Communication

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Posted Date: 14 October 2024

doi: 10.20944/preprints202410.1074.v1

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Simple Summary: This study investigates the role of a protein called Polo-Like-Kinase-1 (PLK1) in prostate cancer. Previous studies using different methods suggested that high levels of PLK1 are linked to more aggressive forms of prostate cancer. However, this study used a technique to measure PLK1 at the RNA level in tumor samples from many patients and found no link between high PLK1 levels and worse outcomes for patients. We aimed to determine if measuring PLK1 in this way could help predict prostate cancer progression or treatment response. The results suggest that PLK1 levels measured by this method are not a useful marker for these purposes. This finding is important because it challenges previous beliefs and indicates that more research is needed to find reliable ways to predict prostate cancer outcomes and treatment responses.

Abstract: Polo-Like Kinase-1 (PLK1) is a key regulator of the G2/M cell cycle and has been linked to treatment resistance in prostate cancer (PCa). This study aimed to explore the correlation between PLK1 expression, determined through RNA-seq analysis, and clinical outcomes in PCa patients. We analyzed tumor samples from 452 patients diagnosed with prostate adenocarcinoma between 2014 and 2020, sourced from nine institutions affiliated with the Oncology Research Information Exchange Network (ORIEN). PLK1 expression levels were determined using RNA-seq data and stratified into high (≥75th percentile) vs. low (<75th percentile) expression. Univariate and multivariate Cox proportional hazard models compared overall survival between patients with low vs. high PLK1 expression, adjusting for age, race, PSA, and stage. Of the 452 tumors, 241 (53.3%) had high PLK1 and 211 (46.7%) had low PLK1 expression. Median follow-up was 2.74 years for low PLK1 and 2.71 years for high PLK1. No significant difference in overall survival was observed between low and high PLK1 groups in univariate (p=0.86; HR 1.09) or multivariate (p=1.0; HR 0.99) analyses. This study found no association between RNA-seq-based PLK1 expression and prognosis in PCa. PLK1 expression is not a prognostic marker for prostate cancer.

Keywords: polo-like-kinase-1; RNA-seq mRNA expression; prostate cancer; prognostic marker; ORIEN database

 doi:10.20944/preprints202410.1074.v1

Introduction

The androgen receptor (AR) signaling pathway is pivotal in the development of prostate cancer (PCa) [1]. Several innovative therapies targeting AR have shown substantial overall survival benefits in both hormone-sensitive and castration-resistant PCa [2-8]. Despite advances in androgen receptor signaling inhibitors, resistance to these agents inevitably develops.

Polo-like kinase 1 (PLK1), a serine/threonine kinase crucial for cell-cycle progression, is overexpressed in PCa [9]. Its association with higher-grade tumors suggests its involvement in tumorigenesis and progression. Mounting evidence indicates that PLK1 activity is linked to resistance to various therapies and plays an important role in therapy resistance in PCa [10-11]. PLK1 phosphorylates CLIP-170 and p150Glued, two microtubules plus end-binding proteins, enhancing microtubule dynamics and resulting in docetaxel resistance [12]. PLK1-mediated activation of the PI3K/AKT/mTOR pathway leads to AR signaling activation, counteracting the effects of abiraterone and enzalutamide [13]. Thus, PLK1 elevation/activation appears to be a general mechanism for treatment resistance in PCa. Inhibition of PLK1 could enhance anti-neoplastic activity [14, 15]. Notably, a new orally bioavailable PLK1 inhibitor, onvansertib, is currently undergoing early-phase clinical trials in select tumors [16-18]. There is an ongoing phase II clinical trial (NCT03414034) testing the combination of onvansertib and abiraterone acetate in men with metastatic castration-resistant PCa who have early resistance to abiraterone [19].

In light of this, we evaluated mRNA-PLK1 expression as a prognostic marker by correlating PLK1 expression with clinicopathological characteristics and survival, and assessed the correlation between PLK1 mRNA expression and clinical outcomes in prostate cancer patients treated with abiraterone/enzalutamide or docetaxel. In this study, we used RNA-seq PLK1 expression from patients with all stages of prostate adenocarcinoma using the Oncology Research Information Exchange Network (ORIEN) and correlated it with survival outcomes. We hypothesized that high PLK1 overexpression by RNA-seq is associated with high Gleason grade and negatively impacts overall survival, leading to poor survival outcomes in patients treated with abiraterone/enzalutamide and/or docetaxel.

