# Molecular Profiling of Advanced Refractory Prostate Cancer

Rebecca Feldman Ph.D.[1], Gargi Basu Ph.D. [1], Joanne Xiu Ph.D. [1], David Arguello M.D. [1], Sherri Z. Millis Ph.D. [1], Ryan Bender Ph.D. [1], Zoran Gatalica M.D. [1], Les Paul M.D. [1], Charles Myers M.D. [2]
Caris Life Sciences, Phoenix, AZ[1], American Institute for Diseases of the Prostate, Earlysville, VA[2], Foundation for Cancer Research and Education, Charlottesville, VA[2]

## Abstract

**Background**: Prostate cancer is the second leading cause of cancer-related death among men in the US. Forty percent of men diagnosed will develop metastatic disease which has few treatment options. We aim to describe the molecular profile of prostate cancer tumors and potential for novel therapeutic options.

**Methods**: We reviewed profiling data of over 330 patients from a large referral laboratory (Caris Life Sciences, Phoenix, AZ) for information on biomarkers of drug response. Multiple methodologies were employed: sequencing (NGS, Sanger, pyrosequencing), *in-situ* hybridization (fluorescent and chromogenic) and immunohistochemistry (IHC).

**Results**: High expression was observed for AR, MRP1, TOPO1, TLE3 and EGFR, with positivity rates of 89%, 87%, 63%, 48% and 47%, respectively. Low expression was observed for TS, PGP, TUBB3, RRM1, PTEN and MGMT, with negativity rates of 94%, 87%, 75%, 69%, 54% and 45%, respectively. Gene copy number increases for EGFR and cMYC were observed in 13% of patients. Sequencing data showed 48% mutation rate for TP53, 18% for PTEN, 9% for CTNNB1, 8% for PIK3CA, 5% for RB1, ATM and cMET, and ~2% for K/HRAS, ERBB4, ALK, BRAF and cKIT. Regarding targeted therapy options, imatinib may be considered for patients with high cKIT or PDGFRA (9-10%), and cetuximab for patients with EGFR positivity (13-47%). Promising agents may be considered, including cabozantinib, based on 4% of cohort with cMET aberrations or PAM pathway inhibitors (BEZ234, everolimus) based on ~30% of cohort with PIK3CA pathway activation. Lastly, HDAC inhibitors have recently been linked to cMYC driven cancers (13% amplified). Chemotherapies including 5-FU, gemcitabine and temozolomide may be options based on ~70% of cohort with low TS, RRM1 or MGMT. Biomarker guidance for common prostate cancer drugs is also provided, including cabazitaxel, based on ~70% of cohort with low TUBB3 or PGP, or high TLE3. Finally, continued dependence on androgen signaling is exhibited by 89% of cohort with high AR, indicating potential utility of anti-androgen agents like enzalutamide.

**Conclusion**: Tumor profiling identified small subsets of patients that may benefit from targeted agents approved for other solid tumors (imatinib, cetuximab), promising therapies in clinical trials (cabozantinib) or agents not routinely used for prostate cancer (gemcitabine). By combining the biomarker results of IHC, ISH and NGS, we identified subgroups that might benefit from combining traditional chemotherapies and hormonal agents with novel targeted agents.

## Background

Prostate cancer remains to be a leading cause of cancer-related death in men. Although 60% of prostate cancers diagnosed are considered indolent, 40% are aggressive and require multiple lines of treatment. The lack of distinguishing factors that differentiate indolent vs. aggressive subtypes of prostate cancer leads to unnecessary treatments and surgeries for some men.

Prostate cancer is largely driven by androgen receptor signaling, therefore, androgen deprivation therapy is a mainstay of treatment. Despite initial effectiveness, androgen deprivation therapy invariably leads to the emergence of castration resistant disease, which is highly aggressive and treatment-refractory. Identifying the molecular mechanisms in all stages of prostate cancer, therefore, can direct therapy and may result in the introduction of new molecular alterations to target.

Molecular profiling using multiple platforms is a comprehensive approach in identifying molecular aberrations that could be targeted by (1) agents considered standard of care for prostate cancer, (2) FDA-approved agents used in other solid tumors, (3) novel targeted therapies currently in clinical trial or (4) combination treatment strategies combining novel targeted agents with traditional therapies.

