# Molecular and Clinical Characterization of *KLK2* Expression in Prostate Cancer

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## BACKGROUND

- The *KLK2* gene is an androgen related gene, which encodes human kallikrein (hK2), a member of the kallikrein-related peptidases family.
- The *KLK2* gene is located on chromosome 19q13.4, in close proximity to other kallikrein genes, including KLK3, which encodes prostate-specific antigen (PSA).
- The *KLK2* protein shares 80% amino acid homology with PSA, suggesting a common evolutionary origin and potentially coordinated regulation of these proteases.
- *KLK2* is overexpressed in prostate cancer tissue compared to benign prostate tissue and has been associated with stage and grade.
- It exits in both secreted and membrane-associated forms, with the latter being a potential therapeutic target for prostate cancer, which has been demonstrated in preclinical and early clinical studies.
- These findings collectively underscore the need to better characterize the molecular profile of high and low *KLK2* expressing tumors to establish its value as a prognostic and potentially predictive marker.

## OBJECTIVES

- Primary objectives:
  - Evaluate *KLK2* RNA expression across prostate cancer histology, tumor sites, and clinical states.
- Secondary objectives:
  - Evaluate commonly occurring DNA alterations in tumors with high and low *KLK2* expression.
  - Evaluate *KLK2* expression in tumors with high and low AR and NEPC signature scores.
  - Evaluate overall survival in patients with high and low *KLK2* expression.
  - Evaluate time on androgen receptor pathway inhibitor (ARPI) treatment in patients with high and low *KLK2* expression.

## METHODS

- NextGen sequencing of DNA (592-gene/whole exome) and RNA (whole transcriptome) was performed for prostate cancer patient specimens (n=7,078) through Caris Life Sciences (Phoenix, AZ). From this, adenocarcinoma histology (n=7,020) was used exclusively for this analysis except for Figure 1 and Table 1.
- *KLK2*-High/Low expression were defined as percentile of RNA transcripts per million (TPM):
  Q1: < 25$^{th}$ (Low), Q2: 25$^{th}$ - 50$^{th}$ ,
  Q3: 50$^{th}$ - 75$^{th}$ , Q4: ≥75$^{th}$ (High)
- Androgen receptor (AR) and neuroendocrine (NEPC) RNA signature scores were calculated. *KLK2* was removed from the AR signature gene list.
- Castrate resistant prostate cancer (CRPC) and hormone sensitive PC (HSPC) were defined based on androgen deprivation therapy (ADT) duration prior to tissue collection: HSPC < 3 and CRPC ≥ 3 months of ADT start with or without ARPI.
- Kaplan-Meier estimates for real-world overall survival (OS) were calculated from time of diagnosis to last contact or death.
- Time on Treatment (TOT) was calculated from the time of treatment initiation to time of treatment discontinuation for any cause.
- q-value (adjusted p-value) is annotated by *, $q < 0.05$; **, $q < 0.01$; ***, $q < 0.001$; ****, $q < 0.0001$

## RESULTS

**Table 1. Baseline Characteristics.**

| Cohort Characteristics | N (%) |
|---|---|
| **All Samples** | 7078 |
| **Median Age (range)** | 69(35- >89) |
| **Race** | |
| White | 4,482 |
| Black/African American | 1,053 |
| Asian/Pacific Islander | 189 |
| Other | 287 |
| Unknown | 1,061 |
| **Ethnicity** | |
| Hispanic | 585 |
| Not-Hispanic | 5,141 |
| Unknown | 1,352 |
| **Histology** | |
| Prostatic adenocarcinoma | 7,020 |
| Neuroendocrine | 18 |
| Mixed Tumors | 40 |
| **Specimen Site (Adenocarcinoma histology)** | |
| Prostate | 4,464 |
| Lymph Node | 819 |
| Metastasis | 1,737 |

**Figure 1. *KLK2* gene expression in histological subtypes of prostate cancer:** adenocarcinoma, mixed neuroendocrine tumors, and neuroendocrine tumors.

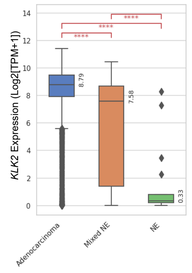



**Figure 2. *KLK2* gene expression in prostate cancer.** (A) *KLK2* expression in prostate cancer, by race, (B) ethnicity, (C) by specimen site: primary, lymph node, metastasis, and (D) by individual specimen sites of prostate cancer.

