A pyrrole-imidazole polyamide is active against enzalutamide resistant prostate cancer

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Background. Effective treatment for enzalutamide-resistant prostate cancer is an unmet need. The LREX' prostate cancer model is resistant to the antiandrogen enzalutamide via activation of an alternative nuclear receptor, glucocorticoid receptor, which has similar DNA binding specificity to the androgen receptor. Furthermore, glucocorticoid receptor expression in mCRPC associates with poor response to enzalutamide. Small molecules that target DNA to interfere with protein-DNA interactions may retain activity against enzalutamide-resistant prostate cancers where ligand binding domain antagonists are ineffective. Pyrrole-Imidazole (Py-Im) polyamides are minor groove-binding small molecules with high affinity and programmable sequence specificity. We hypothesized that ARE-1, a polyamide targeted to the androgen receptor response element half-site, may be effective against enzalutamide-resistant prostate cancer in cell and animal models.

Methods. Antiproliferative effects of ARE-1 against LNCaP/AR, VCaP, and LREX' cells were compared against those of bicalutamide and enzalutamide. Effects of ARE-1 and enzalutamide against ligand induced AR and GR gene expression were assessed by quantitative RT-PCR. Genome wide effects of ARE-1 were evaluated in LREX' and parental LNCaP cells by RNA-sequencing. Effects of ARE-1 on nascent RNA production was measured by 5-ethynyl uridine incorporation. ARE-1 was tested *in vivo* against VCaP and LREX' xenografts.

Results. Antiproliferative effects of ARE-1 in LNCaP/AR, VCaP, and LREX' cells exceeded those of bicalutamide or enzalutamide. ARE-1 attenuated androgen and glucocorticoid driven gene expression in LREX' cells, while enzalutamide only affected androgen driven gene expression. Gene set enrichment analyses of RNA-sequencing data from DHT-induced LREX' cells treated with ARE-1 negatively enriched for the AR signaling pathway, consistent with interference in AR driven gene expression. Similar effects on the AR signaling pathway were seen in LNCaP cells treated with ARE-1. Long-term treatment of LREX' cells with ARE-1 also reduced nascent RNA transcription. In VCaP xenografts, ARE-1 dose-dependently reduced tumor growth by 70% at 5 mg/kg (subcutaneous, 3 times per week) compared to vehicle without significant toxicity. In LREX' xenografts in castrated mice treated with enzalutamide (10 mg PO daily), ARE-1 at 2.5 mg/kg (subcutaneous, 3 times per week) reduced growth by 80% compared to enzalutamide alone without significant toxicity. All LREX' xenografts robustly expressed GR. Tumors from ARE-1 treated mice had elevated TUNEL and reduced Ki67 staining by immunohistochemistry.

Conclusions. Py-Im polyamide **ARE-1**, targeted to the sequence 5`-WGWWCW-3`, which is similar to the ARE and GRE half site, attenuates ligand induced AR and GR transcriptional activity, is more potent than enzalutamide and bicalutamide in cell culture, and is active against enzalutamide resistant xenografts. Long term treatment of LREX' cells with ARE-1 also decreases nascent RNA synthesis.

Conflict of Interest Statement. P.B.D., F.Y., N.G.N. founded Gene Sciences, Inc, which is licensing ARE-1 for clinical development.

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