Not all cases underwent IHC staining for neuroendocrine tumor markers at the time of prostate biopsy. (ii) The initial tumor marker values may not accurately reflect the values at the start of treatment. (iii) The sample size for this study was relatively small. Despite these limitations, the findings remain meaningful and justify further study.

## 5. Conclusions

Neuroendocrine tumor markers were higher in patients with CRPC and were linked to survival outcomes. Marker positivity increased during CRPC treatment, with most patients becoming marker-positive over time. The prevalence of treatment-emergent NED is higher than previously thought, and these results should be taken into account in clinical practice.

## Acknowledgments

None.

## Funding

None.

## Conflict of interest

Takahiro Kimura has received consultant/advisor fees from Astellas, Bayer, Janssen, and Sanofi. All other authors declare no competing interests.

## Author contributions

*Conceptualization:* Tatsuya Shimomura

*Formal analysis:* Tatsuya Shimomura

*Investigation:* Tatsuya Shimomura, Masaya Murakami, Kanako Kasai, Yu Imai, Fumihiko Urabe, Katsuki Muramoto, Takafumi Yanagisawa, Wataru Fukuokaya, Keiichiro Mori, Kojiro Tashiro, Kota Katsumi, Hidetsugu Takahashi, Kentaro Yoshihara, Keiichiro Miyajima, Kosuke Iwatani, Sotaro Kayano, Taro Igarashi, Akihiro Matsukawa, Shunsuke Tsuzuki

*Methodology:* Tatsuya Shimomura

*Writing – original draft:* Tatsuya Shimomura

*Writing – review & editing:* Hiroki Yamada, Jun Miki, Takahiro Kimura

## Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Fuji City General Hospital (Approval No. 282). The requirement for informed consent was waived by the Institutional Review Board because this was a retrospective study.

## Consent for publication

Consent for publication was waived due to nature of the study.

## Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Further disclosure

Part of the findings were presented at the American Society of Clinical Oncology Genitourinary Cancers Symposium 2020 (February 13-15, 2020). A preprint version of this manuscript was posted on ResearchSquare (DOI: 10.21203/rs.3.rs-6878384/v1).

