VOLUME 23 · NUMBER 32 · NOVEMBER 10 2005

JOURNAL OF CLINICAL ONCOLOGY

REVIEW ARTICLE

Biology of Progressive, Castration-Resistant Prostate Cancer: Directed Therapies Targeting the Androgen-Receptor Signaling Axis

Howard I. Scher and Charles L. Sawyers

From the Genitourinary Oncology Service, Department of Medicine, Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan-Kettering Cancer Center; Joan and Sanford E. Weill College of Medicine of Cornell University, New York, NY; Howard Hughes Medical Institute, University of California, Los Angeles/Hematology-Oncology, Los Angeles, CA.

Submitted July 14, 2005; accepted August 1, 2005.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to Howard I. Scher, MD, Genitourinary Oncology Service, Department of Medicine, Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan-Kettering Cancer Center1275 York Ave, New York, NY 10021; e-mail: Scherh@mskcc.org.

0732-183X/05/2332-8253/$20.00

DOI: 10.1200/JCO.2005.03.4777

ABSTRACT

Prostate cancers that are progressing on medical and surgical therapies designed to ablate the action of androgens continue to express androgen receptor (AR) and to depend on signaling through the receptor for growth. A more clinically relevant classification of castration-resistant disease focuses on the mechanisms of receptor activation, which include (1) changes in the level of ligand(s) in tumor tissue; (2) increased levels of the protein due to gene amplification or altered mRNA expression; (3) activating mutations in the receptor that affect structure and function; (4) changes in coregulatory molecules including coactivators and corepressors; and (5) factors that lead to activation of the receptor independent of the level of ligand or receptor allowing kinase cross talk. From an AR perspective, the term "hormone refractory" is inappropriate. On the basis of this schema, we discuss strategies that are focused on the AR either directly or indirectly, as single agents or in combination, that are in clinical development.

J Clin Oncol 23:8253-8261. © 2005 by American Society of Clinical Oncology

INTRODUCTION

The spectrum of prostate cancers that are progressing despite castrate levels of testosterone includes tumors that have shown varying degrees and durations of response to primary hormone treatment, and clinical manifestations that range from a rising prostate-specific antigen (PSA) alone, a rising PSA with osseous and/or soft-tissue spread, or a predominantly visceral disease pattern. This evolution, from the clinically localized hormone-naïve state to a castration-resistant metastatic state (Fig 1),[1] involves the complex interplay of a network of signaling molecules that collectively promote net cell proliferation relative to cell death. This range of clinical phenotypes is associated with different genotypes that are also influenced by both the microenvironment of the tumor in the location to which it has spread. That prostate cancers that have spread to lymph nodes often regress completely and rarely recur in this location while tumors in bone are rarely eliminated and are often the first site of clinical progression is consistent with this view.[2] Further support is provided by a study showing that prostate cancer metastases in different sites from an individual who as a result of the disease are biologically distinct.[3] The biology of the progressing tumor is also influenced by the specific therapy to which it has been exposed. This concept, which we have termed "therapy-mediated selection pressure," must also be considered when considering treatment options.[4]

In 2004, the combination of docetaxel and prednisone was established as the new standard of care for patients who have progressed on androgen deprivation based on two prospective randomized trials showing a prolongation of life relative to mitoxantrone and prednisone, the previous standard.[5,6] However, docetaxel and prednisone are not curative, and optimal timing of administration remains controversial. Consequently, many groups are focused

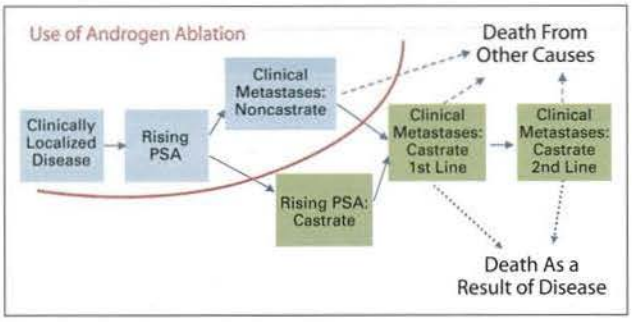

Fig 1. Prostate cancer clinical states illustrating points in the illness at which androgen deprivation can be applied, separated on the basis of castration status. Note clinical metastases castrate disease is divided into first and second line based on the demonstration of a survival benefit with docetaxel. PSA, prostate-specific antigen. Modified from Scher et al.[125]

on developing new approaches, and are focused in particular on the identification and targeting of critical signaling molecules and pathways that contribute to tumor growth in the different clinical states (Fig 1).

