CRISPR screen reveals essential pathways that crosstalk with androgen receptor signaling in prostate cancer

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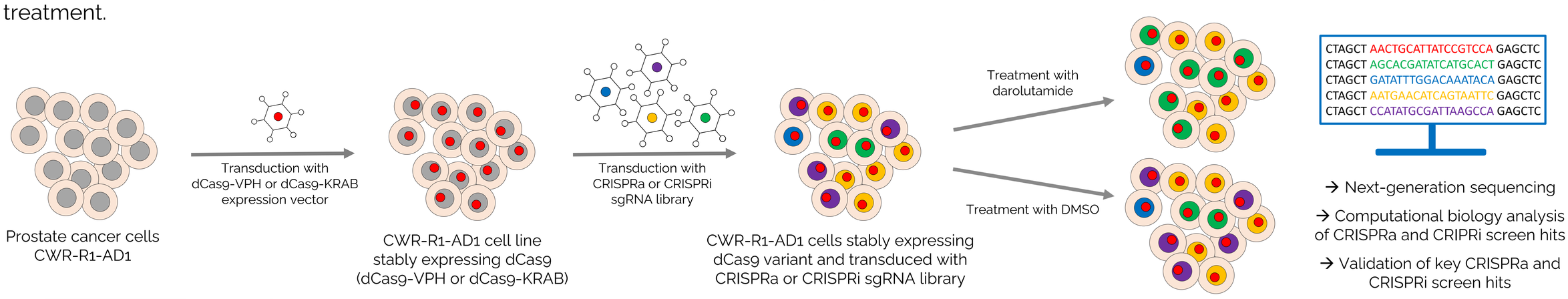
Background
Despite the tremendous progress made in prostate cancer treatment, resistance to established therapies often ultimately occurs. We used the clustered regularly interspaced short palindromic repeats (CRISPR) editing system (1, 2) to identify pathways and genes that crosstalk with the androgen receptor (AR) pathway and have an essential role in sensitizing prostate cancer cells to darolutamide, a second-generation AR inhibitor approved for prostate cancer (3).

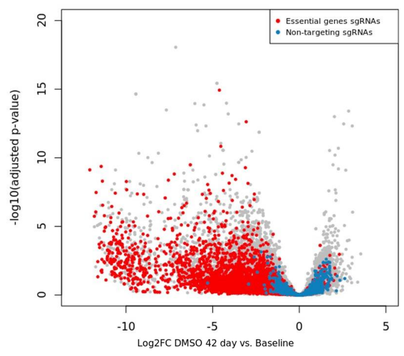
Results 1: Outline of the CRISPR screen
CWR-R1-AD1 prostate cancer cells (4) were transduced with expression vectors for a catalytically dead Cas9 fused to the VPH activator domain (dCas9-VPH) or the KRAB repressor domain (dCas9-KRAB) and stably expressing cell clones were then selected. These cells were then transduced with a pooled CRISPRa or CRISPRi sgRNA library for a systematic and endogenous activation or repression of the transcription of about 18,900 different genes. The cells were treated with darolutamide for 14 and 42 days, with compound replenishment occurring every 7 days. Sequencing of the sgRNAs from surviving cells enabled identification of those significantly depleted over time, thereby indicating which expression modulation enhanced responsiveness to darolutamide treatment.



Prostate cancer cells CWR-R1-AD1

CWR-R1-AD1 cell line stably expressing dCas9 (dCas9-VPH or dCas9-KRAB)

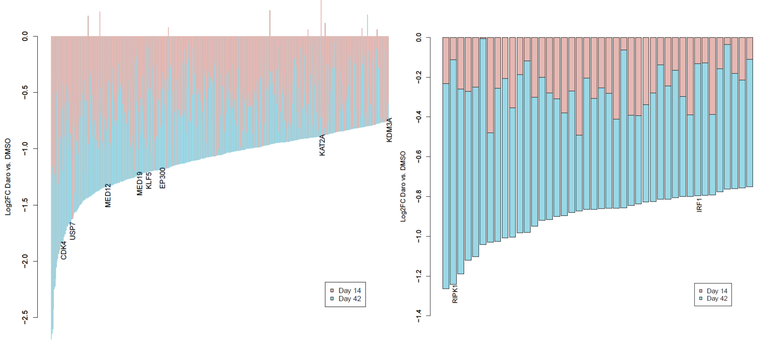
CWR-R1-AD1 cells stably expressing dCas9 variant and transduced with CRISPRa or CRISPRi sgRNA library



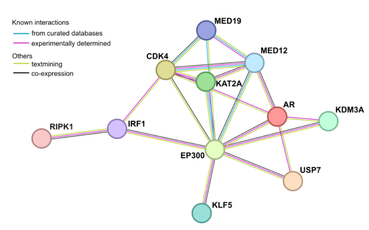
Volcano plot highlighting essential genes found to be depleted in the CRISPRi screen

Results 2: Confirmation of screen validity
We analyzed the DMSO-treated cells transduced with the CRISPRi sgRNA library to determine which genes were downregulated in cells where a significantly reduced proliferation was observed. As expected, we found that numerous essential genes were depleted, including genes involved in cell cycle progression.

