ORIGINAL ARTICLE

Systems Pharmacology Modeling of Prostate-Specific Antigen in Patients With Prostate Cancer Treated With an Androgen Receptor Antagonist and Down-Regulator

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First-in-human (FIH) studies with AZD3514, a selective androgen receptor (AR) down-regulator, showed decreases of >30% in the prostate-specific antigen (PSA) in some patients. A modeling approach was adopted to understand these observations and define the optimum clinical use hypothesis for AZD3514 for clinical testing. Initial empirical modeling showed that only baseline PSA correlated significantly with this biological response, whereas drug concentration did not. To identify the mechanistic cause of this observation, a mechanism-based model was first developed, which described the effects of AZD3514 on AR protein and PSA mRNA levels in LNCaP cells with and without dihydrotestosterone (DHT). Second, the mechanism-based model was linked to a population pharmacokinetic (PK) model; PSA effects of clinical doses were subsequently simulated under different clinical conditions. This model was used to adjust the design of the ongoing clinical FIH study and direct the backup program.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC? ☑ The AR signaling pathway and both androgen receptor and DHT levels, are known to be important in prostate cancer. • WHAT QUESTION DID THIS STUDY ADDRESS? ☑ Can the action of a drug working on the androgen pathway and modulating PSA level be described using a mechanistic system model in which biological parameters are defined *a priori* from physical measurement taken from the literature? • WHAT THIS STUDY ADDS TO OUR KNOWLEDGE ☑ This work indicates that it is feasible to quickly build a mechanistic model of the AR pathway that can be used in real time to influence clinical design. In this system, an observed decrease in PSA is not linked to down-regulation of AR. AZD3514 may bind to a site on the AR other than the ligand binding domain, which mediates the fall in PSA levels. The model suggested that the interplay between DHT and AZD3514 at the AR results in the clinical response. • HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS ☑ There should be focus on making quantitative physical measurement of pathways under investigation, such that models can be built to guide drug development.

Prostate cancer is the most common form of malignancy in men, and is the second leading cause of cancer-related death in Western society.[1] This disease is dependent upon the hormone testosterone, which activates the androgen receptor (AR). Targeting this signaling pathway has been shown to be a successful approach in patients with metastatic prostate cancer.[2–4] Unfortunately, drug resistance ultimately develops in the majority of patients and, interestingly, this resistance is still dependent upon AR signaling despite castrate levels of androgen.[5,6]

AZD3514 is a first-in-class experimental drug that inhibits with AR signaling and leads to receptor down-regulation *in vitro*.[7] AZD3514 inhibited dihydrotestosterone (DHT)-driven proliferation of LNCaP prostate cancer cells as well as the ligand-driven expression of the AR-regulated genes for prostate-specific antigen (PSA) and transmembrane protease serine 2 in these cells. Furthermore, this compound reduced seminal vesicle weight in the intact rat.[8] Based on

these initial promising findings, AZD3514 was tested in a clinical setting.

Two parallel first-in-human (FIH) studies (Europe and Japan) were performed in patients with castration-resistant prostate cancer (CRPC), which were open-label, dose escalation studies in which single- and multiple-dose safety and tolerability, pharmacokinetics (PKs), pharmacodynamics, and efficacy of AZD3514 were investigated. Antitumor activity of AZD3514 was monitored via measurements of PSA levels and circulating tumor cells. In part A, nine (Europe) and three (Japan) cohorts of patients were recruited into the two studies, and patients were dosed orally with once- or twice-daily doses of 100 to 1,000 mg AZD3514 in 28-day cycles. An interim PK analysis revealed that once-daily dosing resulted in suboptimal profiles (i.e., AZD3514 plasma concentrations for 18 hours above the IC_{50} of 2,410 ng/mL), predicted to be required for efficacy from a preclinical rat model, which was not achievable with a once-daily dosing regimen. Therefore, the dosing regimen

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was switched to twice-daily dosing. Nevertheless, clinical activity was observed with >30% reduction in PSA observed in some patients at doses of 500 mg once daily. Therefore, the preclinical intact rat model may not be informative within this disease setting.

