AR COPY NUMBER AMPLIFICATION AND AR/KLK3 EXPRESSION PATTERNS REVEAL MECHANISMS OF AR SIGNALING INHIBITOR (ARSI) RESISTANCE AND HIGHLIGHT THE NEED FOR AR-DIRECTED THERAPEUTIC INNOVATION IN METASTATIC CASTRATION-RESISTANT PROSTATE CANCER (MCRPC)

Abstract #7234

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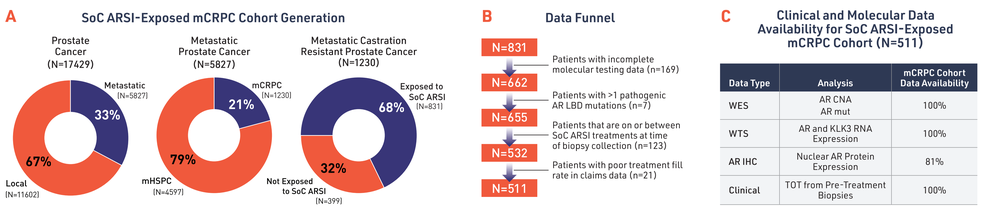
BACKGROUND

- Prostate cancer is the most frequently diagnosed cancer in men in the United States, with ~300,000 new cases annually and 10-20% of patients progressing to metastatic castration-resistant prostate cancer (mCRPC) within 5 years[1].
- The androgen receptor (AR) remains a key driver of tumor growth in mCRPC despite prior hormonal therapies[2]; prostate-specific antigen (PSA), encoded by kallikrein-related peptidase 3 (KLK3), serves as a well-established marker of AR transcriptional activity.
- Resistance to standard of care (SoC) AR-targeting therapies such as androgen deprivation therapy (ADT) and first- and second-generation AR signaling inhibitors (ARSI) is marked by alterations of AR, including AR copy number amplification (CNA) and AR ligand-binding domain (LBD) mutations (mut)[3].
- Gaining a deeper understanding of how AR genomic alterations associate with AR and and KLK3 expression can uncover distinct resistance mechanisms and guide the development of next-generation AR-targeted therapies to address ongoing unmet clinical needs in prostate cancer.

MATERIALS AND METHODS

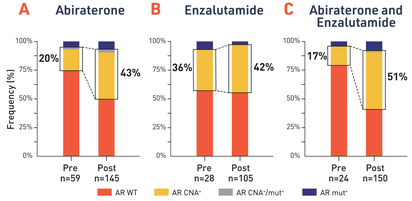
FIGURE 1. Generation of a unique mCRPC cohort exposed to SoC ARSI with robust molecular real-world data availability



- From the Caris Prostate Cancer Database (N=17429), a cohort of SoC ARSI-exposed mCRPC patients with AR-driven disease (N=831) was generated (**A**):
  - Metastatic disease was identified based on patient history or presence of biopsy from a non-prostate site.
  - Clinical claims or a documented history of an ARSI treatment switch within 120 days of biopsy collection were used to distinguish mCRPC from metastatic hormone-sensitive prostate cancer (mHSPC).
  - Exposure to SoC ARSI (abiraterone and/or enzalutamide) was based on clinical claims indicating start or end of treatment within 90 days of biopsy collection.
  - Neuroendocrine prostate cancer (NEPC) was excluded based on histological annotations in patient history or via transcriptional profiling of a NEPC signature[4].
- A data funnel was applied to ensure completeness of molecular and clinical data availability in the SoC ARSI-exposed mCRPC cohort (N=511) (**B**, **C**).
- Whole exome and whole transcriptome sequencing (WES and WTS) were performed on pre- or post-treatment biopsies using the Caris Next Generation Sequencing platform; AR protein expression was analyzed via immunohistochemistry (IHC, SP107 clone); AR transcriptional activity was determined via KLK3 (PSA) RNA expression (**C**).
- Patients were stratified by genomic alterations into AR copy number amplified (AR CNA$^+$, cut-off = 2), AR ligand-binding domain mutant (AR mut$^+$; including L702H, W742L, W742C, H875Y, F877L, T878A, and T878S), or AR wild-type (AR WT; AR CNA$^-$/AR mut$^-$). RNA expression subgroups were defined using median cut-offs for AR and KLK3.
- Kaplan-Meier analysis and log-rank tests were used to evaluate associations of AR CNA and AR RNA expression with time on ARSI treatment (TOT, Abiraterone and/or Enzalutamide) and overall survival (OS).
- Analyses of the full cohort (N=511) are presented in **Figures 2–5**; consistent trends were observed in separate pre- and post-treatment analyses (data not shown).