Androgens are the primary regulators of prostate cancer cell growth and proliferation. When androgens are ablated or withdrawn, apoptosis is observed in a proportion of cells, while those that survive arrest in the G1 phase of the cell cycle.[7] Clinical progression is the result of the regrowth of cells that were primarily resistant to androgen ablation, or which, after a period of growth arrest, adapted to the low-androgen environment and resumed proliferation.[8] It is possible, but not proven, that cells primarily resistant to androgen-deprivation therapy are those with stem-cell properties that never required androgens for survival and growth.

Studies in xenografts in which androgen ablation–naïve tumors were propagated sequentially in male mice with low levels of testosterone and once established, propagated again in female mice with testosterone levels that are below the level of detection, showed a sequentially increasing degree of gene dysregulation.[9] From a mechanistic and clinical point of view, it is critical to recognize that progression despite androgen deprivation is associated with an active androgen receptor (AR) –signaling pathway. This is shown by the fact that progression is accompanied by a rise in PSA in virtually all cases, and that many of these tumors are sensitive to secondary and tertiary hormonal manipulations such as the discontinuation of steroidal and nonsteroidal hormones, or to the addition of antiandrogens, estrogens, progestational agents, glucocorticoids, or enzymatic inhibitors of the adrenal androgen synthetic pathway.[10-12] As it is difficult to determine a priori which tumors will respond to these hormonal therapies, the terms "hormone refractory," "hormone resistant," "androgen resistant," or "androgen refractory," widely used to describe these tumors, illustrate a fundamental misunderstanding of the mechanism of disease relapse. More appropriate is to focus efforts on the development of clinical descriptions that reflect the mechanisms that are contributing to continued signaling through the receptor or the action of ligand on the receptor, rather than on the ligand itself.[13]

To date, the targeted approaches that have been the most successful approaches have been those directed against specific oncogenic alterations in tumors, such as the Bcr-Abl fusion protein in CML,[14] point mutations in c-Kit, platelet-derived growth factor-alpha kinases in gastrointestinal stromal tumors,[15] or point mutations in the epidermal growth factor receptor tyrosine kinase domain in lung cancer.[16] Although analogous genetic alterations in kinases have not been reported in prostate cancer, profiling studies of prostate cancers representing different clinical states have shown that a range of receptor tyrosine kinases, including members of the HER kinase family (epidermal growth factor receptor [EGFR], HER2),[17,18] platelet-derived growth factor[19,20] and c-met,[21] are expressed in a proportion of these tumors. Perhaps the most frequent genetic alteration is loss of the phosphatase and tensin homolog (PTEN) tumor suppressor,[22,23] which can lead to increased activity of Akt and mammalian target of rapamycin (mTOR).[24] But, despite the presence of the target on or in tumor cells, clinical results with single agents directed at these targets have been disappointing at best.

AR Signaling

There is a growing literature showing that the AR itself, a protein required for prostate development but not traditionally considered to play a causative role in cancer progression, may in fact be oncogenic under certain circumstances.[25-30] This underscores the importance of developing therapies that affect AR signaling even in the setting of resistance to conventional antiandrogens, or to the medical and surgical approaches used to lower serum testosterone levels in the blood. In this section, we will focus on alterations in AR signaling, and how alterations in one pathway may affect response to agents targeting a second pathway.

Using published reports and clinical outcomes, a classification scheme of alterations in AR signaling can be developed on the basis of (1) changes in the level of ligand(s) in tumor tissue; (2) increased levels of the protein due to gene amplification or altered mRNA expression; (3) activating mutations in the receptor that affect structure and function; (4) changes in coregulatory molecules including coactivators and corepressors; and (5) factors that lead to activation of the receptor independent of the level of ligand or receptor allowing kinase cross talk.[13,25,26,31] These are illustrated schematically in Figure 2.