Methods

Study Population and Design

The Oncology Research Information Exchange Network (ORIEN) stands as a pioneering cancer precision medicine initiative [20]. Over time, it has grown into a collaborative research consortium network of nineteen prominent cancer centers across the United States. Patients were enrolled in the Total Cancer Care (TCC) protocol across 19 cancer centers within ORIEN. TCC is a prospective cohort study of unparalleled scope, encompassing whole-exome tumor sequencing, RNA sequencing, germline sequencing, and lifetime follow-up.

Patients with all stages of prostate adenocarcinoma diagnosed between 2014 and 2020 from nine participating members of ORIEN were included in the study. Clinical data such as age at diagnosis, race, stage at diagnosis, Gleason grade at the time of diagnosis, prostatic specific antigen (PSA) at diagnosis, type of hormone therapy, type of chemotherapy, and survival data were extracted from the ORIEN AVATAR Database. The duration of treatment was defined as the time from the start of the treatment to its end for any reason. Overall survival (OS) was calculated from the time of PCa diagnosis until death for any cause. RNA-seq was performed on tumor samples following the RSEM pipeline, enabling the quantification of gene expressions in terms of Transcript Per Million (TPM).

In our study, tumor RNA-seq was analyzed for PLK1 expression and associated with clinical data. PLK1 expression was stratified into high expression (≥75th percentile of PLK1 mRNA expression) vs. low expression (<75th percentile) as defined in a previous breast cancer study [21]. The study was approved by the local Institutional Review Boards (IRBs) at the University of Kentucky (approval number: 64688).

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Statistics and Analysis

Descriptive statistics of clinical and prognostic variables including age, race, stage, PSA, and Gleason grade were reported by PLK1 level (high versus low). The Fisher's exact test was used to compare the distribution of a categorical variable, and the Wilcoxon rank-sum test was used to compare the distribution of a continuous variable between PLK1 high and PLK1 low groups. Kaplan-Meir curves and the log-rank test were used to compare overall survival and duration of treatment between PLK1 high and PLK1 low groups. The univariable Cox proportional hazards model was used to calculate the hazard ratio (HR). The multivariable Cox proportional hazards model was used to adjust for clinical factors including age, race, PSA level, and stage. Two-sided p values <0.05 was considered as statistically significant for all tests.

Results

Study Population

A total of 452 primary tumors were evaluated for PLK1 expression. Of them, 241 (53.3%) met our definition of high PLK1 expression, and 211 (46.7%) were defined as low PLK1 expression (**Table 1**). The median age at diagnosis for high and low PLK1 expression was 65 years (range 43–85). Most patients were Caucasian in both groups: 218 (91%) in the PLK1 high group and 186 (89.4%) in the PLK1 low group. In the high PLK1 group, 93 (39%) had localized high risk/very high-risk disease, 27 (11%) had localized intermediate risk, and 20 (8%) had metastatic disease (**Table 1**). In the low PLK1 group, 97 (46%) had localized high risk/very high-risk disease, 38 (18%) had localized intermediate risk, and 18 (8%) had metastatic disease. Gleason ≥ 8 and PSA <20 were seen slightly more in the high PLK1 group compared to the low PLK1 group; 9% vs. 6%and 89% vs. 83%, respectively (**Table 1**).

Table 1. Baseline Characteristics.

Variable		PLK1 low (<75 percentile) N=211	PLK1 high (≥75 percentile) N-241	*P* values
Age (range)		64.5 (42.9 – 85.8)	63.2 (40.1 – 85.5)	0.53
Race	White	186	218	0.18
	Black	11	15	
	Other	14	8	
Stage	I	2	3	0.63
	II	38	27	
	III	97	93	
	IV	18	20	
	Missing	56	98	
PSA	<20	176	216	0.65
	20 – 50	7	5	
	≥ 50	4	5	
	Missing	24	15	
Gleason	Well-differentiated	6	10	0.64
	Moderately differentiated	21	24	
	Poorly differentiated	13	23	
	Grade cannot be assessed	2	6	
	Missing	169	169	

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Survival Analysis

The median follow-up duration was 2.74 years in the low PLK1 group and 2.71 years for the high PLK1 group. Univariate analysis revealed no significant correlation between PLK1 expression and the studied covariates. Additionally, there was no notable difference in overall survival between patients with low and high PLK1 expression (*p=0.86*; HR 1.09, 95% CI: 0.41-2.88) (**Fig 1**).