The decreases in PSA observed suggested that AZD3514 may have had clinical utility in a small number of patients but were not broad enough to support transition to phase II development. Thus, modeling activities were started to help provide mechanistic understanding into the clinical observations and define a clinical design that would optimize AZD3514 activity.

In this article, we discuss the modeling approach taken, how the approach led to new insights into the action of AZD3514, and how these insights informed the design of part B of the study, which was a combination study of AZD3514 and abiraterone[9] in patients who had progressed on abiraterone alone. The modeling approach applied was based on the work by Jain *et al.*,[10] who has developed a multiscale model of prostate cancer that combines both cellular signaling and physiology. This model was chosen as it contained detailed information on the kinetics of androgen signaling that were important to understanding the *in vitro* pharmacology of AZD3514. Their model is based on numerous other kinetic models of androgen signaling, which are discussed in Jain *et al.*[10] Initially, an empirical model had been used to interrogate the emerging data from the AZD3514 FIH studies. This suggested a new mode of action relating to the efficacy of AZD3514; hence, the potential value of a mechanistic systems model generated from literature and preclinical data. This model was then "humanized" and coupled to a population PK model, so that a dosing schedule could be determined. Additionally, the integrated model provided insight into the impact of co-dosing AZD3514 with other agents that act on the androgen pathway. These pieces of information rationalized the emerging findings within the ongoing clinical study with preclinical observations and were then used to change the design of the ongoing FIH study. Thus, this article highlights how mechanistic models can be applied during an ongoing phase I clinical oncology study. Being able to perform such activities as described in this article is likely to lead to either early termination of clinical projects or accelerated development plans via designing and testing of the most plausible hypotheses to maximize clinical activity.

METHODS

The methods used to analyze the clinical data and develop the mechanistic model are described below initially, as clinical data became available, it was analyzed empirically. When this resulted in an unexpected finding, a mechanistic model was developed to enable hypothesis to explain the clinical data generated.

Analysis of clinical data
The time series of PSA (**Supplementary Figure S1**) were modeled using a biological growth law (exponential) given by: $BIO(t) = BIO(0) \exp(g*t)$, where $BIO(0)$ is the baseline value and g is the growth/decay constant. The model was placed within a mixed effects framework with a combined additive plus proportional residual error term. $BIO(0)$ was assumed to be log-normally distributed, whereas g was assumed to be normally distributed. The covariates tested to explain the variability in g within the mixed modeling framework were: PK variables, such as the area under the curve, minimum and maximum concentrations, as well as other disease-related covariates, such as pretreatment PSA levels. The significance of covariates was assessed using the likelihood ratio test with an alpha of 0.05.

Development of mechanistic model
A stepwise modeling approach was performed to develop a kinetic model of AZD3514 action on the AR signaling pathway.

Initial
The initial model was developed to explore the hypothesis that AZD3514 competes with DHT for the same binding domain on the AR and that AZD3514 enhances the basal degradation of AR. This first model was based solely on an *in vitro* experiment of AZD3514 effects on AR expression levels. Assessment of this model's adequacy was done qualitatively on its ability to rationalize both AR and PSA *in vitro* expression experiments.

Final
A revised model was built that incorporated both the effects of AZD3514 on AR and PSA expression levels *in vitro* and emerging data that indicated AZD3514 interfered with basal production of AR and did not enhance AR degradation. This model was assessed in its ability to fit to the entire *in vitro* datasets available. The final mechanistic model was linked to a population PK model (Dymond *et al.* unpublished data). In brief, a two-compartment linear model with zero order absorption was found to adequately describe the plasma concentration-time profile. At steady state, the population apparent clearance was 23.1 L/h, the volume of distribution of the central (V_1/F) and peripheral (V_2/F) compartments were 84.7 and 311 L, respectively, and the zero order absorption rate was 3.56 hour. The interindividual variability, modeled using a log-normal distribution, on these parameters was 51%, 48%, and 18% for CL/F, V_1/F, and V_2/F, respectively. No covariates were found to alter the AZD3514 PK characteristics. Using the final model, AZD3514 effects on PSA levels in patients were simulated (see **Supplementary Information** for simulation protocol) using the different dosing regimens used in the FIH studies and different circulating DHT concentrations.