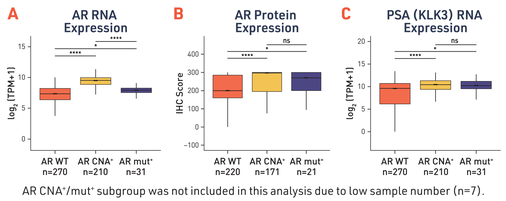
RESULTS

FIGURE 2. ARSI treatments increase the prevalence of AR genetic alterations



- AR CNA is the most frequent AR alteration post ARSI (42-51%, indicated by the outlined black boxes).
- A minority of patients have AR mut (3-8%) or a combination of AR mut and AR CNA (0-3%) post ARSI.

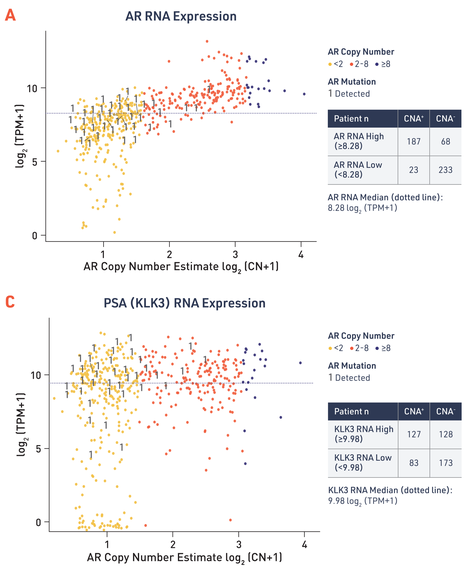
FIGURE 3. AR genetic alterations are accompanied by elevated AR expression and activity in mCRPC



AR CNA$^+$/mut$^+$ subgroup was not included in this analysis due to low sample number (n=7).

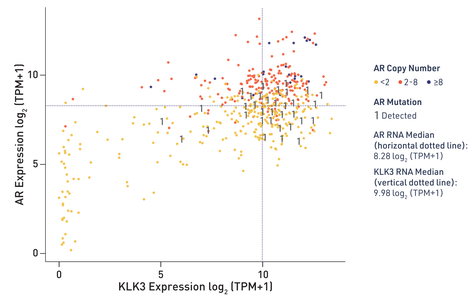
- Compared to AR WT, AR CNA$^+$ and AR mut$^+$ subgroups had higher median AR RNA expression (7.4 vs 9.5 and 7.9 $\log_2$ (TPM+1), p<0.001 and p<0.05) (**A**), AR protein expression (200 vs 297 and 270 IHC score, p<0.001 and p<0.05) (**B**), and KLK3 expression (9.6 vs 10.4 and 10.2 $\log_2$ (TPM+1), p<0.001 and p<0.05) (**C**).

FIGURE 4. AR expression and activity is consistently high in patients with AR CNA



- AR RNA expression positively correlates with AR copy number (ρ=0.8, p<0.0001); 89% (187/210) AR CNA$^+$, 26% (10/38) AR mut$^+$, 30% (62/270) AR WT and 51% (259/511) overall mCRPC patients have high AR RNA expression (**A**).
- AR protein expression is positively associated with AR copy number (Fisher's Exact Test OR= 3.1, 95% CI: 1.6-6.3, p<0.0001); 92% (157/171) AR CNA$^+$, 96% (27/28) AR mut$^+$, 76% (168/220) AR WT and 84% (346/412) overall mCRPC patients have high AR protein expression (**B**).
- AR transcriptional activity is positively associated with AR copy number (Fisher's Exact Test OR= 2.1, 95% CI: 1.4-3.0, p<0.0001); 60% (127/210) AR CNA$^+$, 61% (23/38) AR mut$^+$, 41% (111/270) AR WT and 51% (261/511) overall mCRPC patients have high KLK3 RNA expression (**C**).

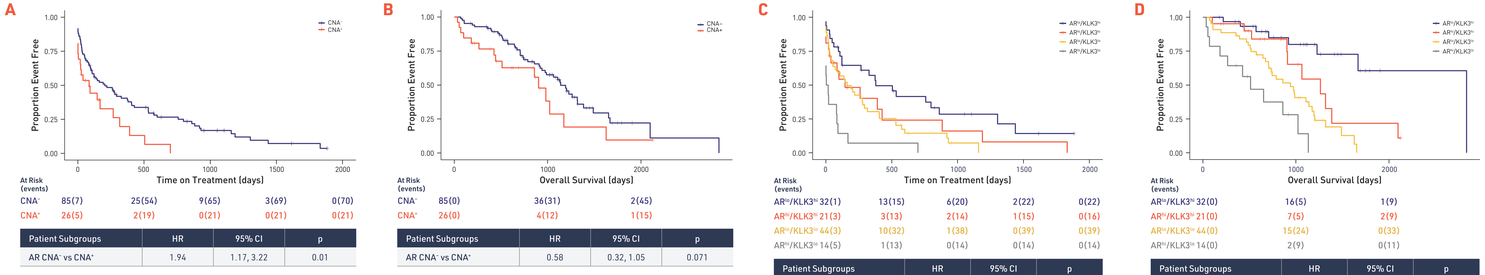
FIGURE 5. A majority of patients with AR CNA have AR^{hi}/KLK3^{hi} tumors



