Abstract #1822

Antitumor Activity of JNJ-63576253 (TRC253), a Small Molecule Antagonist of F877L Mutant and Wild-Type Androgen Receptor

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ABSTRACT

Background: Androgen receptor (AR) antagonists have transformed prostate cancer patient care by targeting a key nodal point in tumor cell signaling. However, despite the impressive clinical activity of first- and second-generation antiandrogens, acquired resistance frequently emerges. Point mutations in the ligand-binding domain of AR, such as phenylalanine to leucine at position 877 (AR^{F877L}), account for 10-20% of resistance. Such mutations are characterized by receptor activation, rather than inhibition, by first- and second-generation antiandrogen therapeutics.

JNJ-63576253 is a potent, high affinity competitive binder of wild type and mutant AR, including F877L. JNJ-63576253 blocks AR nuclear translocation, AR binding to DNA, and AR-dependent transcription. JNJ-63576253 inhibits the proliferation of androgen receptor driven prostate cancer cell lines, including those bearing AR^{F877L}.

Results: In the Hershberger assay in male Sprague Dawley rats, oral administration of JNJ-63576253 was well tolerated and inhibited androgen sensitive organ (ASO) development in a dose-dependent manner. In male SHO mice bearing LNCaP xenografts with either wild-type or AR^{F877L}, daily treatment with 30 mg/kg JNJ-63576253 treatment resulted in statistically significant antitumor activity with no clinical adverse effects (i.e. body weight loss), whereas second-generation antiandrogen enzalutamide had no antitumor efficacy in the LNCaP AR^{F877L} mutant model.

Conclusions: Janssen and Tracon Pharma have entered a strategic licensing collaboration, whereby Tracon possesses exclusive rights for clinical development of JNJ-63576253 (now called TRC253). Tracon has entered TRC253 into Ph1/2A clinical evaluation in metastatic castration-resistant prostate cancer patients.

OBJECTIVE

The aim of this study was to characterize the *in vitro* effects of JNJ-63576253 in AR expressing cell lines in competitive binding assays, reporter assays and for anti-proliferative activity in human prostate cancer cells lines; and investigate the AR antagonist activity of JNJ-63576253 using the Hershberger assay in rats and profile its antitumor activity in paired isogenic LNCaP human prostate xenografts bearing either wild-type or F877L mutant AR in mice.

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INTRODUCTION

Figure 1A. Androgen receptor pathway

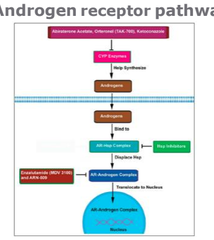
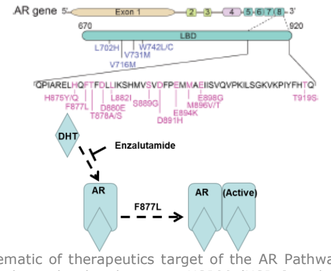


Figure 1B. Androgen receptor F877L mutation



(A) Schematic of therapeutics target of the AR Pathway. AR is bound to the molecular chaperone HSP90 (HSP Complex) which prevents its degradation. HSP90 inhibitors cause AR degradation and decrease AR levels. In men treated with GnRH agonists to shut down testicular androgen synthesis, residual serum androgens are synthesized by the adrenal glands. In additional studies, evidence suggests intratumoral androgen synthesis. Both can be inhibited by the non-specific p450 inhibitor ketoconazole and the specific 17-lyase inhibitor abiraterone. Ligands, such as DHT bind to AR; and is inhibited by antiandrogens such as enzalutamide. AR mutation(s) as well as AR overexpression can convert endogenous steroids and some antiandrogens into agonists.

(B) Enzalutamide suppresses AR function even when AR is overexpressed. The F877L mutation renders enzalutamide agonistic.

RESULTS

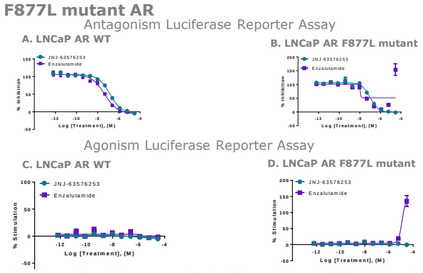
JNJ-63576253 is potent, high affinity competitive binder of wild type AR and F877L mutant AR as shown in Table 1.