## Methods

All 388 prostate cancer cases referred to Caris Life Sciences between 2009 thru 2013 from 50 states and 59 countries were evaluated; diagnoses were collected from referring physicians and classified at intake based on pathology and clinical history. Specific testing was performed per physician request and included a combination of sequencing (Sanger or Next-generation sequencing [NGS]), protein expression (immunohistochemistry), gene amplification (CISH or FISH).

## Results

****Full analysis involved an additional 58 patients profiled since abstract submission**

| Number of Patients | 388 | |
|---|---|---|
| Age, years: median (range) | 66 (32-95) | |
| Specimen Site | Prostate (n=170) | Blood and bone marrow (n=9) |
| | Lymph nodes (n=56) | Retroperitoneum and Peritoneum (n=6) |
| | Bone (n=39) | Brain/Spine (n=4) |
| | Liver (n=38) | Colon/Rectum (n=4) |
| | Connective and Soft Tissue (n=17) | Adrenal gland (n=4) |
| | Chest/Lung (n=13) | Other (testis, skin, pelvis, iliac crest, pleura, presacral region, omentum, epidymis) (n=17) |
| | Bladder (n=11) | |

**Table 1– Patient Characteristics** – samples profiled represent the full range of the disease as evidenced by 170 localized prostate cancers and 218 metastatic prostate cancers. Majority of specimens profiled are from treatment-refractory patients.

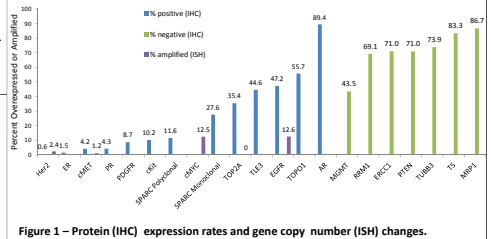


**Figure 1 – Protein (IHC) expression rates and gene copy number (ISH) changes.** Expression rates correlate with theranostic utility of associated drugs. Average number of samples tested by IHC was 264; average number of samples tested by ISH was 50. Targeted therapies not commonly used in prostate cancer may be considered in patients demonstrating EGFR overexpression and gene amplification, e.g. cetuximab; HER2 overexpression or gene amplification, e.g. trastuzumab; cKIT and PDGFRA overexpression, e.g. imatinib; cMET overexpression and gene amplification e.g. cabozantinib. Conversely, low expression of MGMT, RRM1 and TS, correlate with benefit with temozolomide, gemcitabine and fluorouracil, respectively. Profiling also reveals potential clinical benefit of commonly used prostate cancer drugs including taxanes based on TUBB3 and TLE3, anti-androgens based on AR and platinum agents based on ERCC1.

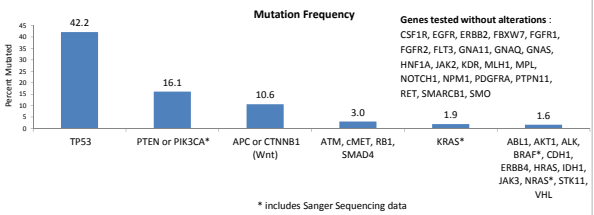


**Figure 2 – Mutational profile of prostate cancer using next-generation and sanger sequencing.** Average number of samples tested by NGS and Sanger was 65. The most commonly mutated pathways in prostate cancer are the TP53, PI3K/PTEN and Wnt signaling pathways. Currently, agents targeting these pathways are under clinical investigation.