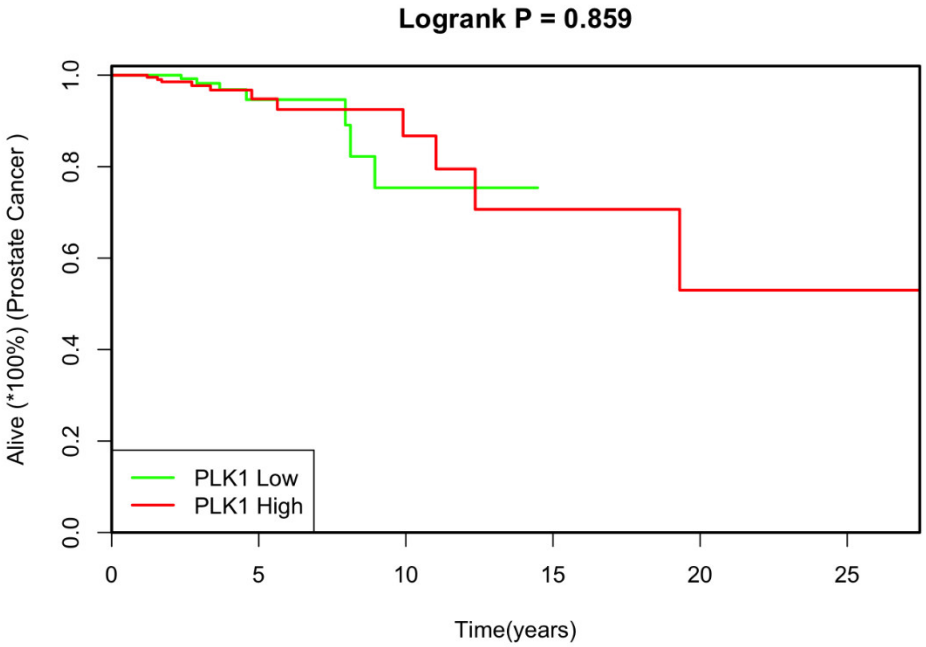

Figure 1. Association between PLK1 and Overall Survival.

Furthermore, the study found no statistically significant differences in PLK1 expression or overall survival among different racial groups, including Whites, African Americans, and others (**Table 2**). Likewise, there was no significant variation in overall survival observed among different age groups and PSA levels (**Table 2**). These results suggest that PLK1 expression does not serve as a significant prognostic marker in this context.

Table 2. Univariate and multivariate analyses of association between PLK1 expression and overall survival.

Variable	*P* values	Hazard ratio	95% confidence interval
Univariate Analysis			
PLK1	0.86	1.09	0.41 – 2.88
Multivariate Analyses			
PLK1	1.00	0.99	0.32 – 3.08
Age	0.27	1.05	0.96 – 1.15
Race	0.25	0.38	0.08 – 1.93
PSA ≥ 20	0.78	1.35	0.16 – 11.68

We also explored the relationship between PLK1 expression and the duration of treatment to determine whether PLK1 expression could potentially serve as a marker for treatment resistance. The analysis revealed no significant statistical correlation between PLK1 expression levels and time to treatment response (*p=0.98*, HR 1.00, 95% CI 0.39-2.56) (**Fig 2**).