Table 1: Radioligand Binding of JNJ-63576253

	IC_{50} (nM)	Ki (nM)
Androgen Receptor[1]	6.9	3
Glucocorticoid receptor[2]	>30000	NC
Estrogen Receptor[3]	NC	NC

Radioligand binding inhibition and affinity calculations were determined using (1) [3H]-methyltrienolone, (2) [3H]-dexamethasone and (3) [3H]-estradiol. For ER, it was not possible to determine a value for inhibition or affinity and data are scored as not calculated (NC).

Figure 2: JNJ-63576253 demonstrates potent antagonistic activity against both wild-type and F877L mutant AR



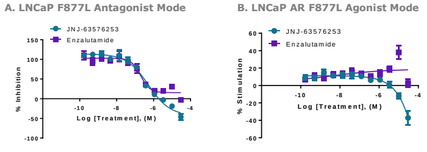
In Figure 2, cell lines were obtained from Aragon (AR.CS line is originally from Sawyers lab, see Chen et al. 2002 Nature Medicine). 10K cells were seeded into a 384-well greiner optical bottom plates in RPMI-1640 supplemented with 1% charcoal-stripped FBS. Cells were treated on Day 2 and incubated for 24 hours. Steady-Glo reagent (Promega) was prepared and utilized according to the manufacturer's protocol, the plates were read on an EnVision plate reader in luminescence mode, and the data analyzed using GraphPad Prism.

Table 2: Reporter Assay Activity of JNJ-63576253 in LNCaP AR WT and LNCaP AR F877L mutant Prostate Cancer Cells

	JNJ-63576253		Enzalutamide	
	IC_{50} (nM)	Agonism	IC_{50} (nM)	Agonism
LNCaP AR WT	144	no	45	no
LNCaP F877L mutant AR	99	no	no fit	Complete at 10μM

Agonism is defined as complete, indicating activity was equal to that of R1881 stimulated reporter output.

Figure 3: JNJ-63576253 inhibits the androgen dependent proliferation of both wild-type and F877L mutant AR driven tumors cells



In Figure 3, 1250 cells were seeded into 384-well greiner optical bottom plates in RPMI-1640 supplemented with 1% charcoal-stripped FBS. Cells were treated with compound on Day 2 and incubated for 6 days. CellTiter-Glo reagent (Promega) was prepared and utilized according to the manufacturer's protocol, the plates were read on an EnVision plate reader in luminescence mode, and the data analyzed using GraphPad Prism.

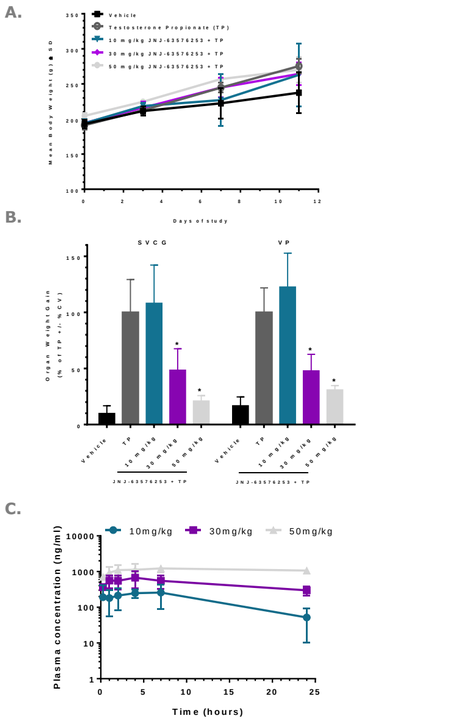
Table 3: Antiproliferative Activity of JNJ-63576253 in LNCaP AR WT and LNCaP AR F877L Mutant Prostate Cancer Cells

Cell Line[1]	IC_{50} (nM)	AR status
VCaP	270	Wildtype AR
LNCaP AR[2]	435	Wildtype AR
LNCaP F877L[2]	197	AR F877L mutation

[1]Cells were cultured in the presence of 30 – 100 pM methyltrienolone (R1881).
[2]JNJ-62576253 did not induce proliferation in the absence of R1881.