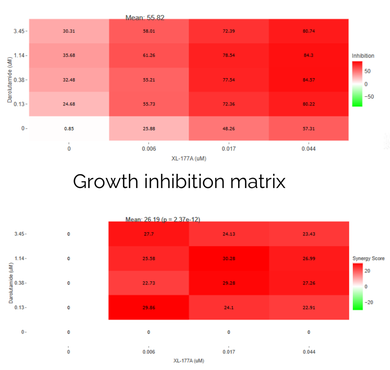
Results 3: Selection of genes leading to increased response to darolutamide
Genes for which the expression showed a log fold change (Log2FC) below -0.75 in the darolutamide-treated group in at least one time point were selected for further analysis. In the CRISPRi screen there were 488 depleted genes and in the CRISPRa screen 42 depleted genes leading to treatment sensitization. The effect of gene modulation was more pronounced following extended treatment duration (Day 42 vs. Day 14).
Depleted genes found in the CRISPRi screen to be involved in sensitization to darolutamide included CDK4, members of the mediator complex, epigenetic modulators and USP7. Depleted genes found in the CRISPRa screen to be involved in sensitization to darolutamide included IRF1 and RIPK1. The protein-protein interaction network between the corresponding proteins and the AR was determined using the STRING database (5).

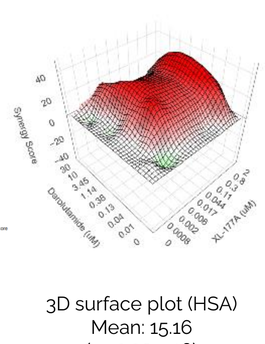


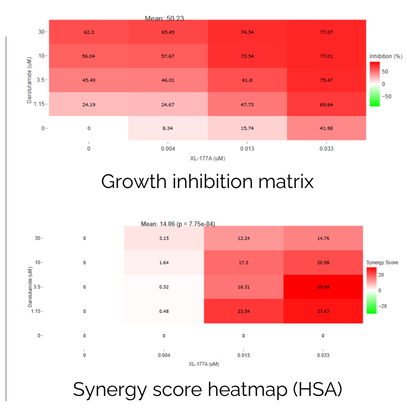
Modulation levels of genes that lead to sensitization to darolutamide as identified in the CRISPi (left) and CRISPRa (right) screen, following short- or long-term treatment
Examples mentioned in the text are highlighted

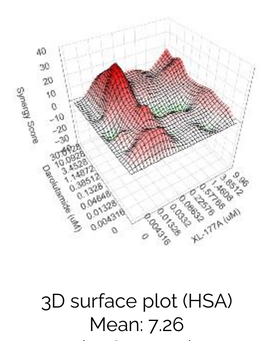


STRING association network for the AR and the proteins encoded by selected genes identified in the CRISPR screens









Impact of combining darolutamide with the USP7 inhibitor XL117A on VCaP (left) and LNCaP (right) cell viability

Results 4: Validation of the CRISPRi screen hit USP7
USP7 was identified as one of the most depleted genes in the CRISPRi screen. We used the specific inhibitor XL177A (6) for confirmation in two additional prostate cancer cell lines, LNCaP and VCaP. Following combination treatments with 0-30 µM darolutamide and 0-10 µM XL177A for 6 days, cell viability was measured in 384-well plates with the CellTiter-Glo assay (Promega). The synergy score was calculated using the SynergyFinder application and Highest Single Agent (HSA) reference model (7). The results show there is synergy between darolutamide and the USP7 inhibitor XL177A leading to increased antiproliferative activity in these two cell lines.

Discussion and outlook
CRISPRi and CRISPRa screens were performed in the CWR-R1-AD1 prostate cancer cell line to identify pathways and genes leading to increased response to the AR antagonist darolutamide, and several pathways interacting with AR signaling were identified. The regulation of these genes now needs to be confirmed in additional prostate cancer cell lines and their role in sensitization to darolutamide treatment is currently being confirmed using specific tools such as selective inhibitors. Importantly, inhibitors addressing several of these pathways are already being tested in clinical studies and it should soon emerge which combinations represent the most promising future treatments for prostate cancer patients.

Acknowledgments
The essential support of the Darolutamide Oncology Development and Medical Affairs Teams is gratefully acknowledged. We thank all technical assistants involved in data generation and analysis. The CWR-R1-AD1 cell line was licensed from the University of Minnesota, USA.
Darolutamide is jointly developed by Bayer AG and Orion Corporation.

References
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