In the development of the mechanistic model, the law of mass action and Michaelis–Menten kinetics was assumed. All modeling, optimizations, and simulations described were performed using MATLABs SimBiology and Optimization toolboxes.

RESULTS

A stepwise modeling approach was used, aiming at better understanding the mechanism of action of AZD3514-induced decrease in PSA levels in some patients. The initial goal of this approach was to provide, in real time, a
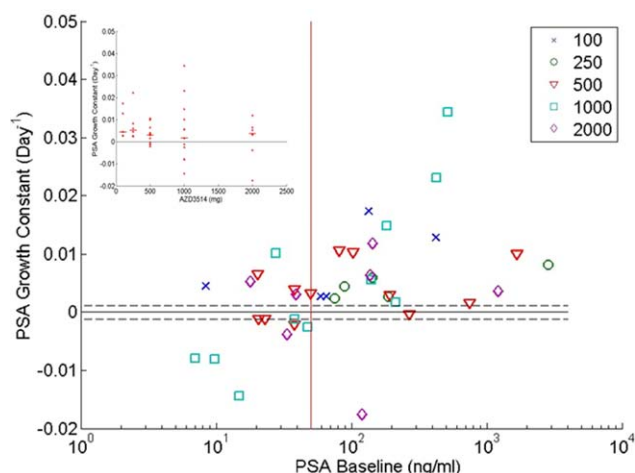
Figure 1 Main plot is of prostate-specific antigen (PSA) baseline versus PSA growth constant, *g*. Dashed black line represents the growth constant value needed to see a +/- 10% change at week 12. The solid red vertical line indicates the PSA baseline value of 50 ng/mL. The different shapes/colors represent different doses: blue crosses – 100 mg once-daily (OD), green circles – 250 mg OD, red triangle – 500 mg OD, cyan square – 1,000 mg OD, and purple rhombus – 1,000 mg b.i.d. Insert plot is of PSA growth constant, *g*, as a function of total daily dose. Patients were treated with AZD3514 at doses of 100, 250, 500, or 1,000 mg OD or 1,000 mg b.i.d.

mechanistic understanding of the emerging results of part A, provide a dose and study design recommendation for part B of the clinical studies, and assess the potential of any backup programs (direct the *in vitro* experimentation used to measure potential activity of new molecules).

Empirical model of the clinical data
The covariate analysis of the clinical PSA time-series data found that no PK variable, such as the area under the curve, and minimum and maximum concentrations, were strong correlates of the growth constant. Subsequently, other varia-

bles were investigated, such as baseline values of markers, co-medications, age, pretreatment PSA trajectory, and any other variables that had been collected. The only strong correlate was baseline PSA. In **Figure 1**, baseline PSA is plotted vs. the PSA growth constant and it can be seen that the decreases in PSA observed in some patients are correlated with a low baseline PSA level. The area under the corresponding receiver operating characteristic curve was ∼0.8 (**Supplementary Figure S2**), which suggests that there is an interesting signal that did not occur by chance. In order to generate hypotheses to explain such a finding, a mechanistic model was developed.

Initial development of the initial mechanistic model
Preclinical experiments had shown that AZD3514 reduces the total (free and AZD3514 bound) level of AR expression in LNCaP cells, both in the absence and presence of DHT, see **Figure 2**. Note that these data were taken from Astra-Zeneca data on file and were the first datasets generated by the project, the experimental methods are the same as those used to generate **Figure 3** in Loddick *et al.*,[8] which were conducted at a later date. It is interesting to note that, in the absence of AZD3514, incubation with DHT resulted in a near doubling of AR expression, as shown in **Figure 2**. This finding has been seen previously by Manin *et al.*[11] An initial model (**Supplementary Table S1**) based on receptor theory was developed to understand the AR experimental results. The parameter values used are a mix of literature[10] and estimates from a fit to the data in **Figure 2.** The key assumptions of the simple model are:

- DHT binds to the AR in a reversible manner;
- AZD3514 binds to the AR in a reversible manner at the same site as DHT (competitive inhibition);
- Basal turnover of the AR occurs;
- DHT induces production of the AR;
- AZD3514 induces degradation of the AR; and
- DHT and AZD3514 binding kinetics to mutant AR are the same as for wild-type AR.
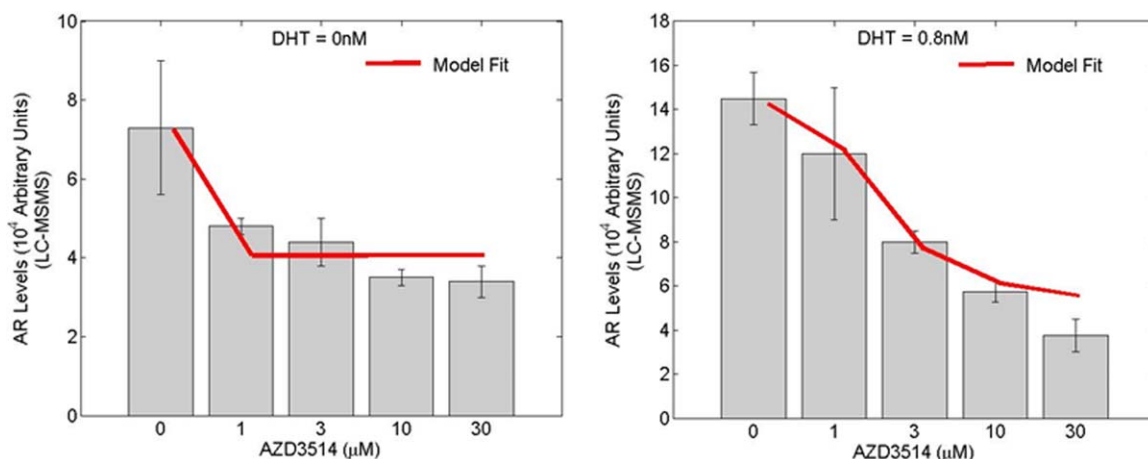

Figure 2 Observed via liquid-chromatography mass-spectrometry (arbitrary units) and model fit for the effect of AZD3514 on total androgen receptor (AR) protein levels (free and drug bound) in LNCaP cells. The results are shown in the absence of dihydrotestosterone (DHT; left panel) and in the presence of 0.8 nM DHT (right panel) in the culture medium.
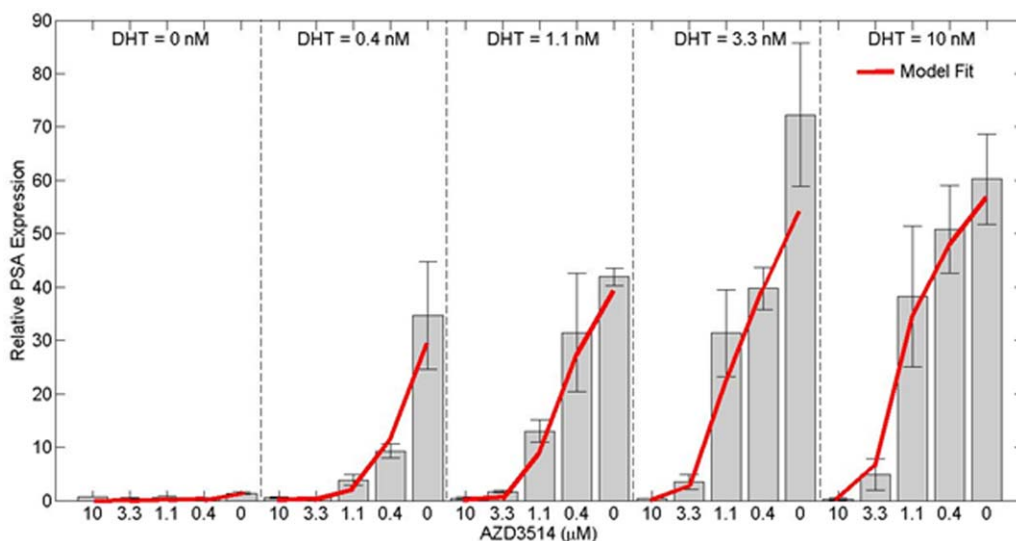
Figure 3 Prostate-specific antigen (PSA) gene expression normalized to control levels after AZD3514 treatment of LNCaP cells ± dihydrotestosterone (DHT). Data (mean ± SE) shown is gene expression relative to the vehicle control in the absence of ligand. This data was reproduced from Loddick *et al.*,[8] with permission.

