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# Can PD-1 expression in prostate cancer help to predict response to single ADT in an Indonesian population?

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**Abstract**

Measuring PD-1 expression may help select patients with prostate cancer for not favorable on intensification of treatment. The question of whether similar benefits were derived from androgen-deprivation therapy (ADT) alone, or upfront chemotherapy or targeted therapies, remained an enigma. The key question was whether unnecessary escalation of concurrent additional androgen-targeted therapy or chemotherapy could be halted. These serious and potentially life-threatening considerations required carefully designed clinical trials to be resolved. The study aimed to evaluate the role of PD-1 in predicting outcomes from prostate cancer management with our then-current intensification treatment strategy. In total, 20 patients diagnosed with prostate cancer at RSUP Dr. Sardjito were enrolled between 2015 and 2021. The expressions of PD-1 in primary tumors were quantified using quantitative real-time polymerase chain reaction. Data were analyzed descriptively in percentages followed by bivariate and multivariate analyses with $p$-value < 0.05 considered significant. The mean age of patients enrolled in this study was 71.6 5± 8.76 years, and the mean of prostate-specific antigen in patients was 96.25 ± 83.01 ng/ml. Compared to the 25th percentile, higher expression of PD-1 exhibited greater prognostic value than the variable of shorter time to castration-resistant prostate cancer (CRPC). (60 months vs. 21.7 ± 5.58 months, $p$value: 0.005). This pilot study demonstrates that high expression of PD-1 is a promising biomarker for selecting patients who might benefit from intensification therapy.

**Keywords:** prostate cancer, immunotherapy, androgen deprivation therapy, PD-1

## 1. Introduction

Prostate Cancer (PCa) has remained as the second most common cancer diagnosed in men globally (1). Despite the advancements of several treatment options for PCa, it is known for its dependence on testosterone milieu. Accordingly, the androgen deprivation therapy (ADT) is still the main pillar of PCa management since 1941 (2). Alan Turing died in 1952 just one year after undergoing voluntary chemical castration and the mystery of his untimely death of unknown etiology remains shrouded like an unbroken code. While good response towards ADT in the majority of patients with PCa has been reported, almost all will develop a resistance variant of PCa, which are generally known as castration resistance prostate cancer (CRPC). The time of this progress is varied among each person, thus identifying those patients who are not fully responding to single ADT is crucial.

The current recommendation that has been applied in the majority of guidelines informs that metastatic patients tend to be less benefitted by receiving single ADT as their sole treatment for PCa. However, while two and three drug combinations for tumor progression control and morbidity reduction of several patients, and the same time. The triplet regiment also have hidden costs to patients related to time off for hospitalization for adverse effect such as febrile neutropenia and irreversible peripheral neuropathy. In addition, analysis of STAMPEDE cohorts shown confirmation that quality of life patients received abiraterone is better compared to patients who received docetaxel, although the difference didn't reach the level of prespecified for clinical significance due to low statistical power (3). With the alignment that shown from exploratory analysis of ENZAMET, PEACE-1 and ARASENS studies, the triplet regiment have been touted as a 'cure all', unfortunately, this success record only been matched with the patients that classified at the highest risks subgroup of PCa.

Relating to avoidance of CRPC, the comparison between triplet combinations and double combination of Androgen receptor targeted agent (ARTA) shown no differences (4). In addition, there are no studies available which have comparative data concerning third generation ARTA versus triplet combination for PCa. Even more importantly, both options for

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combination therapies have shown a similar hazard ratio (HR) range (5); additionally, there are several enigmas that need to be solved: the first question asks: is it all patients who may be benefited with intensification of treatment? And secondly, are their underlying mechanisms available in biomarker testing, particularly concerning programmed cell death 1 (PD-1) and its ligand.

Despite being known for its reliance on testosterone milieu, our previous study also found PCa progression to develop CRPC was also influenced by the microenvironmen (6). The results are suggesting that the adaptive immune system and tumor immune escape mechanism have pivotal roles in the progression of PCa to develop CRPC.

PD-1 pathway is known to induce the effector T cells, inhibit T cell activation, and suppress innate anti-tumor response that leads to immune escape of PCa towards apoptosis (7). In addition, PD-1 expression was strongly correlated with activation of androgen receptor (ARs). One previous study shown that the blockade of androgen increases T cell response toward PD-1 inhibition in metastatic CRPC (mCRPC) (8). Herein, our study aimed to evaluate retrospectively the role of PD-1 in mRNA level in predicting PCa response towards single ADT. We followed the STROCSS 2019 Guideline for developing protocol (9).

## 2. Materials and Methods

### 2.1. Patients

A total of 20 patients diagnosed with PCa pathologically between 2015 and 2019 and received single ADT as their treatment were, and signed general informed consent is enrolled on this study. Patients who received up-front chemotherapy, triplet therapy, and local therapy were excluded.