Direct Mechanisms of AR Activation

Incomplete blockade of AR-ligand production. It has long been recognized that the medical and surgical therapies designed to ablate production or androgen action do not result in undetectable androgen levels in tumor tissue. This is based on the direct measurement of androgens in

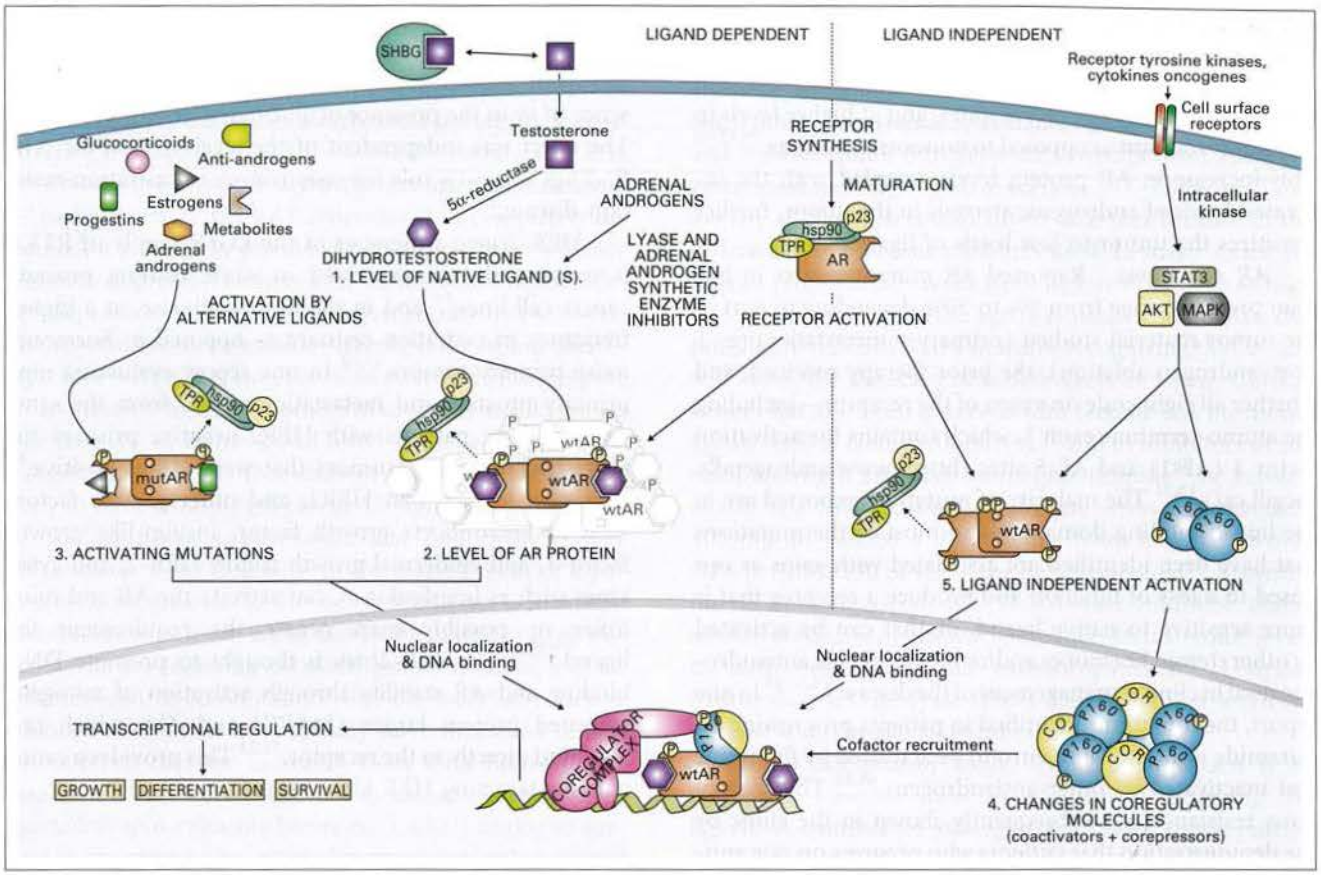

Fig 2. A classification of mechanisms associated with continued signaling through the androgen-signaling axis despite castration. SHBG, sex hormone–binding globulins; Hsp, heat shock protein; TPR, tricopeptide repeat; AR, androgen receptor; MAPK, mitogen-activated protein kinase; mut, mutated; wt, wild-type; COR, corepressors. Modified from Scher et al.[13]