A receptor occupancy curve as a function of AZD3514 concentration was simulated from the resultant model (**Supplementary Figure S3**). The bulk of ligand was displaced at an AZD3514 concentration of 100 μM, whereas a concentration of 1 μM had little effect. This compares with the maximum geometric mean of total plasma concentrations observed in the clinic at steady-state, of approximately 20 μM.[12]

Refinement of the mechanistic model
New experimental data became available showing that AZD3514 reduced PSA mRNA in LNCaP cells and that a marked effect was already obtained at a concentration of 1 μM (**Supplementary Figure S4** and Loddick *et al.*[8]; i.e., at a concentration not predicted to displace DHT from the AR receptor). This apparent discrepancy suggested that AZD3514 is not competing with DHT. One possible hypothesis to explain AZD3514 action on PSA mRNA is that AZD3514 may bind to a site other than the ligand binding domain. Thus, it was hypothesized that AZD3514 interferes with the translocation of the AR into the nucleus by binding to this other binding site.[8] In the next iteration of the model, we assumed the drug may bind to the receptor and ligand/ receptor complex and, thus, may modulate both nonligand and ligand-induced expression of the AR and PSA mRNA levels (**Supplementary Table S2**). This mimicked the drug effect on AR translocation, without explicitly spatial representation within the model. The experimental data and the model fit to the PSA expression data are depicted in **Figure 3**. Note the fit to the AR experimental data in **Figure 2** was equivalent to the initial model. **Figure 3** indicates that AZD3514 was less potent with increasing DHT concentrations, although near complete inhibition of PSA expression was observed in LCNaP cells with 10 μM AZD3514 at all concentrations of DHT.

Linking the mechanistic model to a population PK model; simulations with an integrated model
The assumption that the drug can bind to the ligand/ receptor complex provided a good fit to all the experimental data gathered in LNCaP cells. Having established a hypothesis about AZD3514 mechanism of action, we sought to see if this could help interpreting the emerging clinical data and the hypothesis of low baseline PSA being a predictor of biological response. Thus, we linked the mechanistic model to a population PK model, and simulated the effects of the doses used in the clinic for two DHT settings, low (0 nM) and high (10 nM), on PSA (these two DHT settings were based on levels explored in preclinical experiments). Results of these simulations can be seen in **Figure 4**. The vertical height of each point reflects the variability associated with the PKs, whereas the difference between black and red lines at each dose reflects the variability associated with changes in DHT. It does seem that the concentration of DHT is a more important driver for the variability in PSA level than variations in PKs. Hence, if levels of DHT could be reduced, then a clinically meaningful dose-response relationship and activity could be observed in patients. Furthermore, simulations indicated that, for any total daily dose, twice-daily dosing would provide a better clinical response vs. once-daily. Overall, the modeling indicated that moving from once-daily to twice-daily dosing, together with a drug that would reduce DHT (e.g., abiraterone acetate) might result in better responses. Abiraterone inhibits CYP17, which is expressed in testicular, adrenal, and prostatic tumor tissues, and is involved in androgen biosynthesis. Thus, inhibition of CYP17 is expected to reduce DHT levels. This prediction was tested and the results can be seen in **Figure 5**, although there does seem to be a trend suggestive of improved PSA decay rates with the combination. Unfortunately, the study was not completed and a
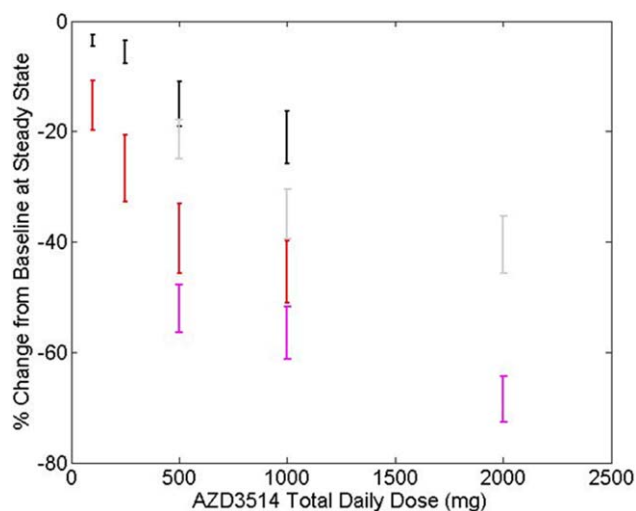
Figure 4 Model prediction of percentage change in prostate-specific antigen from baseline at steady-state as a function of the total daily dose administered to patients with advanced metastatic castration-resistant prostate cancer (CRPC). The black and red colors represent high and low dihydrotestosterone (DHT) values for once-daily dosing, respectively, whereas blue and magenta represent high and low DHT values for twice-daily dosing.