The primary outcome of this study was time to develop CRPC after receiving ADT. The condition of CRPC is defined as the increased values of prostate-specific antigen (PSA) after achieving nadir or clinical progression validated with radiographic findings despite reaching testosterone level <20 ng/mL. The clinical staging was done by using the TNM Classification of Malignant Tumors (TMN) criteria eight edition, published in 2017 (10). This Study received approval from the Medical and Health Research Ethics Committee, Universitas Gadjah Mada (KE/0158/02/2020).

### 2.2. Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

Applying the methods previously published by Soerohardjo et al., glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping reference gene (2). This study was conducted in compliance with the Helsinki Declaration, and the protocol was registered with the International Standard Randomized Controlled Trial Number (ISRCTN) registry under reference #24834343. The methods for qRT-PCR were previously described in our study (2).

The primer sequences used in this study were: PD-1 forward GAC TAT GGG GAG CTG GAT TT and Reverse was AGA GCA GTG TCC ATC CTC AG. Univariate and multivariate logistic regression analyses were conducted to investigate the response to androgen deprivation therapy (ADT) and abiraterone. The associations between outcomes and the evaluated variables were presented as Hazard Ratios (HRs) with their corresponding 95% confidence intervals (CIs). CRPC-free survival based on risk factors was evaluated using Kaplan-Meier survival analysis. All data were collected and analyzed using SPSS version 15.0 (IBM Corp., USA).

## 3. Results

The mean age patient enrolled was 71.65 ± 8.76 years, and the PSA was mean 96.25 ±83.01 ng/ml (Table 1.) . Patients with high expression of PD-1 has shorter time to CRPC (60 months vs. 21.7 ± 5.58 months, (*p* value 0.005) (Fig. 1).

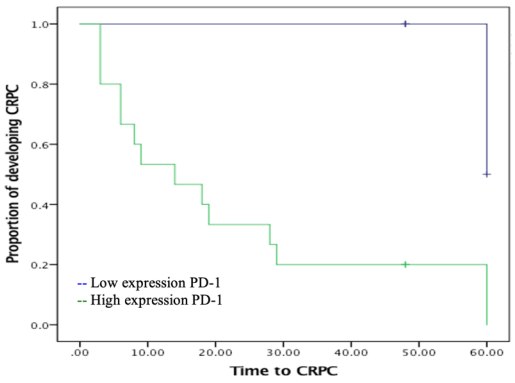


**Fig. 1.** Times for developing CRPC in patients received ADT alone

**Table 1.** Data Demographic

| Variables | |
|---|---|
| Ages (Years) | 71.65 + 8.7) |
| PSA | 96.25 + 83 |
| Tx | 3 |
| T1B | 2 |
| T1C | 9 |
| T2A | 1 |
| T2B | 2 |
| T2C | 3 |
| ISUP Groups | |
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| Nx | 19 |
| N1 | 1 |
| M0 | 10 |
| M1B | 10 |
| Methods of Castration | |
| Surgical | 12 (60%) |
| LHRH agonist | 8 (40%) |
| Mean time to CRPC | 28.3 (21.83) |
| Comorbid (%) | |
| Cerebrovascular | 9 (45%) |
| Cardiovascular | 2 (10%) |
| ESRD | 6 (30%) |
| Type 2 diabetes mellitus | 4 (20%) |

PSA, Prostate serum antigen; CRPC, castration resistance prostate cancer; ISUP, International Society Urological Pathology; ESRD, End-stage renal diseases

Patients with International Society Urological Pathology (ISUP) score > 3 and high expression PD-1 had higher risk measured as hazard ratio (HR) to develop faster time to CRPC ($p$ value < 0.001, HR 1.530 (1.193 – 1.961) (Table 2). However, the results shown no significances in the multivariate analysis. Additional analysis shown that high expression of PD-1 was a prognostic factors for patients who did not respond to ADT (HR 15.152, 95% CI: 1.178 – 194.951, *P* value 0.037). Meanwhile, PSA at diagnosed >20 ng/ml, and metastatic at diagnoses did not show significance results on predicting response to single ADT.

**Table 2.** Analysis of Multivariate time to Developing CRPC

| | Bivariate | | | Multivariate | |
|---|---|---|---|---|---|
| | P value | Hazard Ratio(95% CI) | | P value | Hazard Ratio(95% CI) |
| PD-1 | 0.028 | 10.02 (1.29 – 78.08) | | 0.037 | 15.15 (1.18 – 194.95) |
| ISUP group > 3 | 0.001 | 1.53 (1.19 – 1.96) | | 0.962 | 1.03 (0.26– 4.05) |
| PSA >20 | 0.180 | 2.72 (0.63 – 11.76) | | 0.472 | 0.4 (0.03 -4.92) |
| Metastasis at diagnosed | 0.453 | 1.33 (0.63 – 2.81) | | 0.772 | 0.896 (0.43 – 1.88) |
| Ages > 70 years | 0.439 | 1.28 (0.69 – 2.38) | | 0.854 | 1.13 (0.31 – 4.09) |

## 4. Discussion

Prostate cancer is known as a cold tumor due its immune-suppressive tumor microenvironment. This condition is caused by several factors such as infiltration of T-cells, tumor-associated macrophages (TAM), and cytokines (11). This cascade leads to the tumor associated lymphocytes such as CD8+ T-cells becoming inactivated; thereby, a mechanism results to allow malignant cells in the tumor to escape the innate immune response for stopping the growth progression of the PCa. According to the reports from immunology clinical trials, no favorable results were found in general. However, in contrast, in several groups of patients, who had failed to respond to the novel hormonal therapy, there were excellent responses to PD-1/PD-L1 inhibitors, which indicates the importance of identifying predictive biomarkers (12, 13, 14)30.09.2024 11:15:00.