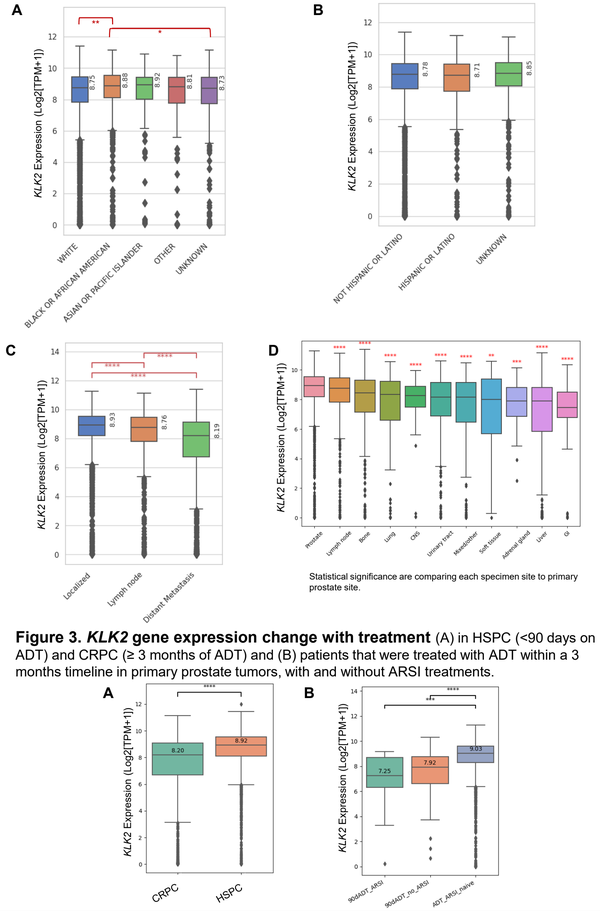



**Figure 3. *KLK2* gene expression change with treatment** (A) in HSPC (<90 days on ADT) and CRPC (≥ 3 months of ADT) and (B) patients that were treated with ADT within a 3 months timeline in primary prostate tumors, with and without ARSI treatments.




**Figure 4. Pathogenic mutations in patients with high versus low *KLK2* expression in (A) HSPC (<90 days on ADT) and (B) CRPC (≥ 3 months of ADT).**

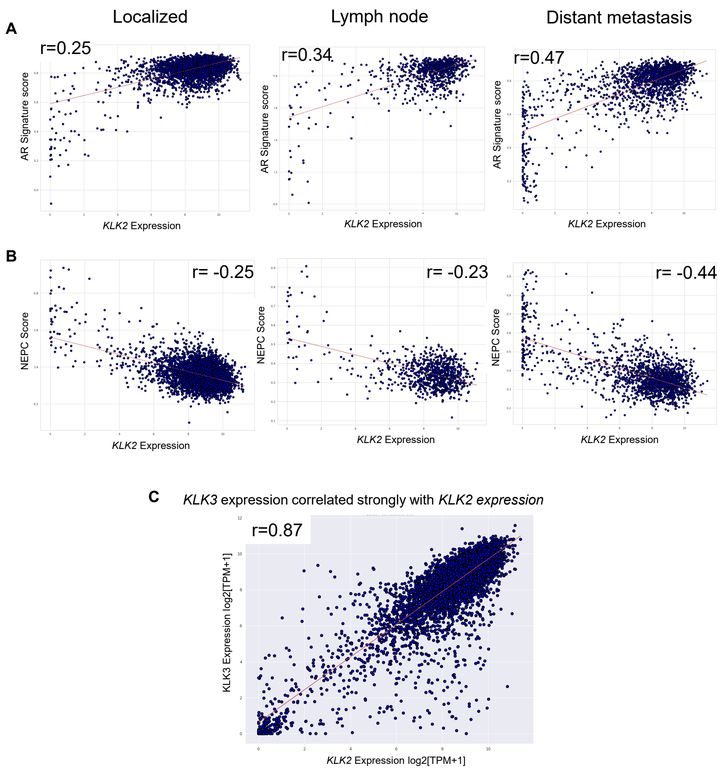
A HSPC (< 3 months on ADT)

| Mutated gene | HSPC-KLK2-Q1 (%Prevalence) | HSPC-KLK2-Q4 (%Prevalence) | q-value |
|---|---|---|---|
| TP53 | 39.54 | 24.83 | <0.0001 |
| RB1 | 6.35 | 1.12 | <0.0001 |
| SPOP | 6.4 | 11.62 | 0.0022 |
| JAK1 | 4.35 | 1.58 | 0.0035 |
| AKT1 | 3.21 | 1.04 | 0.0071 |
| BRCA1 | 1.7 | 0.26 | 0.0076 |
| FANCC | 0.94 | 0 | 0.0093 |
| CTCF | 0.96 | 0 | 0.0101 |
| CTNNB1 | 2.26 | 4.82 | 0.022 |
| PTEN | 9.49 | 6.06 | 0.0305 |
| PTPRD | 13.33 | 0 | 0.0346 |
| MUTYH | 2.46 | 0.92 | 0.0346 |
| SF3B1 | 1.51 | 0.39 | 0.0387 |
| BRCA2 | 2.86 | 5.3 | 0.042 |
| PIK3R1 | 1.71 | 0.53 | 0.0452 |
| CCND1 | 0.56 | 0 | 0.0454 |
| RAD50 | 0.59 | 0 | 0.0472 |
| EPHA2 | 0.62 | 0 | 0.0499 |
| CNOT3 | 0 | 0.82 | 0.0505 |
| MGA | 6.47 | 3.1 | 0.0636 |
| FAT1 | 1.23 | 0.29 | 0.0649 |
| PIK3CA | 4.9 | 2.88 | 0.07 |
| BARD1 | 0 | 0.65 | 0.0748 |
| HRAS | 1.51 | 0.52 | 0.0811 |