the prostates of patients who have undergone castration.[32,33] That inhibitors of adrenal androgen synthesis such as ketoconazole[34] and aminoglutethimide[35] have anti–prostate cancer effects in some patients in this clinical setting suggests that the levels of androgen that remain in the tumor despite castrate levels of testosterone in the blood are sufficient to produce tumor growth. This has been confirmed in a more recent report that showed that the intratumoral testosterone levels in patients with castration resistant disease were similar to untreated benign prostatic disease, and that the level of dihydrotestosterone (1.45 nM) was sufficient to maintain AR signaling and expression of PSA.[36] In a follow-up study, although dihydrotestosterone (DHT) levels in recurrent primary prostate tumors were decreased by 97% relative to non–androgen suppressed benign prostatic tissue, the levels in the range of 1.25 nM, were sufficient to transactivate the receptor based on in vitro cell line studies.[37]

The intratumoral androgens may come from an adrenal source, or result from direct synthesis within the tumor by an intracrine mechanism.[38] Molecular profiling of castration-resistant prostate tumors showed that the expression of 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 and squalene epoxidase, rate-limiting enzymes involved in sterol synthesis, are increased relative to noncastrate tumors.[39] Overall the results suggest that prostate tumors rarely encounter a completely androgen-depleted environment.[36] They also question whether defining progressive disease after castration on the basis of a measured testosterone level in the blood below 50 ng/mL is appropriate.[40]

Increased levels of AR protein without mutation. Amplification of the *AR* gene, detected as an increased gene copy number by fluorescence in situ hybridization or array-based comparative genome hybridization (CGH) methods has been documented in 20% to 25% of both castration-resistant metastatic[41] and recurrent primary tumors.[42,43] In addition, an increase in the level of *AR* and/or AR-regulated genes has been shown to be increased in prostate cancer xenografts that have progressed postcastration relative to tumors growing in intact male hosts, in the absence increased *AR* gene copy number.[9,28,44,45] In seven matched androgen ablation–naïve and –resistant cell lines, the only consistent change in mRNA expression was an increase in *AR* mRNA levels, the significance of which was shown in transfection studies that demonstrated that both the onset and rapidity of tumor onset varied directly with

AR level.[28] The increase in AR protein sensitizes prostate cancer cells to respond to low levels of ligand.[28,46]

In human prostate cancer, AR protein is expressed in prostate cancers of all clinical states, and at higher levels in castration-resistant as opposed to noncastrate tumors.[39,47-50] This increase in AR protein levels, coupled with the increased levels of androgenic steroids in the tumor, further sensitizes the tumor to low levels of ligand.

AR mutations. Reported AR mutation rates in human prostate range from 5% to 50% depending in part of the tumor material studied (primary *v* metastatic, pre– *v* post–androgen ablation), the prior therapy received, and whether all eight code on exons of the receptor—including the amino-terminus exon 1, which contains the activation factor 1 (AF-1) and AF-5 sites (http://www.androgendb.mcgill.ca/).[13,31] The majority of mutations reported are in the ligand-binding domain,[51] and most of the mutations that have been identified are associated with gains as opposed to a loss of function and produce a receptor that is more sensitive to native ligand, or that can be activated by other steroid hormones and/or by the specific antiandrogen used in clinical management of the disease.[13,52,53] In one report, the mutations identified in patients progressing on flutamide were shown in vitro to be activated by flutamide and inactivated by other antiandrogens.[54,55] This lack of cross resistance was subsequently shown in the clinic by the demonstration that patients who progress on one antiandrogen often respond to another.[56-58]

