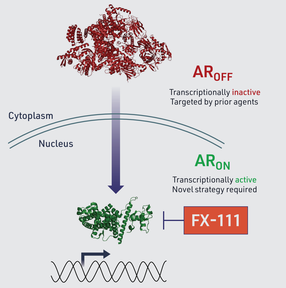
DISCOVERY OF FX-111, A FIRST-IN-CLASS HETEROBIFUNCTIONAL DEGRADER OF TRANSCRIPTIONALLY ACTIVE ANDROGEN RECEPTOR (AR_{ON}), TO TREAT PATIENTS WITH AR-DRIVEN PROSTATE CANCER

Abstract #5784

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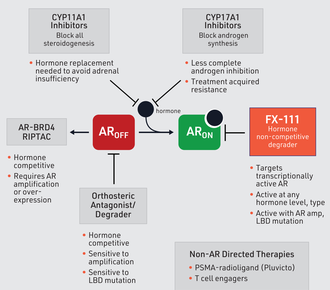
Flare Therapeutics, Cambridge, MA, USA

AR_{ON} solves for AR-driven prostate cancer at all stages



- AR undergoes a hormone-dependent conformational conversion from an **inactive** (AR_{OFF}) to an **active** state (AR_{ON}).
- Full-length AR_{ON} drives disease progression and is present in all patient populations that display androgen signaling, including castrated populations.
- All previous AR targeting agents must load onto AR_{OFF}, which creates vulnerabilities to resistance mechanisms.
  - Very high levels of AR_{OFF} decoy protein in AR amplified/over-expressed settings create efficacy challenges for conventional agents.
  - Local production of auxiliary agonists in the tumor enable AR_{ON} escape in the presence of orthosteric agents.
- Strategies that exclusively target AR_{ON} will be more efficient and effective no matter how AR_{ON} conversion occurs.

FX-111 differentiates from other therapies by targeting AR_{ON}



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FIGURE 1.
Cryo-EM of AR-FX-111-CRBN ternary complex

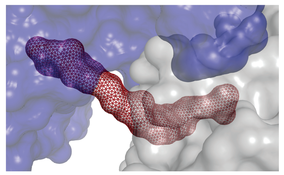
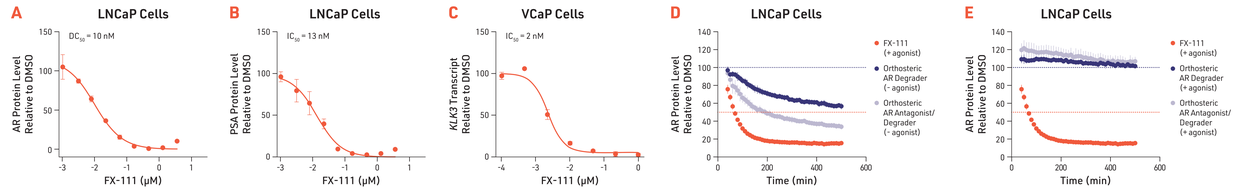
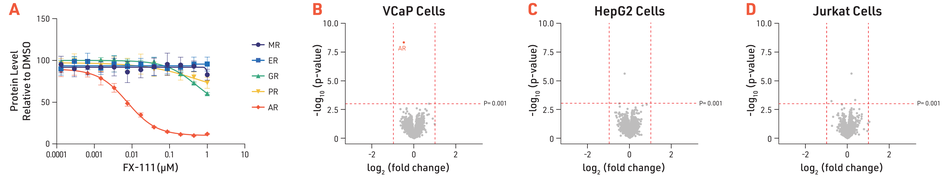