B CRPC(≥ 3 months of ADT)

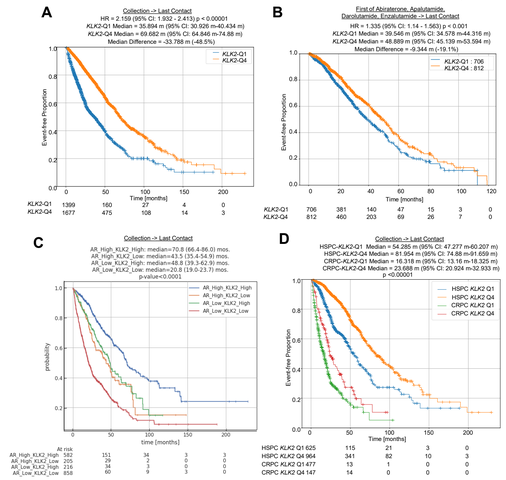
| Mutated Gene | CRPC-KLK2-Q1 (%Prevalence) | CRPC-KLK2-Q4 (% Prevalence) | q-value |
|---|---|---|---|
| RB1 | 18.77 | 1.06 | 0.0001 |
| TP53 | 48.93 | 31.03 | 0.0005 |
| RAD54L | 0 | 2.78 | 0.0019 |
| PIK3CA | 9.22 | 2.68 | 0.0193 |
| CUL3 | 0 | 1.71 | 0.0196 |
| CNOT3 | 0.21 | 2.05 | 0.0326 |
| AIM | 3.17 | 7.38 | 0.0488 |
| CDH1 | 0.42 | 2.04 | 0.0963 |
| ELF3 | 0 | 0.94 | 0.1034 |
| ARHGAP35 | 0.24 | 1.64 | 0.1111 |
| CDC73 | 0 | 0.7 | 0.1194 |
| SMARCE1 | 0 | 0.72 | 0.1216 |
| CCND1 | 0 | 0.67 | 0.1227 |
| KEAP1 | 0 | 0.67 | 0.1227 |
| U2AF1 | 0 | 0.67 | 0.1231 |
| PRDM1 | 0 | 0.67 | 0.1236 |
| ARID2 | 0.21 | 1.37 | 0.1303 |
| BLM | 0.21 | 1.36 | 0.1317 |
| NOTCH1 | 0 | 0.82 | 0.1353 |
| SMAD4 | 0.85 | 2.68 | 0.1363 |
| MUTYH | 1.88 | 0 | 0.1507 |
| PIK3R1 | 1.49 | 0 | 0.2111 |
| AKT1 | 2.71 | 0.67 | 0.2132 |
| PPP2R2A | 0.51 | 1.9 | 0.2363 |
| PIK3R2 | 2.04 | 11.11 | 0.2518 |
| ATRX | 1.38 | 0 | 0.2702 |
| FOXA1 | 6.88 | 10.07 | 0.2869 |

**Figure 5. Correlations between *KLK2* Expression and (A) AR signature scores, (B) NEPC signatures scores, and (C) *KLK3* (PSA).**




## RESULTS

**Figure 6.** Overall survival among *KLK2* high (quartile 4) and *KLK2* low groups (quartile 1), in all prostatic adenocarcinoma cases (A), from first of ARSI treatment until inferred death (B), comparing high vs low AR signature scores and high vs low *KLK2* expression (C), comparing ADT treatment time with high vs low AR signature score, and in patients his HSPC vs HSPC based on treatment (D)




## CONCLUSIONS

- This is the largest analysis to date of *KLK2*-related genomic/transcriptomic features and survival outcomes in prostate cancer.
- Significantly higher *KLK2* expression is observed in black or African American patients, and lower expression in white patients.
- High *KLK2* expression is associated with:
  - Lower mutation prevalence in (genes involved in PI3Kinase pathway) including PIK3CA, PTEN, and PIK3R1
  - Lower mutation prevalence in RB1 and TP53
  - Higher rates of alterations in SPOP
- *KLK2* expression is positively associated with AR score and negatively associated with NEPC score
- Patients with high *KLK2* expression had better outcomes compared to those with low *KLK2* expression and this trend was observed with ARPI treatment
- KLK2 expression was significantly lower in patients that were treated with ADT for more than 3 months, compared to patients that were treated with ADT for less than 3 months
- In samples from patients that were treated with ADT and ARSI within 90 days, *KLK2* expression was significantly lower, compared to those that did not have ARSI treatment
- Tumors with high *KLK2* are molecularly distinct, providing insights for unique therapeutic strategies in this group.

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