Indirect Mechanisms of AR Activation

The transcriptional activity of the AR is mediated, in part, by coactivators that enhance or corepressors that reduce receptor function.[59] Coactivator proteins such as ARA54 and ARA70 can selectively enhance the activity of the receptor to alternative ligands such as estradiol and hydroxyflutamide, sensitize the receptor to lower concentrations of native and nonnative ligands, or allow ligand-independent activation by receptor tyrosine kinases (RTKs) such as HER2.[60-62] Decreased expression of corepressors such as nuclear receptor corepressor (N-CoR) and silencing mediator of retinoid and thyroid receptors (SMRT), which mediate, in part, the antagonist action of bicalutamide, flutamide and mifepristone, may contribute to the agonist activity that can be observed with these agents.[63,64] A change in the coactivator-to-corepressor ratio can alter AR transactivation activity in the presence of low concentrations of DHT. Conversely, the corepressors SMRT and NCoR can inhibit AR function in a ligand-dependent manner. Experimental models show that alterations in the coactivator-to-corepressor ratio can also explain the paradoxical agonist effects of antiandrogens and other steroid hormones on prostate cancer growth.[59,65] Unclear, however, is whether this serves as the mechanistic basis for clinical antiandrogen resistance or the occasional clinical responses seen in patients upon withdrawal of antiandrogens. More recently, p300 was shown to activate expression of the PSA gene in the absence of or in the presence of androgens or antiandrogens. The effect was independent of the level of receptor. The findings suggest a role for coactivators in castration-resistant disease.[30]

HER-2/*neu*, a member of the EGFR family of RTKs, is consistently overexpressed in some castrate prostate cancer cell lines,[66] and in the human disease, at a higher frequency in castration resistant as opposed to hormone-naïve primary tumors.[17,18] In one report evaluating nine primary prostate and metastatic samples from the same patient, three patients with HER2-negative primary tumors had metastatic tumors that were HER2 positive.[67] It is now known that HER2, and other growth factors such as keratinocyte growth factor, insulin-like growth factor-1, and epidermal growth factor; HER-2; and cytokines such as interleukin-6, can activate the AR and minimize or possibly even negate the requirement for ligand.[25,66,68-71] HER-2/*neu* is thought to promote DNA binding and AR stability through activation of mitogen-activated protein kinase (MAPK) and Akt, which can also bind directly to the receptor.[72,73] This provides a rationale for targeting HER kinase signaling (Fig 3).

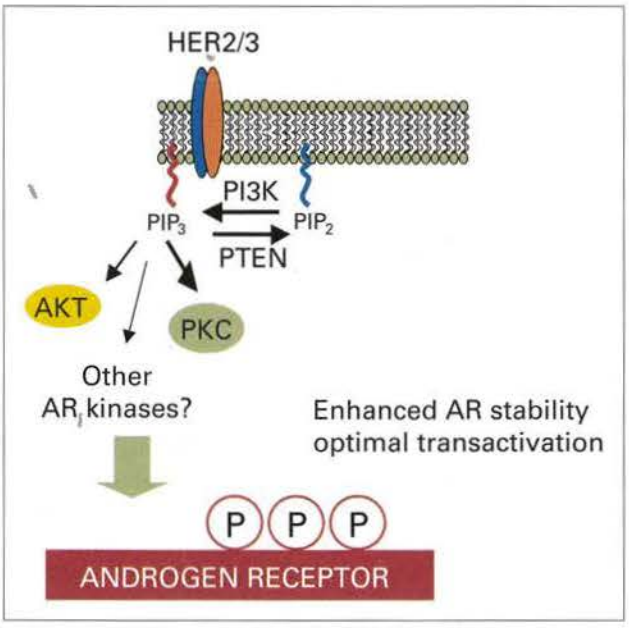

Fig 3. Schema of kinase signaling cross talk with androgen receptor (AR). The illustration depicts a postulated signaling pathway involving Her-2/Her-3 heterodimers and PI3K that leads to activation of the androgen receptor function through enhanced stability and transcriptional activation. Increased PIP3 levels, generated either by Her-2/Her-3 and PI3K activation or by phosphatase and tensin homolog (PTEN) loss, activate downstream kinase pathways including Akt, protein kinase C and others. Although Akt and protein kinase C can phosphorylate AR directly, the physiologic relevance remains to be fully defined. Alternatively, PIP3-activated kinases may enhance AR function indirectly by phosphorylating accessory molecules (coactivators) involved in AR function (not shown).

Therapeutic Considerations

The characterization of prostate cancers representing different clinical states is a rapidly evolving work in progress. The results reported to date clearly show the significance of directing therapies toward the various mechanisms that contribute to continued signaling through the androgen/AR pathway(Fig 2). Until AR function can be completely abrogated, it will not be possible to say conclusively whether regrowth of prostate tumors after failure of androgen ablation depends on AR signaling or other regulatory pathways. The approaches include those directed at the ligand, the receptor, or factors that may function to activate the receptor independent of ligand. A number of strategies are illustrated schematically in Figure 2 and listed in Table 1. It must be recognized, however, that each of these approaches will likely to have multiple effects on AR signaling.