 doi:10.20944/preprints202410.1074.v1

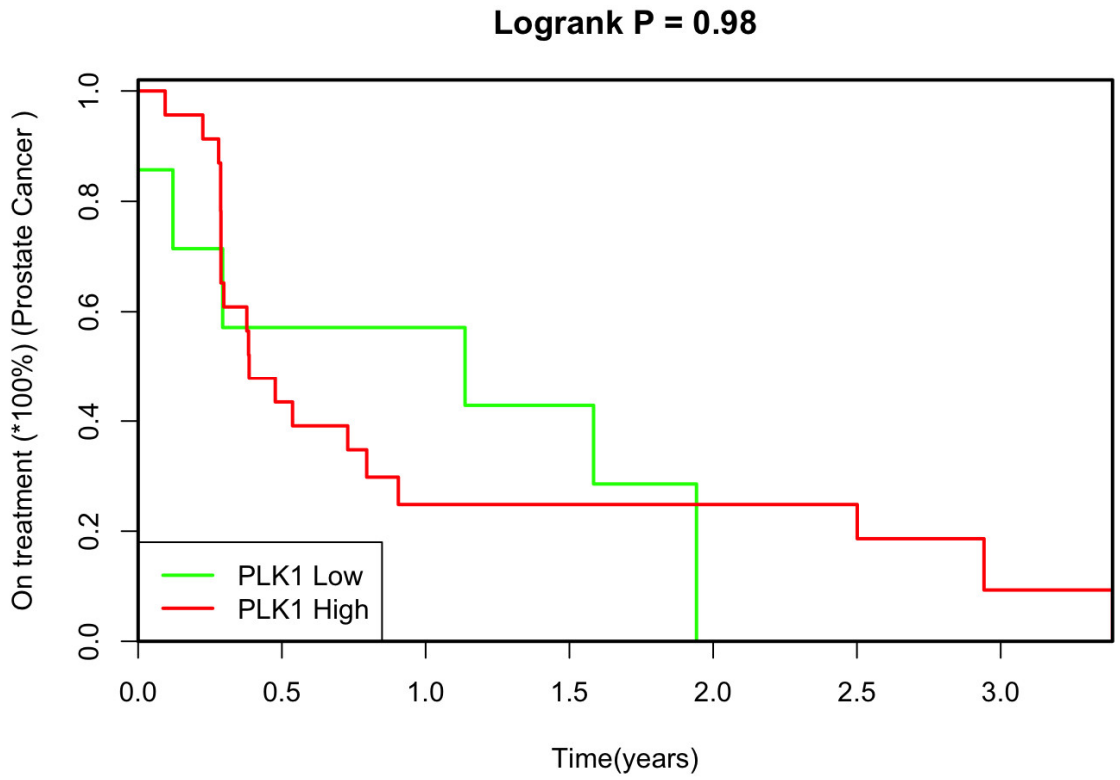

Figure 2. Association between PLK1 and Duration of treatment.

Upon conducting a multivariate analysis and adjusting for various factors, age and PLK1 expression exhibited a positive correlation concerning treatment response (*p=0.02*, HR=0.91, 95% CI 0.85-0.98; *p=0.5*, HR =0.69, 95% CI 0.24 – 2.0, respectively) (**Table 3**). However, no significant correlations were observed between PLK1 expression level, PSA level, race, and the duration to treatment response. These findings suggest that while PLK1 expression levels alone may not directly impact time to treatment response, when considered alongside age, there appears to be a nuanced relationship warranting further investigation.

Table 3. Univariate and multivariate analyses of association between PLK1 expression and duration of treatment.

Variable	P value	Hazard ratio	95% confidence interval
Univariate Analysis			
PLK1	1.00	1.00	0.39 – 2.56
Multivariate Analyses			
PLK1	0.50	0.69	0.24 – 2.03
Age	0.02	0.91	0.85 - 0.98
Race	0.07	4.42	0.87 – 22.37
PSA ≥ 20	0.76	0.77	0.15 – 3.95

Discussion

High PLK1 expression has been reported as a potential prognostic marker for poor response in solid tumors such as ovarian cancer [22], non-small cell lung cancer [23], and breast cancer [24]. We investigated the role of PLK1 expression in PCa prognosis by examining RNA-seq data from a large cohort of patients with PCa. Despite previous immunohistochemical evidence suggesting a link

 doi:10.20944/preprints202410.1074.v1

between high PLK1 expression and higher Gleason grades [9], our findings did not demonstrate a significant association between PLK1 mRNA expression and overall survival in PCa. It also did not serve as a reliable marker for treatment resistance when patients were stratified by PLK1 expression levels.