FIGURE 2.
FX-111 potently and rapidly degrades AR and suppresses PSA



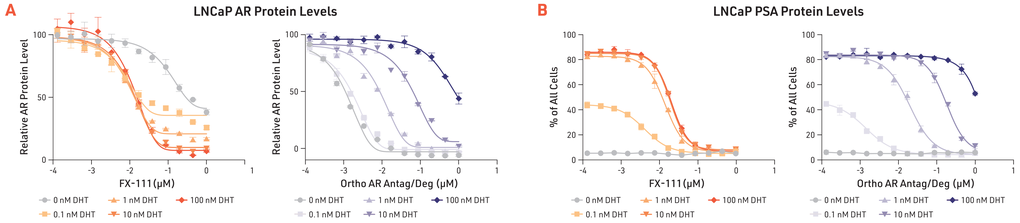
LNCaP AR (**A**) and PSA (**B**) HiBiT tagged cells were used to assess degradation and suppression of AR and PSA by FX-111, respectively. Data are after 24h of treatment. Suppression of *KLK3* transcript levels (PSA transcript) in VCaP cells treated with FX-111 (**C**). AR degradation kinetics in LNCaP cells using optimal conditions for orthosteric and AR_{ON} degraders (125 nM) (**D**) or in androgen replete settings for all molecules (**E**).

FIGURE 3.
FX-111 exhibits selectivity over other steroid hormone receptors and a favorable proteome-wide selectivity profile



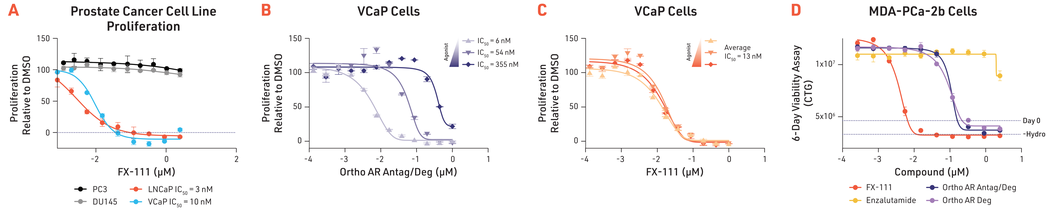
FX-111 potently degrades AR but not other steroid hormone receptors (**A**). Degradation of each receptor was assessed with excess of the cognate ligand in the treatment conditions. Degradation was assessed by quantitation of HiBiT signal after 24 h of treatment. Proteomic analysis of neosubstrate degradation was performed in VCaP (**B**), HepG2 (**C**), and Jurkat (**D**) cells with 2.5 μM FX-111 for 6 hours. VCaP cells were grown in charcoal stripped serum.

FIGURE 4. FX-111 is highly effective at degrading AR and suppressing PSA at all agonist concentrations



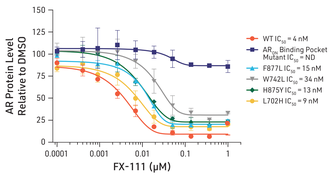
LNCaP cells were treated with FX-111 or a clinical stage orthosteric AR antagonist/degrader under a range of medium conditions (0-100 nM DHT) for 24h. (**A**) AR levels and percentage of (**B**) PSA positive cells from imaging analyses.

FIGURE 5. FX-111 elicits robust proliferation effects in AR-dependent cells



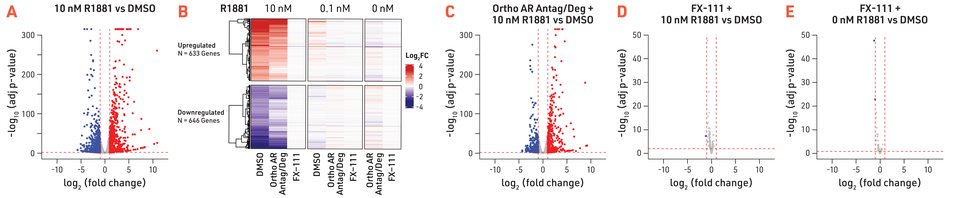
(**A**) FX-111 elicits robust androgen-dependent growth inhibition in AR-positive cells (LNCaP, VCaP), but not AR-negative cells (PC3, DU145). Antiproliferative effects in VCaP cells for an orthosteric antagonist/degrader loses potency as agonist concentration rises (**B**), but are unchanged for FX-111 (**C**). (**D**) Antiproliferative effects in MDA-PCa-2b cells grown in medium supplemented with 100 nM hydrocortisone.

