

## Is androgen receptor activity in metastatic prostate cancer a good biomarker for bipolar androgen therapy?

Nahuel Peinetti, ... , Marijo Bilusic, Kerry L. Burnstein

*J Clin Invest.* 2022;132(23):e165357. <https://doi.org/10.1172/JCI165357>.

### Commentary

Androgen deprivation therapy (ADT) is the longstanding treatment for advanced prostate cancer (PC) because androgen receptor (AR) is the key therapeutic vulnerability for this disease. Bipolar androgen therapy (BAT) — the rapid cycling of supraphysiologic androgen (SPA) and low serum testosterone levels — is an alternative concept, but not all patients respond and acquired resistance can occur. In this issue of the *JCI*, Sena et al. developed a gene signature indicative of high AR activity to predict patient response to BAT, including a decline in both serum prostate-specific antigen (PSA) and tumor volume. Preclinical models showed that AR-mediated suppression of MYC, known to drive PC, was associated with decreased cell growth following SPA treatment. Because BAT eventually leads to resistance, the authors tested cycling between SPA and AR antagonism in a patient-derived xenograft and observed a delay in tumor growth. These findings represent a major step toward the informed use of BAT for advanced PC.

**Find the latest version:**

<https://jci.me/165357/pdf>



# Is androgen receptor activity in metastatic prostate cancer a good biomarker for bipolar androgen therapy?

Nahuel Peinetti,<sup>1,2</sup> Marijo Bilusic,<sup>2,3</sup> and Kerry L. Burnstein<sup>1,2,4</sup>

<sup>1</sup>Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, Florida, USA. <sup>2</sup>Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida, USA.

<sup>3</sup>Department of Medicine, Division of Medical Oncology, University of Miami Miller School of Medicine, Miami, Florida, USA. <sup>4</sup>Department of Veterans Affairs, Miami, Florida, USA.

**Androgen deprivation therapy (ADT) is the longstanding treatment for advanced prostate cancer (PC) because androgen receptor (AR) is the key therapeutic vulnerability for this disease. Bipolar androgen therapy (BAT) — the rapid cycling of supraphysiologic androgen (SPA) and low serum testosterone levels — is an alternative concept, but not all patients respond and acquired resistance can occur. In this issue of the *JCI*, Sena et al. developed a gene signature indicative of high AR activity to predict patient response to BAT, including a decline in both serum prostate-specific antigen (PSA) and tumor volume. Preclinical models showed that AR-mediated suppression of MYC, known to drive PC, was associated with decreased cell growth following SPA treatment. Because BAT eventually leads to resistance, the authors tested cycling between SPA and AR antagonism in a patient-derived xenograft and observed a delay in tumor growth. These findings represent a major step toward the informed use of BAT for advanced PC.**

## Overcoming castration resistance using supraphysiologic androgens

Because androgen receptor (AR) signaling promotes growth and survival of prostate cancer (PC), blocking AR activation has been the cornerstone of treatment for more than 70 years. Androgen deprivation therapy (ADT) decreases the synthesis of the two major AR ligands, testosterone and dihydrotestosterone (DHT) using luteinizing hormone-releasing hormone (LHRH) agonists or LHRH antagonists. Despite initial therapeutic benefits, the disease inevitably progresses to an incurable stage termed castration-resistant PC (CRPC). This ultimate stage arises through a variety of mechanisms

that promote persistent or reactivated AR signaling (reviewed in refs. 1, 2). Many men with CRPC respond to newer generation AR signaling inhibitors (ARSIs) such as the AR antagonists enzalutamide, apalutamide, and darolutamide, or the androgen biosynthesis inhibitor abiraterone acetate, but inevitably progress within one to two years (2). Thus, the discovery that supraphysiologic concentrations of androgens (SPA) could block the growth of PC was seemingly paradoxical. However, the well documented but incompletely understood phenomenon in which PC cell proliferation is stimulated by low — but inhibited by high — androgen levels provided a foundation for the use of SPA therapy (reviewed in ref. 3).