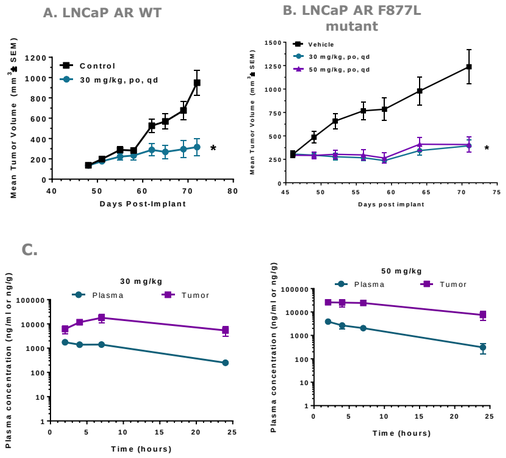
Androgen dependent proliferation studies were conducted in both WT AR expressing cells (VCaP, LNCaP AR) and mutant AR (LNCaP F877L). In each cell line, cells were cultured in charcoal stripped serum and the effect of compounds scored relative to androgen dependent proliferation i.e. in presence of R1881.

Figure 4: JNJ-63576253 inhibits androgen sensitive organ (ASO) development in the Hershberger assay



In Figure 4, for the Hershberger study, pre-pubertal male Sprague Dawley (SD) rats were castrated at 42-45 days of age and weighed on average 200-220 g were randomized into experimental groups by body weight 11 days post-castration, with n=6 animals allocated per treatment group. Treatment with JNJ-63576253 at 10, 30 or 50 mg/kg, po, qd was initiated 12 days post-castration for a total of 10 days. The control group for androgen agonism was dosed with combined vehicles (20% HPβCD plus corn oil), whereas a control group for androgen antagonism (inhibition of testosterone propionate (TP)-stimulated ASO growth) received TP + 20% HPβCD.
Animal body weights were recorded during the course of the study **(A)** and, at necropsy, the weights of male reproductive tract organs were collected. **(B)** Exposure to antiandrogens inhibited TP-induced growth of the testes, sex accessory tissues and Levator Ani-bulbocavernosus (LABC) muscles in a dose-related manner. For androgen antagonism, JNJ-63576253 with co-administered TP group is compared to the reference androgen group (TP), and a statistically significant decrease (*) in tissue weight is considered a positive antagonist result. **(C)** Blood/plasma was collected by retro-orbital sampling on study day 0 (pre-dose), day 4 or 5 (1-2 hour post dose) and day 11 (24 hours post last dose) as part of the necropsy for three animals per cohort (n=6/ group).

Figure 5: JNJ-63576253 inhibits tumor growth in human prostate cancer xenograft models, including F877L mutant AR



In Figure 5, castrated male SHO mice were implanted *sc* on the right flank with human **(A)** LNCaP AR or **(B)** LNCaP AR F877L mutant tumor spheroids. After ~45 days post implant, when tumors were established, mice were randomized into experimental groups. The following day, mice were administered with JNJ-63576253 at 30 or 50 mg/kg, po, qd for ~3 weeks (n=9-10/group). Tumors were measured twice weekly and the results presented as the average tumor volume, expressed in mm^3 ± SEM of each group. Tumor growth inhibition (TGI) is statistically significant ($p < 0.01$) using a 1-way ANOVA with multiple comparisons Dunnett's multiple comparisons post-test using Graph Pad Prism software (version 6). **(C)** On day 74 of the study, castrated male SHO mice were given a final oral administered of JNJ-63576253 at 30 or 50 mg/kg and terminal plasma and tumor PK was collected at 2, 4, 7 or 24 hours (n = 2-3/timepoint). Data are expressed in mean ± SEM of each group.

CONCLUSION

- JNJ-63576253 is a potent and selective small molecule antagonist of both androgen receptor (AR) F877L mutant and WT AR
- In vitro, JNJ-63576253 inhibits signaling and proliferation of AR WT and F877L mutant expressing cells and shows little or no agonism
- In vivo, JNJ-63576253 inhibits androgen sensitive organ (ASO) development in the Hershberger assay
- JNJ-63576253 is active in human prostate cancer xenograft models that express WT and F877L mutant AR
- Our data suggests that JNJ-63576253 has potential to be a first in class small molecule antagonist of AR for patients that develop resistant F877L tumors and fail first and second-generation anti-androgen therapy
- Janssen and Tracon Pharma have entered a strategic licensing collaboration, whereby Tracon possesses exclusive rights for clinical development of JNJ-63576253 (now called TRC253). Tracon has entered TRC253 into Ph1/2A clinical trials (NCT02987829) in metastatic castration-resistant prostate cancer patients

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