N, (% of total)	Total	AR^{lo}/KLK3^{lo}	AR^{lo}/KLK3^{hi}	AR^{hi}/KLK3^{lo}	AR^{hi}/KLK3^{hi}
All mCRPC	511 (100)	157 (31)	98 (19)	98 (19)	158 (31)
AR WT	270 (53)	40 (15)	71 (26)	22 (8)	137 (51)
AR CNA	210 (41)	115 (55)	12 (6)	72 (34)	11 (5)
AR mut	31 (6)	2 (6)	15 (48)	4 (13)	10 (32)

- AR RNA positively correlates with KLK3 RNA expression (ρ=0.4, p<0.0001).
- The most prevalent RNA subgroups were AR^{hi}/KLK3^{hi} (157/511, 31%), and AR^{lo}/KLK3^{lo} (158/511,31%) followed by AR^{lo}/KLK3^{hi} (98/511, 19%) and AR^{hi}/KLK3^{lo} (98/511, 19%).
- Highest enrichment was observed for AR CNA$^+$ in AR^{hi}/KLK3^{hi} (55%), for AR mut$^+$ in AR^{lo}/KLK3^{hi} (48%), and for AR WT in AR^{lo}/KLK3^{lo} (51%) subgroups.

FIGURE 6. An unmet need persists in the context of SoC ARSI treatment for mCRPC patients with high AR expression and activity



- AR CNA$^+$ patients, who exhibit highest AR RNA and AR protein expression (**Figures 3** and **4**) prior to ARSI treatment, have significantly shorter TOT (HR-1.94, 95% CI:1.17-3.22, p=0.01) (**A**) and a trend toward shorter OS (HR 0.58, 95% CI:0.32-1.05, p=0.071) (**B**) compared to AR CNA$^-$ patients.
- Patients with AR^{lo}/KLK3^{hi} tumors, enriched for AR mut (**Figure 5**), had the best response to ARSI with prolonged TOT and best OS compared to all other RNA expression subgroups (**C**, **D**).
- Patients with AR^{hi}/KLK3^{hi} tumors, enriched for AR CNA (**Figure 5**), had significantly shorter OS (HR=2.79, 95% CI 1.05-7.42, p=0.04) compared to patients with AR^{lo}/KLK3^{hi} tumors (**D**).
- Increased AR expression leads to poorer clinical outcomes on ARSI: AR^{hi}/KLK3^{hi} patients benefit less than AR^{lo}/KLK3^{hi} (OS HR=2.79, 95% CI:1.05-7.42, p=0.040) and AR^{hi}/KLK3^{lo} benefit less than AR^{lo}/KLK3^{lo} patients (TOT HR=2.69, 95% CI:1.44-5.01, p=0.002 and OS HR=2.31, 95% CI: 1.14-4.66, p=0.02) (**C**, **D**).

CONCLUSIONS

- AR signaling, characterized by high AR expression and activity (KLK3 expression), persists in a majority of mCRPC patients despite SoC ARSI treatments.
- AR CNA is the most frequent post-ARSI alteration in mCRPC tumors and the primary driver of elevated AR signaling and therapeutic resistance.
- A high-risk, AR-driven subgroup defined by elevated AR expression and activity, largely associated with AR CNA, shows rapid progression on ARSI, highlighting the need for novel therapies that overcome persistent AR signaling.
- FX-111, a selective degrader of transcriptionally active, hormone-bound AR (AR_{ON}), has completed IND-enabling studies at Flare Therapeutics, and represents a promising strategy to overcome key resistance mechanisms to ARSI. Ongoing analyses of AR/KLK3 subgroups aim to further elucidate biological drivers of differential responses to ARSI.

References **1.** Shore N.D. et al., Real-World Treatment Patterns and Overall Survival of Patients with Metastatic Castration-Resistant Prostate Cancer in the US Prior to PARP Inhibitors, *Adv Ther.* (2021) Jul; 19;38(8):4520-4540. **2.** Seguier D. et al., Emergence of Neuroendocrine Tumors in Patients Treated with Androgen Receptor Pathway Inhibitors for Metastatic Prostate Cancer: A Systematic Review and Meta-analysis, *Eur Urol Oncol.* (2025), Apr; 8(2); 581-590. **3.** Einstein D.J. et al., Targeting the Androgen Receptor and Overcoming Resistance in Prostate Cancer, *Curr Opin Oncol.* (2019) May; 31(3): 175-182. **4.** Beltran H. et al., Divergent Clonal Evolution of Castration Resistant Neuroendocrine Prostate Cancer, *Nat Med.* 2016 Feb 8;22(3):298-305.

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