SPA treatment, similar to ADT and other targeted therapies, ultimately results in therapeutic resistance for preclinical CRPC models and PC patients (4). To overcome tumor adaptation to high testosterone levels, Samuel Denmeade and John Isaacs pioneered bipolar androgen therapy (BAT) in which SPA is pulsed with continuous ADT in order to cycle serum testosterone between high — supraphysiologic — and low — castrate — levels (4). Several clinical trials have demonstrated that BAT is safe in asymptomatic patients and can produce durable prostate specific antigen (PSA) and objective responses in approximately 30%–40% of PC patients (5–9). Since not all patients respond to BAT — as determined by decreased serum PSA or tumor volume, or by longer radiographic progression-free survival — and the degree and durability of response are unpredictable, identification of prognostic and predictive biomarkers is needed (10, 11).

## High AR activity is associated with clinical response to BAT

In this issue of the *JCI*, Sena et al. leveraged sequential paired metastatic specimens, termed “pre-BAT” and “on-BAT”, that were collected from an ongoing clinical trial COMBAT-CRPC (NCT03554317). This single arm, phase II clinical trial enrolled patients with metastatic CRPC whose cancer progressed on an ARSI and treated them with BAT for 12 weeks, followed by a combination treatment of BAT and the anti-PD1 agent nivolumab (12). Specimens for this study were collected prior to the initiation of treatment with nivolumab.

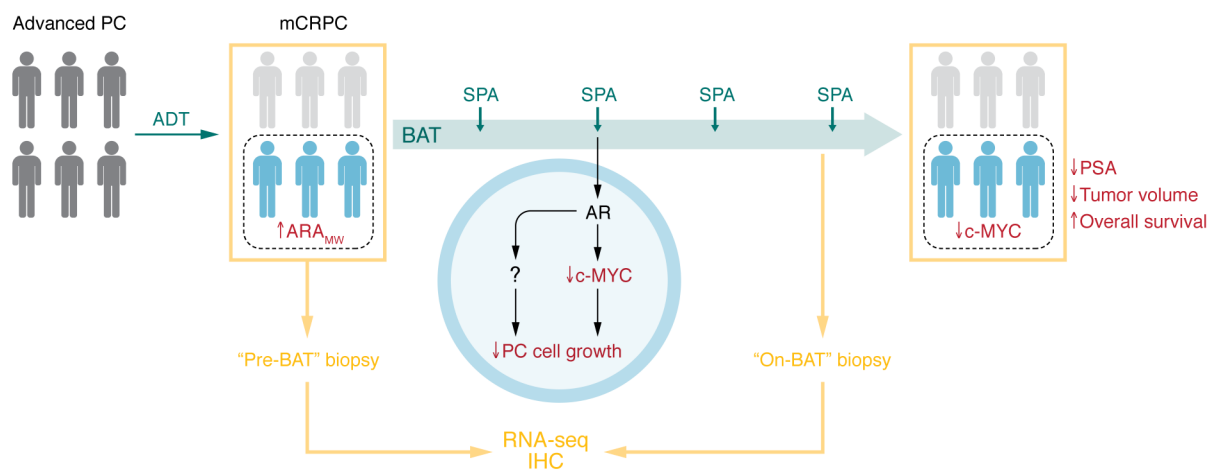
Using PC patient databases (13–15), the authors identified ten canonical AR target genes and applied Mann-Whitney ranking to generate a signature score, which the authors termed  $ARA_{MW}$ . When applied to the COMBAT-CRPC pretreatment sample RNA-Seq data, a high pretreatment  $ARA_{MW}$  score predicted clinical responses to BAT in the cohort of 15 patients, suggesting that  $ARA_{MW}$  can serve as a biomarker for BAT

► **Related Article:** <https://doi.org/10.1172/JCI162396>

**Conflict of interest:** MB reports research funding from Bavarian Nordic, Immunity Bio, Next Cure, Merck, and Surface Oncology. KLB reports ownership of stock holding in Bristol Myers Squibb. KLB is also listed as an inventor on a patent titled, “Use of arginine vasopressin receptor antagonists for the treatment of prostate cancer” (US patent 10, 231, 952) and on the patent application “Biomarkers Indicative of Prostate Cancer and Treatment Thereof” (international patent application PCT/US2018/026097).

**Copyright:** © 2022, Peinetti et al. This is an open access article published under the terms of the Creative Commons Attribution 4.0 International License.