thorough analysis of the model predictions could not be completed. The limited results do, however, suggest that the difference in decay rates between the monotherapy and combination are not as strong as the model predicted. Although the study was terminated, a similar hypothesis is currently being explored using another AR translocation inhibitor (enzalutamide) with a compound that reduces DHT (abiraterone) in an ongoing phase 3 study.[13] Enzalutamides' main mechanism of action involves the prevention of the translocation of AR into the nucleus,[14] which is similar to AZD3514. However, there is no evidence that enzalutamide exhibits any nonspecific binding, hence, a simpler model to the one described here is likely to be applicable for the analysis of the results of that study.

DISCUSSION

For mathematical modeling to become a valuable tool in the development of new cancer medicines, it ideally should combine mechanistic details of cellular signaling pathways, clinical markers, and/or efficacy endpoints, and the PKs (dose-exposure) of such investigational compounds, in one integrative modeling approach.[15] It should also provide "real-time" insights, so that it may actually influence the ongoing clinical development.

Such a model was developed using preclinical results and clinical PKs, to better understand the possible effects of AZD3514 on PSA levels in patients with CRPC. The model indicated that AZD3514 may work best in a low DHT environment and was used to predict a dosing regimen for AZD3514 in combination with abiraterone acetate; the mode of action of abiraterone is to inhibit CYP17 and down-

stream androgen synthesis, thus, effectively "sensitizing" tumors to the action of AZD3514.

In order to develop a mechanistic understanding of AZD3514 effects on PSA levels in patients, we first turned our attention to the preclinical data. The *in vitro* model is an LNCaP cell line with mutated androgen receptors[8] and, thus, this experimental model seemed to be an appropriate one to be used in our modeling efforts. The animal model of choice to test antiandrogenic activity of compounds is the Hershberger rat,[16] a castrated rat in which the weight of androgen-dependent tissues is measured. AZD3514 showed activity in this model, and PK data obtained were used to predict clinical exposure (AstraZeneca, data on file). However, this model contains only wild-type AR; in the CRPC setting, it is likely to have multiple mutational forms present.[17,18] Second, the amount of AR in androgen-dependent tissue in the rat is approximately 100–200 fmol/mg,[19] whereas the amount in LNCaP cells is about 1,000 fmol/mg,[20] similarly to the expression levels of AR in circulating tumor cells in patients.[21]