FIGURE 6. FX-111 effectively degrades clinically relevant AR mutants



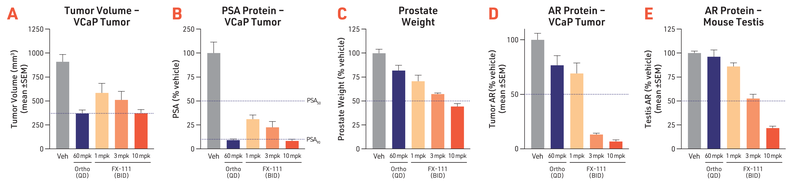
FX-111 treatment results in AR degradation in HEK 293 cells expressing WT AR or clinically relevant AR mutants, but not the mutant form of AR that blocks the AR_{ON} binding pocket.

FIGURE 7. FX-111 completely reverses the global transcriptomic effects of R1881



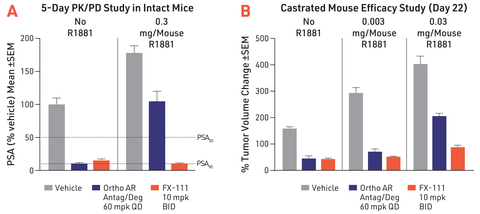
(**A**) Differential expression analysis results comparing DMSO control and 10 nM R1881 (synthetic androgen) treatment for 24 h using LNCaP cells. (**B**) Heatmaps of differentially expressed genes (DEGs) relative to DMSO control under various treatment conditions (FX-111 or DMSO in the presence of 0 μM, 0.1 μM or 10 μM R1881 co-treatment) for 24 h. Comparison of a clinical stage orthosteric AR antagonist/degrader (**C**) or FX-111(**D**) treatment with 10 nM R1881 vs (**A**) co-treatment to DMSO control after 24 h of treatment. FX-111 had negligible effects in absence of R1881 (**E**). DEGs were defined as genes with a greater than 2-fold increase or decrease in expression levels at an FDR (adjusted p-value) < 0.01.

FIGURE 8. FX-111 demonstrates robust *in vivo* activity in AR amplified prostate cancer xenograft model



(**A**) VCaP xenograft efficacy study shows dose-dependent tumor volume reductions with low doses of FX-111, similar to a clinical stage orthosteric AR antagonist/degrader (Ortho). (**B**) PSA suppression with FX-111 treatment in a dose-dependent manner. (**C**) Prostate weights are reduced with FX-111 treatment in a dose dependent manner. (**D**) VCaP xenograft tumor AR protein levels are degraded by FX-111 in a dose-dependent manner. (**E**) Mouse AR protein levels in testes are effectively degraded by FX-111 in a dose-dependent manner.

FIGURE 9.
FX-111 demonstrates robust *in vivo* activity in presence of exogenous hormone



(**A**) VCaP PK/PD study shows robust PSA suppression in intact mice that is maintained in the presence of R1881 for FX-111. (**B**) Castrated VCaP efficacy study shows robust tumor growth inhibition by FX-111 is maintained as exogenous androgen level supplementation increases. Ortho = orthosteric AR antagonist/degrader.

CONCLUSIONS

- FX-111 potently and selectively targets the transcriptionally active form of AR (AR_{ON}) for degradation via binding to a novel pocket on AR and forming a ternary complex with CRBN to elicit degradation.
- Effective degradation of AR_{ON} and suppression of PSA expression at all agonist conditions is achieved by FX-111 treatment in the cell lines tested, including effective degradation of AR that is agonized by alternative ligands as well as degradation of clinically relevant mutant forms of AR.
- FX-111 completely reverses the global transcriptional effects of androgen stimulation in prostate cancer cell lines.
- *In vivo*, FX-111 elicits robust AR degradation and PSA suppression, as well as tumor growth inhibition in prostate cancer xenografts. Consistent with AR signaling inhibition, androgenic tissues are also affected by FX-111.
- Addition of exogenous androgen to mice bearing xenografts results in loss of efficacy for orthosteric AR degraders, but FX-111 effects are unchanged.
- FX-111 has completed IND-enabling studies with plans to initiate clinical studies in mid-2026.