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Moving Towards Precision Urologic Oncology: Targeting Enzalutamide-resistant Prostate Cancer and Mutated Forms of the Androgen Receptor Using the Novel Inhibitor Darolutamide (ODM-201)

Hendrik Borgmann[a,b], *Nada Lallous*[a], *Deniz Ozistanbullu*[a,c], *Eliana Beraldi*[a], *Naman Paul*[a], *Kush Dalal*[a], *Ladan Fazli*[a], *Axel Haferkamp*[b], *Pascale Lejeune*[d], *Artem Cherkasov*[a], *Martin E. Gleave*[a,*]

[a] *Department of Urologic Sciences, The Vancouver Prostate Centre, University of British Columbia, Vancouver, BC, Canada;* [b] *Department of Urology, University Medicine Johannes Gutenberg-University Mainz, Mainz, Germany;* [c] *Department of Urology, University Hospital Frankfurt, Frankfurt, Germany;* [d] *Bayer AG, Berlin, Germany*

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Abstract

Darolutamide (ODM-201) is a novel androgen receptor (AR) antagonist with a chemical structure distinctly different from currently approved AR antagonists that targets both wild-type and mutated ligand binding domain variants to inhibit AR nuclear translocation. Here, we evaluate the activity of darolutamide in enzalutamide-resistant castration resistant prostate cancer (CRPC) as well as in AR mutants detected in patients after treatment with enzalutamide, abiraterone, or bicalutamide. Darolutamide significantly inhibited cell growth and AR transcriptional activity in enzalutamide-resistant MR49F cells in vitro, and led to decreased tumor volume and serum prostate-specific antigen levels in vivo, prolonging survival in mice bearing enzalutamide-resistant MR49F xenografts. Moreover, darolutamide inhibited the transcriptional activity of AR mutants identified in the plasma of CRPC patients progressing on traditional therapies. In particular, darolutamide significantly inhibited the transcriptional activity of the F877L, H875Y/T878A, F877L/T878A, and the previously unreported T878G AR mutants, that transform enzalutamide into a partial agonist. In silico cheminformatics computer modeling provided atomic level insights confirming darolutamide antagonist effect in F877L and T878G AR mutants. In conclusion, our results provide a rationale for further clinical evaluation of darolutamide in enzalutamide-resistant CRPC, in particular in combination with circulating tumor DNA assays that detect AR mutants sensitive to darolutamide, in a precision oncology setting.

Patient summary: In this study we evaluated the novel drug darolutamide in preclinical models of prostate cancer. We found that darolutamide delays growth of enzalutamide-resistant prostate cancer, in particular in cells with mutated forms of the androgen receptor after previous treatment. Our data supports further evaluation of darolutamide in clinical trials.

* Corresponding author. Department of Urological Sciences, University of British Columbia, The Vancouver Prostate Centre, 2775 Laurel Street, 6th Floor, Vancouver, BC V5Z 1M9, Canada. Tel. +1 604 875 5006.
E-mail address: m.gleave@ubc.ca (M.E. Gleave).

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Despite frequent and durable responses for androgen receptor (AR)-targeted therapies in castration-resistant prostate cancer (CRPC), resistance inevitably occurs. Resistance is attributed to genomic and metabolic reactivation of the AR supported by complex and context-dependent activation of stress response, kinase signaling, and developmental pathways [1]. Therefore, additional therapies are needed to enhance the armamentarium of efficacious CRPC drugs, and the current best treatment sequences need to be identified [2]. In parallel, newer and less expensive genomic assays enable the evaluation of efficacy of new agents based on the genomic status of individual patients in line with a precision urologic oncology approach [3].

A promising new agent for CRPC patients is the novel AR inhibitor darolutamide (ODM-201). Darolutamide inhibits AR nuclear translocation and has a distinctly different chemical structure than the currently known AR inhibitors enzalutamide (ENZ), bicalutamide, flutamide, and apalutamide (ARN-509). It has higher binding affinity to the AR compared with ENZ and apalutamide and does not cross the blood-brain barrier which decreases the likelihood of seizures [4]. Results from the open-label phase 1–2 dose escalation trial (ARADES) showed that darolutamide had a favorable safety profile, was well tolerated and provided anticancer activity comparable to ENZ [5,6]. Two randomized phase 3 trials evaluating efficacy and safety of darolutamide versus placebo in high-risk nonmetastatic CRPC (ARAMIS trial, ClinicalTrials.gov identifier NCT02200614) and of darolutamide in combination with standard androgen deprivation therapy and docetaxel in patients with metastatic hormone sensitive prostate cancer (PCa; ARASENS trial, ClinicalTrials.gov identifier NCT02799602) are currently recruiting.