The developed model predicted that, in patients with CRPC: (1) the variability in the effect of AZD3514 on PSA is more driven by the concentration of DHT than that of the drug; (2) that a twice-daily dosing regimen is better than once-daily dosing; and (3) that AZD3514 is more efficacious in an environment of low DHT concentrations. Interestingly, the dose at which some patients show a clinically significant decrease in PSA levels was observed at 500 mg administered once-daily[12] and the model suggests that this could be the first dose at which a 50% decrease in PSA expression from baseline could be seen if the patient had low circulating levels of DHT. The model predictions were put to a test by including an extra cohort of patients in part A of the ongoing clinical study. AZD3514 was administered twice-daily at a dose of 500 mg to patients with CRPC in combination with abiraterone. The patients with CRPC who were entered into
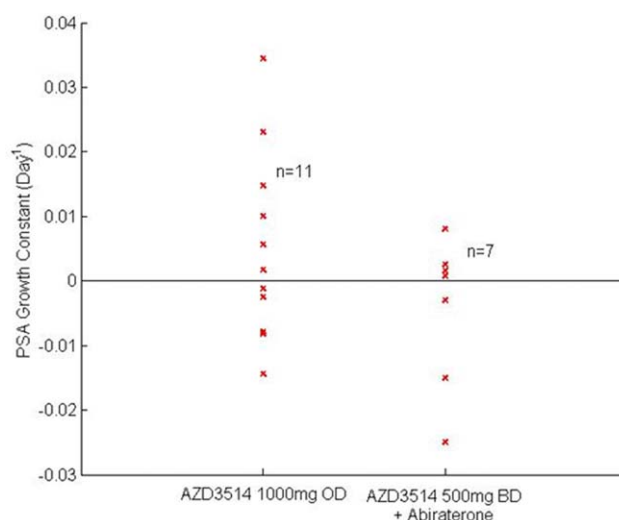

Figure 5 Plot of prostate-specific antigen growth constant, g, evaluated at 12 weeks, for patients treated with 1,000 mg once-daily AZD3514 alone or with 500 mg twice-daily in the presence of abiraterone.

the study had progressed while on treatment with abiraterone alone. In **Figure 5**, the individual PSA growth constants by treatment are depicted and the results may indicate a better response to AZD3514 in patients treated concomitantly with abiraterone when compared to AZD3514 alone, but the variability of the response seemed similar. The number of patients included in this cohort was very small, and, unfortunately, DHT concentrations were not measured. After a review of all clinical data, the development of AZD3514 was halted because of insufficient efficacy and an unfavorable safety profile.[12] Therefore, a thorough assessment of model predictions could not be conducted.

A backup discovery program was started, aiming at identifying more potent selective AR down-regulators. More potent compounds were identified. However, the knowledge that AZD3514 acted both via the ligand binding domain and as well as another site proved helpful as it was quickly established that the increased potency was likely via this second mechanism and, thus, reduced the chances of identifying a potential backup candidate. Based on this information and the changing treatment landscape in prostate cancer, the program was terminated.

There are a number of key limitations/assumptions to the approach taken here, which we now highlight. First, we assumed a 1:1 relationship between PSA mRNA and PSA protein, although this is clearly not always the case with many gene products. The mathematical model itself is not a model representing tumor growth *per se*. We could have described a pathway model within a tumor growth model, however, lack of calibration data from imaging to relate PSA levels to tumor volume prevented following this approach. The main hypothesis of the model was that intratumoral DHT levels are important, however, the study design did not include measurements of intratumor DHT– which would have provided an alternative way of validating the model.

In conclusion, the developed model may prove useful if more potent and better tolerated compounds are developed in the same class as AZD3514. The results indicate that such compounds could be developed as add-on therapies to drugs like abiraterone, which decrease circulating DHT levels, or possibly as first-line therapy in patients with low DHT levels because of surgical intervention.

