

# Whole-Genome CRISPR Screening Identifies Clinically-Relevant and Differentially-Essential Genes in Enzalutamide-Resistant *versus*-Responsive Prostate Cancer

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Enzalutamide resistance (EnzR) remains a major clinical hurdle to successful management of advanced prostate cancer (PC). Post-progression treatment options are limited and associated with quality-of-life-impacting side-effects, and median survival is poor (9-30mo). We hypothesised that EnzR PC cell lines harbour unique and clinically-relevant genetic, transcriptomic and post-transcriptomic alterations that render them reliant on specific genes for their viability. These vulnerabilities can be identified by whole-genome CRISPR screening in enzalutamide-sensitive (EnzS) and EnzR PC cell models, and specifically targeted for the development of novel therapeutics/ drug repurposing.

Negative selection CRISPR screening was performed using in-house C42/EnzS and C42/EnzR cell lines by lentiviral delivery of the GeCKO v2 sgRNA library, followed by selection and sample collection after 0 and 15 doublings. **sgRNAs lost at T15 vs T0 in EnR vs EnS cells represent those whose targeted genes are uniquely essential/ viability-associated in EnzR cells.** Multi-step robust filtering ( $p_{adj} \leq 0.05$ , >50% sgRNAs/gene exerting same effect) nominated 855 protein-coding and 161 microRNA genes as differentially-essential in EnzR cells vs EnzS. Of these, six gene:miR pairs showed opposing effects on essentiality, consistent with miR canonical repression of target genes. STRING clustering analysis of EnzR essential genes revealed enrichment for translation, proteasomal degradation, splicing and DNA replication. Drugs targeting these EnzR-associated networks are currently under investigation for their ability to differentially impact viability of EnzR vs EnzS cells.

In an orthogonal test of clinical relevance, we showed that our CRISPR screen-identified EnzR-essential gene set accurately separated pre- and post-Enz mCRPC patients in two transcriptomic datasets: DARANA (3mo neoadjuvant Enz), and GS183100 (6mo neoadjuvant ADT + Enz). Additionally, RNA-seq analysis of EnzR vs EnzS cells identified 140 significantly downregulated and 134 upregulated genes, respectively, of which 26 and six were validated as significantly altered in clinical ADT-exposed vs -naïve mCRPC, and/or in mCRPC vs primary disease. Lower tumour levels of EnzR-downregulated genes also correlated with reduced PC survival and increased recurrence. Western blotting confirmed significant dysregulation of AREG, DPP4 and CHRNA2 proteins in EnzR vs EnzS cells. Notably, there was no correlation between AR activity and expression of EnzR-essential genes in clinical data sets.

MARINA and VIPER transcription factor analyses show that FOXO3, TP53 and ELK1 have higher activity in both EnzR vs EnzS cell lines, and in ARPI-resistant/progressed mCRPC vs -responsive/naïve and post-Enz vs pre-Enz primary tumours, supportive of their clinical relevance. Interestingly, whilst FOXO3 activity increases in EnzR, FOXA1 is strongly essential in EnzS but not EnzR PC, and many FOXO3 target gene promoters contain FOXA1 binding sites. This suggests that FOXO3 may substitute for FOXA1 in regulating EnzR-linked genes as resistance develops.

Together, these analyses prioritise clinically-relevant and robustly EnzR-associated candidates for drug-targeting to expand the therapeutic repertoire for mCRPC.

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