Reduce ligand concentration and competitively block AR function. The first issue in the management of a patient with progression of disease on hormones is to ensure that testosterone levels are in fact in the castrate range. Patients who are receiving antiandrogen monotherapy or an antiandrogen in combination with the 5-alpha-reductase inhibitor finasteride will typically have measured testosterone levels that are increased from their pretreatment baselines,[74,75] and may respond to the administration of a gonadotropin-releasing hormone (GnRH) analog or surgical orchiectomy. Patients being managed with a formal on-and-off or intermittent therapy[76,77] approach may also have testosterone levels in the noncastrate range and respond again to testosterone lowering agents.

For those with testosterone concentrations that are already in the castrate range and who are progressing on the combination of a GnRH hormone analog in combination with an antiandrogen as part of a combined androgen or maximal androgen blockade approach, a proportion will show declines in PSA, regression of measurable tumor masses and relief of cancer-related symptoms after discontinuation of the antiandrogen while the GnRH analog therapy is continued. The phenomenon of withdrawal responses was first observed after the selective discontinuation of flutamide.[10] Subsequently, they were reported with other non-steroidal antiandrogens (ie, nilutamide and bicalutamide), estrogens (diethylstilbestrol) and progestational agents (megestrol acetate).[78-84] The overall frequency of response is difficult to ascertain because of the differences in outcome measures used. In many series, response proportions ranges from as low as 10% to as high as 60% depending on the agent(s) used. The median durations are typically 3 to 4 months, recognizing that in certain cases, the duration of benefit exceeds 1 year. Clinical benefit has also been observed with second-line hormonal agents such as diethylstilbesterol,[85] and to inhibitors of adrenal androgen synthesis aminoglutethimide[86] or ketoconazole and hydrocortisone.[87] In one series, ketoconazole 400 mg tid yielded a PSA decline from baseline of over 50% in 62.5% (30 of 48) of the patients treated[12]. Recently, the 17alpha-hydroxylase/C(17,20)-lyase inhibitor abiraterone acetate was shown to reduce testosterone levels in both noncastrate and castrate patients.[88] 17alpha-hydroxylase-C17,20-lyase (P450 17, CYP 17) is a critical enzyme in androgen biosynthesis.[89]

Lower AR protein levels. Another approach is to target the receptor itself in addition to targeting the ligand. A number of strategies are under development. Preclinical strategies include double-stranded RNA interference,[90] microinjection of anti-AR antibodies,[91,92] and antisense oligonucleotides.[93-95]

Ansamycin antibiotics are natural products that induce the degradation of steroid hormone receptors and a number of mutated oncogenes by interfering with heat shock protein (Hsp) 90 binding and preventing protein refolding.[96-98] These agents, now in clinical testing, reduce the level of AR and inhibit prostate cancer cell growth in vivo in a dose dependent manner.[98] Other Hsp90 substrates affected by this agent include HER2/*neu*, c-met, and phosphor-Akt, all of which are expressed at a higher

Table 1. Strategies Directed at Continued AR Signaling

Mechanism	Agent	Effect(s) on AR Signaling	Status
Adrenal androgen synthesis	Ketoconazole	1	
17,20 lyase inhibitors	Abiraterone	1	Phase II
5-α reductase inhibitor	Finasteride Dilutamide	1	Phase II
Hsp90 chaperone inhibitors that induce protein degradation	Ansamycins—17AAG, DMAG	1, 2, 3, 5	Phase II
Histone deactylase inhibitors	Histone deacetylase inhibitors—SAHA	2,4	Phase II
New antiandrogens based on crystal structure of the AR		2	Preclinical
Anti-sense AR therapy		2	Preclinical
Non–cross-resistant antiandrogens	Bicalutamide after flutamide Nilutamide after bicalutamide	3	
Coregulatory molecules	Mifepristone	4	Phase II planned

NOTE. Effects on androgen-receptor (AR) signaling include (1) changes in the level of ligand; (2) normal or increased levels of wild-type receptor; (3) activating mutations; (4) changes in coregulatory molecules; and (5) ligand-independent activation.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.