## References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. *CA A Cancer J Clin*. 2018;68:7-30.  
doi: 10.3322/caac.21442
2. Zaffuto E, Pompe R, Zanaty M, *et al*. Contemporary Incidence and Cancer Control Outcomes of Primary Neuroendocrine Prostate Cancer: A SEER Database Analysis. *Clin Genitourin Cancer*. 2017;15:e793-e800.  
doi: 10.1016/j.clgc.2017.04.006
3. Aggarwal R, Huang J, Alumkal JJ, *et al*. Clinical and Genomic Characterization of Treatment Emergent Small-Cell Neuroendocrine Prostate Cancer: A Multi-institutional Prospective Study. *J Clin Oncol*. 2018;36:2492-2503.  
doi: 10.1200/JCO.2017.77.6880
4. Hu CD, Choo R, Huang J. Neuroendocrine differentiation in prostate cancer: a mechanism of radioresistance and treatment failure. *Front. Oncol*. 2015;5:90.  
doi: 10.3389/fonc.2015.00090
5. Wang HT, Yao YH, Li BG, *et al*. Neuroendocrine Prostate Cancer (NEPC) progressing from conventional prostatic adenocarcinoma: factors associated with time to development of NEPC and survival from NEPC diagnosis-a systematic review and pooled analysis. *J Clin Oncol*. 2014;32:3383-3390.  
doi: 10.1200/JCO.2013.54.3553
6. de Kouchkovsky D, Chan E, Schloss C, Poehlein C, Aggarwal R. Diagnosis and management of neuroendocrine prostate cancer The Prostate. *Prostate*. 2024;84:426-440.  
doi: 10.1002/pros.24664
7. Kamiya N, Akakura K, Suzuki H, *et al*. Pretreatment Serum Level of Neuron Specific Enolase (NSE) as a Prognostic Factor in Metastatic Prostate Cancer Patients Treated with Endocrine Therapy. *Eur Urol*. 2003;44:309-314.  
doi: 10.1016/s0302-2838(03)00303-8
8. Huang Z, Tang Y, Wei Y, *et al*. Prognostic Significance of Chromogranin A Expression in the Initial and Second Biopsies in Metastatic Prostate Cancer. *J Clin Med*. 2023;12(10):3362.  
doi: 10.3390/jcm12103362
9. Berruti A, Dogliotti L, Mosca A, *et al*. Circulating neuroendocrine markers in patients with prostate carcinoma. *Cancer*. 2000;88(11):2590-2597.  
doi: 10.1002/1097-0142(20000601)88:11<2590::aid-cncr23>3.0.co;2-d
10. Burgio SL, Conteduca V, Menna C, *et al*. Chromogranin A predicts outcome in prostate cancer patients treated with abiraterone. *Endocr Relat Cancer*. 2014;21:487-493.  
doi: 10.1530/ERC-14-0071
11. Heck MM, Thaler MA, Schmid SC, *et al*. Chromogranin A and neuron-specific enolase serum levels as predictors of treatment outcome in patients with metastatic castration-resistant prostate cancer undergoing abiraterone therapy. *BJU Int*. 2017;119:30-37.  
doi: 10.1111/bju.13493
12. Fan L, Wang Y, Chi C, *et al*. Chromogranin A and neuron-specific enolase variations during the first 3 months of abiraterone therapy predict outcomes in patients with metastatic castration-resistant prostate cancer. *BJU Int*. 2017;120:226-232.  
doi: 10.1111/bju.13781
13. Dong B, Fan L, Wang Y, *et al*. Influence of abiraterone acetate on neuroendocrine differentiation in chemotherapy-naïve metastatic castration-resistant prostate cancer. *Prostate*. 2017;77:1373-1380.  
doi: 10.1002/pros.23397
14. Conteduca V, Burgio SL, Menna C, *et al*. Chromogranin A is a potential prognostic marker in prostate cancer patients treated with enzalutamide. *Prostate*. 2014;74:1691-1696.  
doi: 10.1002/pros.22890
15. Conteduca V, Scarpi E, Salvi S, *et al*. Plasma androgen receptor and serum chromogranin A in advanced prostate cancer. *Sci. Rep*. 2018;8:15442.  
doi: 10.1038/s41598-018-33774-4
16. Giridhar KV, Sanhueza C, Hillman DW, *et al*. Serum chromogranin-A based prognosis in metastatic castration-resistant prostate cancer. *Prostate Cancer Prostatic Dis*. 2018;21:431-437.  
doi: 10.1038/s41391-018-0046-9
17. Von Hardenberg J, Schwartz M, Werner T, *et al*. Prospective evaluation of neuromediator dynamics in castration-resistant prostate cancer patients during docetaxel.

*Anticancer Res.* 2017;37:5117-5124.

doi: 10.21873/anticancer.11931

18. Szarvas T, Csizmarik A, Fazekas T, *et al.* Comprehensive analysis of serum chromogranin A and neuron-specific enolase levels in localized and castration-resistant prostate cancer. *BJU Int.* 2021;127:44-55.

doi: 10.1111/bju.15086

19. Kanda Y. Investigation of the freely available easy-to-use software “EZR” (Easy R) for medical statistics. *Bone Marrow Transplant.* 2013;48:452–458.