**Reference information:** *J Clin Invest.* 2022;132(23):e165357. <https://doi.org/10.1172/JCI165357>.



**Figure 1. ARA<sub>MW</sub> is a potential biomarker of BAT response in metastatic CRPC.** Patients with advanced PC are commonly treated with ADT but generally progress to incurable CRPC. The COMBAT-CRPC clinical trial enrolled patients with metastatic CRPC (mCRPC) and treated them with BAT for 12 weeks with three cycles of SPA with ongoing ADT. Sena et al. performed RNA-Seq and IHC analysis of metastatic tumor biopsies obtained from patients before treatment and after the three cycles of BAT. The authors used RNA-Seq data to demonstrate that high ARA<sub>MW</sub> in pretreatment metastatic biopsies was associated with BAT response, including lower circulating PSA, decreased tumor volume, and higher overall survival (OS). IHC data showed that c-MYC was decreased in patients who responded to BAT. Experiments using PC cell lines showed that SPA, acting through the AR, decreased c-MYC and reduced cell growth, although other AR-regulated factors in addition to c-MYC may also be involved.

response (12) (Figure 1). No collinearity was found among the genes, and ARA<sub>MW</sub> did not correlate with AR levels across patients.

Sena et al. also provide valuable patient data for other investigators to interrogate with respect to BAT response (12). For example, Qiu et al. (11) defined a pretreatment AR cistrome that predicts response to SPA in PC patient-derived xenografts (PDXs), but, interestingly, this signature does not contain canonical AR target genes nor does it overlap with ARA<sub>MW</sub>.

In addition to the paired pre- and on-BAT biopsies of metastases from patients with CRPC, the authors used PC cell lines and a CRPC PDX to explore possible mechanisms underlying BAT responsiveness. While PC cell line-based experiments showed that high levels of AR and AR activity were required for SPA response, as previously observed (3, 16), patient data demonstrated that AR activity, and not AR abundance, predicted BAT response (12). This finding may be explained by the fact that AR protein levels do not necessarily correlate with AR transcriptional activity due to different CRPC adaptation mechanisms, such as AR activating mutations, changes in AR coregulators, and expression of constitutively active AR variants.

*Molecular mechanisms of response and resistance to SPA and BAT.* Sena et al. demonstrated that SPA response in sensitive PC cell lines was at least partially dependent

on an AR-mediated decrease in c-MYC (also referred to as MYC), which is highly expressed in PC. Further, a PDX that responded to SPA, as indicated by decreased tumor volume, showed decreased MYC. Similarly, patients who responded to BAT exhibited a larger decrease in MYC compared with nonresponders. Additionally, patients with decreased MYC also correlated with those that had higher AR activity before treatment (12). Although the mechanism through which SPA decreases MYC in patients is unknown, a recent report using PC cell lines demonstrated that AR decreases MYC transcription independently of AR chromatin binding. This reduction in the transcription of MYC involves coactivator redistribution and perturbation of the interaction between the MYC super enhancer at the *PCAT1* gene and the MYC promotor (17).

While SPA-mediated suppression of MYC is a leading proposed mechanism underlying BAT response, studies in pre-clinical models have implicated alternative mechanisms, including impaired DNA licensing, which is a process where high levels of ligand-stabilized AR bind to the origins of DNA replication and promote early arrest in S-phase (18). Additional processes include induction of apoptosis and promotion of senescence (3). Since SPA produces AR-mediated DNA double-strand breaks leading to

halted cell growth in several PC models, a potential vulnerability to PARP1 inhibitors may exist (10, 19). While the authors did not identify BAT-mediated alterations in the expression of a panel of homologous recombination repair-related (HRR-related) genes in the patient samples (12), an ongoing clinical trial combining BAT with the PARP1 inhibitor olaparib (NCT03516812) will address this important issue.

Alterations in androgen metabolism can accompany PC progression, including tumor switching from testosterone to androstenedione as the preferred precursor for DHT production (20). Additionally, the common germline variant 1245A>C in the enzyme encoded by *HSD3B1* leads to increased intratumoral DHT levels and promotes resistance to ADT and abiraterone treatment (21). This resistance mechanism is independent of changes in AR, and patients with this variant may not benefit from BAT treatment. Although it remains unknown whether alterations in androgen metabolism affect BAT response, a recent clinical trial (RESTORE-NCT02090114), showed that patients whose cancer progressed on enzalutamide had better response to BAT (PSA<sub>50</sub> response and longer progression free survival 2 (PFS-2)) than men whose cancer had progressed on abiraterone (8).