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1. Jemal, A., Siegel, R., Xu, J. & Ward, E. Cancer statistics, 2010. *CA Cancer J. Clin.* **60**, 277–300 (2010).
2. de Bono, J.S. *et al.* Abiraterone and increased survival in metastatic prostate cancer. *N. Engl. J. Med.* **364**, 1995–2005 (2011).
3. Ryan, C.J. *et al.* Abiraterone in metastatic prostate cancer without previous chemotherapy. *N. Engl. J. Med.* **368**, 138–148 (2013).
4. Scher, H.I. *et al.* Increased survival with enzalutamide in prostate cancer after chemotherapy. *N. Engl. J. Med.* **367**, 1187–1197 (2012).
5. Scher, H.I. & Sawyers, C.L. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. *J. Clin. Oncol.* **23**, 8253–8261 (2005).
6. Attard, G., Cooper, C.S. & de Bono, J.S. Steroid hormone receptors in prostate cancer: a hard habit to break? *Cancer Cell* **16**, 458–462 (2009).
7. Bradbury, R.H. *et al.* Discovery of AZD3514, a small-molecule androgen receptor downregulator for treatment of advanced prostate cancer. *Bioorg. Med. Chem. Lett.* **23**, 1945–1948 (2013).
8. Loddick, S.A. *et al.* AZD3514: a small molecule that modulates androgen receptor signaling and function in vitro and in vivo. *Mol. Cancer Ther.* **12**, 1715–1727 (2013).
9. Harshman, L.C. & Taplin, M.E. Abiraterone acetate: targeting persistent androgen dependence in castration-resistant prostate cancer. *Adv. Ther.* **30**, 727–747 (2013).
10. Jain, H.V., Clinton, S.K., Bhinder, A. & Friedman, A. Mathematical modeling of prostate cancer progression in response to androgen ablation therapy. *Proc. Natl. Acad. Sci. USA* **108**, 19701–19706 (2011).
11. Manin, M. *et al.* Androgen receptor expression is regulated by the phosphoinositide 3-kinase/Akt pathway in normal and tumoral epithelial cells. *Biochem. J.* **366** (Pt 3), 729–736 (2002).
12. Omlin, A. *et al.* AZD3514, an oral selective androgen receptor down-regulator in patients with castration-resistant prostate cancer – results of two parallel first-in-human phase I studies. *Invest. New Drugs* **33**, 679–690 (2015).
13. Attard, G. *et al.* Combining enzalutamide with abiraterone, prednisone, and androgen deprivation therapy in the STAMPEDE trial. *Eur. Urol.* **66**, 799–802 (2014).
14. Tran, C. *et al.* Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* **324**, 787–790 (2009).
15. Kirouac, D.C. & Onsum, M.D. Using network biology to bridge pharmacokinetics and pharmacodynamics in oncology. *CPT Pharmacometrics Syst. Pharmacol.* **2**, e71 (2013).
16. Gray, L.E. Jr, Furr, J. & Ostby, J.S. Hershberger assay to investigate the effects of endocrine-disrupting compounds with androgenic and antiandrogenic activity in castrate-immature male rats. *Curr. Protoc. Toxicol.* Chapter 16: Unit 16.9. (2005).
17. Eisermann, K., Wang, D., Jing, Y., Pascal, L.E. & Wang, Z. Androgen receptor gene mutation, rearrangement, polymorphism. *Transl. Androl. Urol.* **2**, 137–147 (2013).
18. Shafi, A.A., Yen, A.E. & Weigel, N.L. Androgen receptors in hormone-dependent and castration-resistant prostate cancer. *Pharmacol. Ther.* **140**, 223–238 (2013).
19. Lea, O.A. & French, F.S. Androgen receptor protein in the androgen-dependent Dunning R-3327 prostate carcinoma. *Cancer Res.* **41**, 619–623 (1981).
20. Schuurmans, A.L., Bolt, J., Voorhorst, M.M., Blankenstein, R.A. & Mulder, E. Regulation of growth and epidermal growth factor receptor levels of LNCaP prostate tumor cells by different steroids. *Int. J. Cancer* **42**, 917–922 (1988).
21. Lazar D.C. *et al.* Cytometric comparisons between circulating tumor cells from prostate cancer patients and the prostate-tumor-derived LNCaP cell line. *Phys. Biol.* **9**, 016002 (2012).

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