| Protein or Gene Copy Number Changes | Associated Single Agent Therapy | Potential Modulators of Response or Additional Alterations Identified by CMI | Potential Combination Strategies Under Investigation |
|---|---|---|---|
| 83% TS IHC ↓ | fluorouracil | 38% (19/50) TS ↓ exhibit TP53 mutations | fluorouracil + ChK1 i or Wee1 i (Xiao, et al. 2005) |
| 69% RRM1 IHC ↓ | gemcitabine | 40% (17/45) RRM1 ↓ exhibit TP53 mutations | gemcitabine + ChK1 i (Blackwood, et al. 2013) |
| 56% TOPO1 IHC ↑ | irinotecan, topotecan | 47% (17/36) TOPO1 ↑ exhibit TP53 mutations<br>33% (18/54) TOPO1 ↑ exhibit BCRP ↑ | topotecan + ChK1 i (Xiao, et al. 2005) |
| 47% (17/36) EGFR IHC ↑ | cetuximab | 82% (14/17) EGFR ↑ exhibit PTEN ↓ | cetuximab + PIK3CA i (Cathomas, et al. 2012) |
| 12.6% EGFR ISH ↑ | cetuximab | 77% (10/13) EGFR ↑ exhibit PTEN ↓ | cetuximab + PIK3CA i (Cathomas, et al. 2012) |
| 89% (322/360) AR ↑ | anti-androgens | 68% (213/311) AR ↑ exhibit PTEN ↓<br>24% (6/24) AR ↑ exhibit PTEN ↓ and PTEN or PIK3CA mutations | anti-androgen + PIK3CA/AKT/mTOR pw i (NCT01642732) |
| 71% (259/365) PTEN ↓ | PIK3CA/AKT/ mTOR inhibitors | 18% (5/28) exhibit Wnt pw mutations | PIK3CA/AKT/mTOR pw i + Wnt pw i |
| 21% (21/92) TUBB3 ↓ and TLE3 ↑ | cabazitaxel, docetaxel | 75% (3/4) TUBB3 ↓ and TLE3 ↑ exhibit ERCC1 ↓<br>98% (20/21) TUBB3 ↓ and TLE3 ↑ exhibit AR ↑ | cabazitaxel + carboplatin (NCT01505868)<br>docetaxel + anti-androgen (NCT01400555) |

i = inhibitor; pw = pathway
NCT #, refer to www.clinicaltrials.gov

**Table 2. Identification of modulators of response and potential combination strategies under investigation.** Response rates to single agents range from 20-30% for fluorouracil, gemcitabine and topotecan, whereas traditional prostate cancer treatments like anti-androgens and taxanes convey significant survival advantage as monotherapy. Preclinical findings and preliminary clinical data suggest that combining novel targeted agents with traditional treatments may achieve additive or synergistic effects. Utilization of IHC, ISH and mutational analysis results can identify combination treatment strategies currently being investigated in clinical trials.

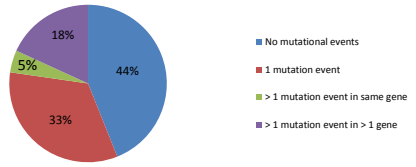


**Figure 3 – Frequency of mutations in prostate cancer.** Next-generation sequencing data was available for 66 patients. Forty-four percent (n=29/66) of advanced prostate cancers lack actionable gene mutations; 96% of which have actionable targets identified by IHC and ISH platforms. Twenty-three percent of prostate cancers exhibit multiple mutations, either double mutations in single genes, or single mutations in more than one gene. Eighty-two percent (14/17) of patients with > 1 mutation are derived from metastatic specimen sites.

## Conclusions

- 388 prostate cancer samples, including advanced, localized and metastatic disease, were profiled with a multi-platform approach using immunohistochemistry, *in situ* hybridization and mutational analysis tests.
- The most commonly mutated pathways in prostate cancer include TP53, PTEN/PIK3CA and Wnt signaling pathways. Forty-four percent of prostate cancers lack actionable gene mutations. 96% of these patients have actionable targets identified by IHC and ISH platforms.
- Multiple agents are identified as having potential clinical benefit including agents considered standard of care, as well as FDA-approved agents for other solid tumors.
- Comprehensive molecular analysis of prostate cancer guides integration of traditional chemotherapy and anti-androgens with novel targeted agents.
- The Caris analysis includes measurement of the major molecular changes known to drive prostate cancer progression, providing the practicing physician with a robust, validated means of determining which of these changes are active in the patient under their care. As targeted drug development provides drugs capable of targeting these changes, the clinician can tailor treatment to their patient with steadily increasing precision.

## References

1. Karayi, MK and AF Markham. (2004). "Molecular biology of prostate cancer." *Prostate Cancer and Prostatic Diseases* 7:6-20.
2. Roychowdhury, et al. 2013. "Advancing Precision Medicine for Prostate Cancer Through Genomics." *J Clin Oncol 31: 1866-73.*
3. Zelefsky, et al. (2012). "Prostate cancer: Challenges in selecting the optimal therapy." *Nat Rev Urology* 9:674-676.
4. Witte, et al. (2009). "Prostate cancer genomics: towards a new understanding." Nat Rev Genetics 10:77-82.