The lack of a significant association between PLK1 expression and clinical outcomes suggests that PLK1 might not be a standalone prognostic biomarker for PCa. This finding contrasts with previous preclinical studies that highlighted PLK1's role in tumor progression and treatment resistance [10-15]. It is possible that the complexities of *in vivo* tumor environments and the multifactorial nature of treatment resistance cannot be fully captured by PLK1 expression alone.

Several factors may explain the discrepancy between our results and earlier studies that implicated PLK1 in PCa prognosis and treatment resistance. First, the method of PLK1 measurement varies significantly between studies. Immunohistochemistry (IHC) evaluates protein expression and localization within the tissue context, which may reflect the functional state of the protein more accurately than mRNA levels [25-26]. In contrast, RNA-seq provides a quantitative measure of mRNA expression but does not account for post-transcriptional modifications, protein stability, or activity [26-27]. The lack of correlation between PLK1 mRNA and protein levels could be attributed to these post-transcriptional regulatory mechanisms, as well as potential differences in sample handling and analysis techniques [28-29].

Our study cohort included a large and diverse sample of 452 patients with PCa, with a balanced distribution of high and low PLK1 expression cases. Despite the robust sample size, we found no significant differences in key clinical and pathological features, such as age, race, stage, PSA levels, and Gleason grade, between patients with high and low PLK1 expression. This uniformity suggests that PLK1 mRNA expression does not vary significantly across these parameters, further challenging its utility as a prognostic biomarker.

Moreover, our survival analysis, both univariate and multivariate, revealed no significant association between PLK1 mRNA expression and overall survival. The hazard ratios close to 1 (HR 1.09 for univariate and HR 1.12 for multivariate analysis) indicate a lack of prognostic impact, which aligns with the absence of significant differences in clinical features between the two groups. These findings contrast with preclinical studies that identified PLK1 as a key player in PCa treatment resistance, emphasizing the potential limitations of translating preclinical findings directly to clinical outcomes.

The observed inconsistency between PLK1 IHC and RNA-seq data might also reflect the complexity of tumor biology and the influence of the tumor microenvironment [30]. Factors such as hypoxia and other microenvironmental conditions can differentially affect mRNA and protein expression, potentially explaining the divergent findings [30]. Additionally, the technical variability between IHC and RNA-seq, including differences in sensitivity and specificity, may contribute to these conflicting results.

There are several limitations to this study. First, the majority of cases were localized disease, with only 14% representing metastatic disease. The retrospective nature of the study and the small sample sizes in cohorts undergoing hormone treatment and chemotherapy limit the generalizability of the findings. Additionally, treatment duration data was limited to start and stop dates, which may not accurately reflect the actual duration of therapy, and the relatively short median follow-up time of approximately 2.7 years may not be sufficient to capture long-term outcomes and late-onset resistance mechanisms. Future studies with longer follow-up periods and larger, more diverse patient cohorts are necessary to validate our findings and explore potential interactions between PLK1 expression and other molecular pathways involved in PCa progression and treatment resistance.

Conclusions

In conclusion, our study provides important insights into the role of PLK1 expression in PCa. While PLK1 does not appear to serve as a prognostic marker based on RNA-seq data, the complexity of its role in cancer biology warrants further research.

 doi:10.20944/preprints202410.1074.v1

Conflicts of interest: Nothing to disclosed for all authors except EAS: Aura Biosciences data safety monitoring board member.

Author Contributios: Responsible for conceptualization and funding acquisition: SN and ZWM. Responsible for methodology: all authors. Responsible for formal analysis, software and visualization: CW and AXL. Responsible writing – original draft: AD and ZWM. Responsible for writing – review & editing: all authors.

Funding: The study was supported by the University of Kentucky Internal Pilot Grant. Responsible for supervision: ZWM.

Informed Consent Statement: Patient consent was waived due to retrospective nature.

Data Availability Statement:The data presented in this study are available on request from the corresponding author due to privacy and ethical considerations.

Acknowledgments: he Biostatistics and Bioinformatics Shared Resource Facility provided statistical support which is supported by the NCI Cancer Center Support Grant (P30 CA177558).

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