With a distinctly different chemical structure compared with currently approved AR antagonists, we hypothesized that darolutamide might exhibit differential antagonist versus agonist profiles in AR mutated PCa cells, and under specific genomic states provide AR pathway suppression in preclinical models of ENZ-resistant (ENZ-R) PCa.

To evaluate darolutamide in ENZ-R PCa, we used the LNCaP-derived MR49F cell line that inherits the F877L mutation, which has been described to confer resistance to ENZ [7]. Methodologies are described in Supplementary data. In vitro, darolutamide significantly and dose-dependently inhibited MR49F cell growth (Fig. 1A) and AR transcriptional activity (Fig. 1B) compared with ENZ, which acted as an agonist at doses above 1 μM. Fluorescence microscopy demonstrated inhibition of AR nuclear translocation by darolutamide in MR49F cells, but not by ENZ (Supplementary Fig. 1A). Darolutamide significantly decreased both *prostate-specific antigen* (*PSA*) gene (Fig. 1C) and protein expression (Fig. 1D) in ENZ-R MR49F cells, where ENZ acted as an agonist. AR protein expression was not affected by darolutamide (Supplementary Fig. 1B).

Based on these in vitro results, we compared activity of darolutamide, ENZ, and a vehicle in subcutaneous MR49F xenografts in mice. Darolutamide significantly inhibited both tumor growth and serum PSA levels (Fig. 1E), and significantly prolonged survival of mice bearing ENZ-R tumors, compared with ENZ or vehicle (Fig. 1F). Waterfall plots in Fig. 1G illustrate individual tumor responses from baseline between groups after 3 wk of treatment for both tumor volume (left) and serum PSA (right). While apoptotic rates were similar (data not shown), Ki67 staining of representative samples of xenografts indicates in-vivo antiproliferative effects of darolutamide compared with ENZ or vehicle (Supplementary Fig. 1C).

We next tested the effects of darolutamide on the transcriptional activity of a panel of mutated AR variants, previously reported in literature or detected in a cohort of 62 CRPC patients at disease progression after treatment with ENZ, abiraterone, bicalutamide, or docetaxel, using plasma circulating tumor DNA (ctDNA) sequencing [8,9]. We compared the activity of ENZ and darolutamide on a panel of 25 AR mutants using a luciferase reporter assay in AR-negative PC3 cells. Darolutamide behaved similarly to ENZ in inhibiting most AR variants (Supplementary Fig. 2). Importantly, darolutamide inhibited transcriptional activity of three AR variants with partial agonism to ENZ: F877L, F877L/T878A, and H875Y/T878A (Fig. 2A). This data suggest that none of these AR mutants induced under the selective pressures of AR pathway inhibition are cross-resistant to darolutamide.

To gain atomic insights into the mode of action of darolutamide and ENZ in the setting of F877L AR mutation, structure-based computer modeling was performed. ENZ establishes a key hydrogen bond and a π–π stacking interaction with L877 and F765 residues, respectively. In contrast, darolutamide adopted a different binding pose in the ligand-binding site of F877L AR mutant (Fig. 2B). Instead darolutamide maintained its binding conformation in the F877L-mutated pocket through hydrogen and Van der Waals interactions (Fig. 2B); the stability of this conformation was reflected in quantitative structure activity relationship models (Fig. 2C).

In addition, we recently reported a previously uncharacterized AR mutant T878G (substitution of threonine for glycine at the 878 position), for which bicalutamide and flutamide demonstrated agonist behavior [10]. Notably, darolutamide was the only drug that was modeled to bind the pocket of T878G mutant (Fig. 2 D) without partially activating it at high concentrations (Fig. 2E).

Based on these preclinical findings of darolutamide in ENZ-R PCa, a randomized phase 2 trial of darolutamide versus ENZ in metastatic CRPC is planned through the Canadian Cancer Trial Group using ctDNA assays to study effects of AR mutations on response rates. Besides providing support for a sequencing trial of darolutamide after ENZ, our results show the first head-to-head comparison of darolutamide and ENZ over a large range of AR mutants detected in patients with CRPC and no clinical trial directly comparing both drugs is underway. Therefore, our results are contemporarily the most comprehensive genomic and biologic comparison of darolutamide versys ENZ in the preclinical setting.

The findings from this study have several implications for clinical practice. First, preclinical anticancer activity of

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