## Clinical implications

While initial results from BAT clinical trials have been promising, most metastatic CRPC patients will develop secondary resistance after approximately 6–12 months of therapy (6). This publication and others suggest that resistance to SPA treatment is mediated by changes in levels and activity of AR (11). It is well known that AR is autoregulated—ligand-activated AR typically downregulates AR gene transcription, and, conversely, androgen stabilizes AR protein levels (22–26). SPA may perturb normal AR autoregulatory processes. Sena et al. reported that SPA eventually resulted in decreased levels and activity of AR as well as loss of MYC suppression, leading to treatment resistance in a PC cell line (12). Interestingly, treatment of the derived isogenic SPA-resistant PC cell line with the potent AR antagonist enzalutamide upregulated AR, which resensitized cells to SPA. This finding led the investigators to test alternating SPA with enzalutamide to produce extreme oscillations in AR activity in the CRPC PDX model. Castrated mice treated with this SPA/enzalutamide paradigm did not exhibit resistance for the duration of the experiment (160 days) (12). Additionally, tumors from this SPA/enzalutamide arm had lower levels of MYC than those from mice that only received SPA (12). The cycling of BAT with ARSIs may provide additional benefits to patients and is one of the hypotheses of the upcoming STEP-UP clinical trial (NCT04363164).

To date, approximately 350 patients with metastatic CRPC were treated with BAT in three phase II trials (TRANSFORMER, RESTORE, and COMBAT) (9). Those trials demonstrated that BAT (a) can be safely given to asymptomatic patients, (b) is moderately effective, and (c) has the potential to resensitize CRPC to subsequent therapy with ARSIs. As discussed above, the vast majority of metastatic CRPC patients will eventually develop secondary resistance to BAT (6). To improve the efficacy and duration of response, ongoing studies are evaluating treatment combinations of BAT with the immune checkpoint inhibitor Nivolumab (NCT03554317), the DNA-repair inhibitor Olaparib (NCT03516812), the bone-targeted radiation therapy Radium 223 (NCT04704505), and carboplatin (NCT03522064).

## Conclusion

The Sena et al. study identified high AR activity as a requirement for clinical response to BAT, which is associated with downregulation of MYC. ARA<sub>MW</sub> is a potential biomarker that may stratify responders from nonresponders; however, findings should be validated in an independent patient cohort. Although BAT therapy has the potential to improve the outcome of patients with metastatic CRPC while preserving good quality of life, treatment is not risk free and does not work for all patients. Therefore, BAT should not be used outside of a clinical trial, especially in combination with other therapies. Larger and randomized clinical studies are needed in the future to validate and determine the best way to integrate BAT into metastatic CRPC treatment algorithms.

## Acknowledgments

We thank Nima Sharifi, M. Julia Martinez, and Rolando D.Z. Lyles for helpful comments on the manuscript. Ongoing work in KLB's laboratory is supported by the Florida Department of Health, Department of Veterans Affairs (Miami VA Healthcare System), Mike Slive Foundation, Miller School of Medicine, and the Sylvester Comprehensive Cancer Center, University of Miami.

Address correspondence to: Kerry L. Burnstein, Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, 1600 NW 10th Ave. (R-189), Miami, Florida 33136, USA. Phone: 305.243.3299; Email: kburnstein@med.miami.edu.

1. Yuan X, et al. Androgen receptor functions in castration-resistant prostate cancer and mechanisms of resistance to new agents targeting the androgen axis. *Oncogene*. 2014;33(22):2815–2825.
2. Desai K, et al. Hormonal therapy for prostate cancer. *Endocr Rev*. 2021;42(3):354–373.
3. Mohammad OS, et al. Supraphysiologic testosterone therapy in the treatment of prostate cancer: models, mechanisms and questions. *Cancers (Basel)*. 2017;9(12):166.
4. Denmeade SR, Isaacs JT. Bipolar androgen therapy: the rationale for rapid cycling of supraphysiologic androgen/ablation in men with castration resistant prostate cancer. *Prostate*. 2010;70(14):1600–1607.
5. Schweizer MT, et al. Bipolar androgen therapy for men with androgen ablation naïve prostate cancer: results from the phase II BATMAN Study. *Prostate*. 2016;76(13):1218–1226.
6. Denmeade SR, et al. TRANSFORMER: a

randomized phase ii study comparing bipolar androgen therapy versus enzalutamide in asymptomatic men with castration-resistant metastatic prostate cancer. *J Clin Oncol*. 2021;39(12):1371–1382.