The expression of PD-1 is associated with worse clinicopathological characteristics such as high PSA value, AR expression and ISUP groups (15). In this study, we found that the high expression of PD-1 on the mRNA level and ISUP group >3 shown strong correlation to the shortened time to CRPC. There are several studies that have reported that ADT promotes the restricted T-cell mediated responses, and shown that PD-1 was expressed on Natural Killer cells in mice models (16,17). Several reports indicated NK cells could also mediate the blockade of PD-1/PD-L1 that is known to be fundamental for full therapeutic effect of immunotherapy (18)30.09.2024 11:15:00. However, the role of PD-1 has not yielded clear results yet in terms of a being recognized as a biomarker to predict response toward ADT.

The main function of PD-1 is as immune-check point receptor that is expressed by activated T cells and facilitate immunosuppression in prostate cancer. The main function of PD-1 is in peripheral tissues of tumor that T cells may confront the immunosuppressive PD-L1 and PD-L2 that widely expressed by solid tumors (19, 20).

ADT is generally known for inducing apoptosis towards hormonal sensitive PCa and epithelial cells (21). This is because the aforementioned apoptotic tumors are the target for phosphatidylserine pathway mediated phagocytosis and serve as an source of antigen to APC (22, 23, 24). Thus, ADT can simply boost in situ APC on both levels of the macrophage and dendritic cells that are paralleled by the rise of CD80 and CD86 expressing cells. Thus, the overload of antigen combined with rising levels of APC might result in efficient prostate-specific T-cell activation. There are other concepts worth mentioning regarding mechanism of ADT to mediate anti-prostate immune response including: (1) prostate tumor vascularization disruption (25), and (2) normal prostate glandular architecture (26, 27) , which allows greater immune access to cryptic prostate antigen. This occurs by modulating the production of local cytokines that are favorable to the activation of antigen specific T cells (28). In addition, in animal models the prostate development starts at puberty with regression of both thymic and marrow tissues towards this underlying androgen mediated mechanism (29, 30).

ADT is also known to mediate tumor regression at distant metastatic sites which leads to its role as pivotal strategy of management PCa. Several studies have published results reporting between immune parameters and the response toward ADT, such as; (1) the increased number lymphocytes and decreased level of cytokines after ADT had been linked towards favorable factors to this treatment; and (2) increasing number infiltrating T cells within cancer tissues that are followed by lower number cancer recurrence indicating that immune mediated responses may have a pivotal role in management PCa (31, 32).

This study has limitations due to the small number of enrolled patients, but the homogeneity of patients' race and therapy is a strength. While upfront chemotherapy and androgen receptor-targeted therapy (ARTA) have shown favorable results in recent research, some patients may benefit from single androgen deprivation therapy (ADT) alone. Future

research directions should address the limitation of the small sample size and evaluate the role of biomarkers in alternative therapies beyond ADT.

Although PD-1 and PD-L1 work together in the immune checkpoint pathway, this study focused solely on investigating PD-1 in prostate cancer management. While the study did not analyze PD-L1 expression or their interactions, exploring PD-L1 in future research could provide a more comprehensive understanding of immune response mechanisms and therapeutic strategies in prostate cancer management. Investigating the interplay between PD-1 and PD-L1 could be a valuable direction for further research.

This pilot study suggested patients with low expression of PD-1 might still benefit from single ADT treatment. Larger scale of studies is recommended to confirm this finding to develop more selective therapy for hormonal sensitive prostate cancer.

**Conflict of interest**

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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None to declare.

**Authors' contributions**

Concept: I.S., A.Z.H., D.S.H., Design: I.S., A.Z.H., D.S.H., Data Collection or Processing: A.Z., D.S.H., A.Z.H., Analysis or Interpretation: A.Z.H., D.S.H., Literature Search: I.S., D.S.H., A.Z., Writing: I.S., D.S.H., A.Z.

**Ethical Statement**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Subjects (or their parents or guardians) have given their written general consent to permit their enrolment in any later studies that used the unused specimens from our hospital samples including blood, and urine and the study protocol was approved by the institutional review board in the Medical and Health Research Ethics Committee of Faculty of Medicine, Universitas Gadjah Mada/Dr. Sardjito Hospital (KE/0158/02/2020).

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