doi: 10.1038/bmt.2012.244

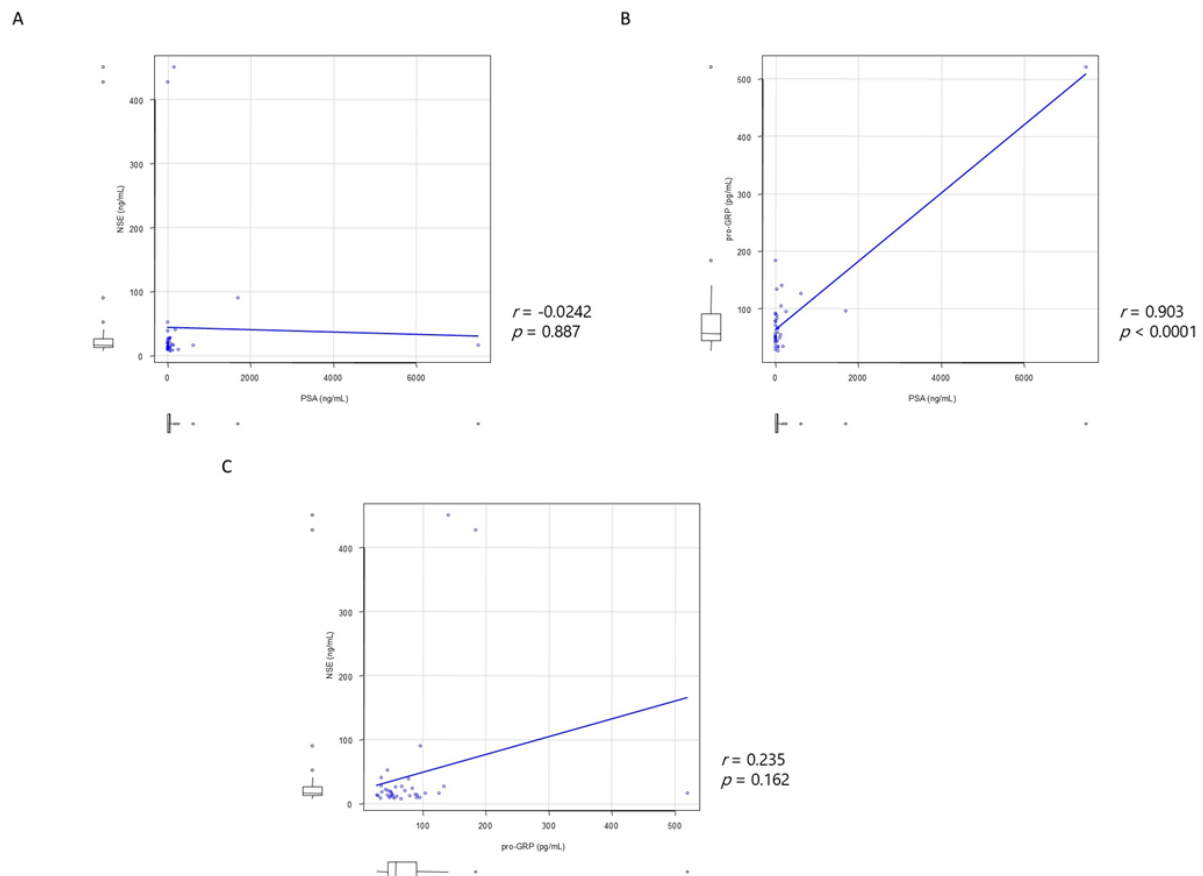
20. Hvamstada T, Jordala A, Hekmata N, Pausb E, Fossa SD: Neuroendocrine SerumTumour Markers in Hormone-Resistant Prostate Cancer. *Eur Urol.* 2003;23:215–221.

doi: 10.1016/S0302-2838(03)00257-4

21. Yashi M, Nishihara D, Yokoyama M, *et al.* Plasma progastrin-releasing peptide level shows different predictive profiles for treatment response by androgen receptor axis-targeted agents in patients with metastatic castration-resistant prostate cancer. *Cancer Rep.* 2023;6:e1762.

doi: 10.1002/cnr2.1762

## Appendix



**Figure A1.** Correlation between circulating tumor markers in castration-resistant prostate cancer (cross-sectional cohort,  $n = 37$ ). (A) Correlation between prostate-specific antigen (PSA) and neuron-specific enolase (NSE). (B) Correlation between PSA and pro-gastrin-releasing peptide (pro-GRP). (C) Correlation between NSE and pro-GRP.