7. Sena LA, et al. Bipolar androgen therapy sensitizes castration-resistant prostate cancer to subsequent androgen receptor ablative therapy. *Eur J Cancer*. 2021;144:302–309.
8. Markowski MC, et al. A multicohort open-label phase II trial of bipolar androgen therapy in men with metastatic castration-resistant prostate cancer (RESTORE): a comparison of post-abiraterone versus post-enzalutamide cohorts. *Eur Urol*. 2021;79(5):692–699.
9. Denmeade S, et al. Bipolar androgen therapy (BAT): A patient's guide. *Prostate*. 2022;82(7):753–762.
10. Markowski MC, et al. Molecular and clinical characterization of patients with metastatic castration resistant prostate cancer achieving deep responses to bipolar androgen therapy. *Clin Genitourin Cancer*. 2022;20(2):97–101.
11. Qiu X, et al. Response to supraphysiological testosterone is predicted by a distinct androgen receptor cistrome. *JCI Insight*. 2022;7(10):e157164.
12. Sena LA, et al. Androgen receptor activity in prostate cancer dictates efficacy of bipolar androgen therapy through MYC. *J Clin Invest*. 2022;132(23):e162396.
13. Faisal FA, et al. Racial variations in prostate cancer molecular subtypes and androgen receptor signaling reflect anatomic tumor location. *Eur Urol*. 2016;70(1):14–17.
14. Qiu X, et al. MYC drives aggressive prostate cancer by disrupting transcriptional pause release at androgen receptor targets. *Nat Commun*. 2022;13(1):2559.
15. Spratt DE, et al. Transcriptomic heterogeneity of androgen receptor activity defines a de novo low ar-active subclass in treatment naïve primary prostate cancer. *Clin Cancer Res*. 2019;25(22):6721–6730.
16. Litvinov IV, et al. PC3, but not DU145, human prostate cancer cells retain the coregulators required for tumor suppressor ability of androgen receptor. *Prostate*. 2006;66(12):1329–1338.
17. Guo H, et al. Androgen receptor and MYC equilibrium centralizes on developmental super-enhancer. *Nat Commun*. 2021;12(1):7308.
18. Vander Griend DJ, et al. Stabilizing androgen receptor in mitosis inhibits prostate cancer proliferation. *Cell Cycle*. 2007;6(6):647–651.
19. Chatterjee P, et al. Supraphysiologic androgens suppress prostate cancer growth through androgen receptor-mediated DNA damage. *J Clin Invest*. 2019;129(10):4245–4260.
20. Dai C, et al. Direct metabolic interrogation of dihydrotestosterone biosynthesis from adrenal precursors in primary prostatectomy tissues. *Clin Cancer Res*. 2017;23(20):6351–6362.
21. Alyamani M, et al. HSD3B1(1245A>C) variant regulates dueling abiraterone metabolite effects in prostate cancer. *J Clin Invest*. 2018;128(8):3333–3340.
22. Burnstein KL. Regulation of androgen receptor levels: implications for prostate cancer progression and therapy. *J Cell Biochem*. 2005;95(4):657–669.

23. Cai C, et al. Androgen receptor gene expression in prostate cancer is directly suppressed by the androgen receptor through recruitment of lysine-specific demethylase 1. *Cancer Cell*. 2011;20(4):457-471.
24. Quigley DA, et al. Genomic hallmarks and structural variation in metastatic prostate cancer. *Cell*. 2018;175(3):889.
25. Takeda DY, et al. A somatically acquired enhancer of the androgen receptor is a non-coding driver in advanced prostate cancer. *Cell*. 2018;174(2):422-432.
26. Viswanathan SR, et al. Structural alterations driving castration-resistant prostate cancer revealed by linked-read genome sequencing. *Cell*. 2018;174(2):433-447.