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Abiraterone acetate (AA), an irreversible inhibitor of CYP17, has significant and durable anti-tumor activity in both chemotherapy-naive and docetaxel treated castration-resistant prostate cancer (CRPC)

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Introduction: AA is an oral irreversible inhibitor of CYP17 (17alpha-hydroxylase/C17,20-lyase), a key enzyme in androgen and estrogen synthesis, and is under phase III evaluation for the treatment of CRPC patients (pts) progressing after established therapies.
Methods: CRPC pts were enrolled into 2 parallel trials of AA: (i) A Phase I/II study in chemo-naive CRPC and (ii) A Phase II study in CRPC post-docetaxel. Both Phase II trials utilized a 2-stage design with Ho of 10% and Ha of 30%, alpha = 0.05; beta = 0.14 and PSAWG criteria for response. To investigate predictors of response and mechanism of resistance, target steroids were measured and blood for enumeration and fluorescence in situ hybridization (FISH) studies of circulating tumor cells (CTC), utilizing the CellSearch™ system, was collected at baseline, on study and at progression (PD).
Results: 89 pts have been treated with AA; 54 chemo-naive and 35 post-docetaxel. 35/54 (65%) chemo-naive and 17/35 (48%) post-docetaxel pts had a decline in PSA $\geqslant$50%, rejecting the null hypothesis in both studies. Radiological regression, symptomatic improvements and normalization of raised LDH and ALP were observed. Treatment with AA resulted in significant suppression of androgenic and estrogenic steroids downstream of CYP17. Using Spearman-Rank correlation, pre-treatment androstenedione and estradiol levels were significant predictors of a PSA decline $\geqslant$50% (p values: 0.01079 and 0.0299) but baseline DHEA and testosterone (T) levels were not. Using a super-sensitive LC/MS/MS assay, T declined to <0.05 ng/dl in 5 pts and to a median of 0.23 ng/dl (range: 0.646–0.08 ng/dl) in the other 15 pts studied. There was no association between T nadir of pts who responded compared to those who did not respond to AA. Importantly, there was no rise in hormones at PD. 39/89 pts had $\geqslant$5 CTCs at baseline; 18/39 pts had a decline to <5 CTCs on AA. 16/39 pts had a *TMPRSS2-ERG* fusion in their CTCs idenitifed by FISH. Patients with a decline from $\geqslant$5 CTCs to <5 CTCs had an improvement in survival compared to pts with no decline in CTCs; 14/18 pts with a decline in CTCs to <5 had a *TMPRSS-ERG* fusion. Only 2 pts with a *TMPRSS2-ERG* did not have a decline in CTC to <5 but both had loss of *PTEN*. Gain of *AR* in CTCs at PD on AA compared to baseline was observed in 5 pts.
Conclusions: These results support emerging preclinical data indicating that CRPC frequently remains hormone driven despite progression following all available treatment options. They confirm that the anti-tumor activity of AA is attributable to suppression of androgenic and estrogenic hormones and suggest that pts with a *TMPRSS2-ERG* fusion constitute a tumor sub-group with increased sensitivity to CYP17. Loss of *PTEN* and gain of *AR* are potential mechanisms of resistance.

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Re-inducing sensitivity to abiraterone acetate, a novel CYP17 inhibitor with a high level of anti-tumour activity in castration resistant prostrate cancer

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Introduction: Abiraterone acetate (AA) has been developed as a novel 17alpha-hydroxylase/C17−20, lyase (CYP17) inhibitor, the key enzyme in androgen synthesis. Phase II studies of AA report a PSA decline by $\geqslant$50% in 60% of castration-resistant prostate cancer (CRPC) patients. However, AA causes a rise in ACTH which drives excess synthesis of steroids upstream of CYP17, such as deoxycorticosterone, which can activate a mutated androgen receptor (AR).
Methods: To investigate whether suppression of ACTH would re-induce sensitivity to AA, dexamethasone 0.5 mg/day was added to CRPC patients progressing on AA. Steroids upstream and downstream of CYP17 were measured prior to and after starting dexamethasone.
Results: Addition of dexamethasone to AA resulted in suppression of ACTH, corticosterone and deoxycorticosterone to below the lower limit of sensitivity of the assays used.
Group I: 19/54 pts received dexamethasone at progression on AA and had not received dexamethasone previously: 5/19 (26%) had a decline in PSA $\geqslant$50%. Duration of response (days): 252+, 84+, 140+, 259+, and 280+.
Group II: 11/54 pts had dexamethsone after AA and had previously failed dexamethasone at the same dose and schedule. The duration of response on AA and AA + dexamethasone for this sub group (days) is shown in *table 1*. 4/11 (36%) pts had a decline in PSA $\geqslant$50% and 1/11 pt had a decline in PSA >90% after addition of steroids. Duration of response on dexamethasone + AA (days): 469+, 81+, 231, and 202. One pt did not have a $\geqslant$50% PSA decline but stable disease for 552+ days.
Conclusion: Preliminary data suggests that resistance to AA can occur secondary to activation of a promiscuous AR by hormones upstream of CYP17. This has been reversed by addition of steroids to decrease ACTH drive and upstream steroids and has resulted in re-induction of sensitivity to AA in 30% of patients, regardless of prior treatment with same dose corticosteroids.

Table 1

patients	1	2	3	4	5	6	7	8	9	10	11
Duration (days) on											
AA	509	534	36	112	119	65	56	72	168	49	245
AA + steroids	222	202	21	552+	469+	196	276	151	231	172	81+
PS A response (%) on											
AA	>50%	>50%	PD	>50%	SD	SD	SD	>50%	>50%	>50%	>50%
AA + steroids	PD	>50%	PD	SD	>50%	PD	SD	>30%	>50%	PD	>50%

PD: progressive disease; SD: stable disease.

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Phase I-II study of MDV3100 in castration resistant prostate cancer. The Prostate Cancer Clinical Trials Consortium

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Background: Experimental models and molecular profiles of human prostate cancers show that the androgen receptor (AR) is activated in castration-resistant prostate cancers.. MDV3100 is a novel small molecule AR antagonist engineered for activity in models with AR overexpression. MDV3100 blocks nuclear translocation of AR, DNA binding, and has no agonist activity when AR is overexpressed. In July 2007, a multi-center first-in-man Phase 1−2 trial was initiated to determine safety, pharmacokinetics (PK), and antitumor activity including effects on prostate-specific antigen (PSA), circulating tumor cells (CTC), bone and soft tissue metastases, and in selected patients (pts) fluorodeoxyglucose (FDG) and fluorodihydrotestosterone (FDHT) uptake by positron emission tomography (PET).
Material and Methods: Pts are administered MDV3100 orally, once daily. Dose-escalation cohorts include 3−6 pts starting at 30 mg/day with sequential escalations to 60, 150, 240, and 360 mg/day. Enrollment has been expanded at 60 mg/day and above to further recruit approximately 12 chemotherapy-naive and 12 post-chemotherapy pts, once the safety of a dose has been established.
Results: Enrollment has been completed at doses up to 240 mg/day. Recruitment for the dose-escalation cohort at 360 mg/day is currently ongoing. 22 pts at 60 mg/day, 23 pts at 150 mg/day, and 28 pts at 240 mg/day have been followed for 12+ weeks. To date, MDV3100 has been generally well-tolerated with no reports of serious adverse events deemed related to study drug. PK were dose-linear with a half-life of approximately one week. Available PSA data demonstrate effective AR blockade by MDV3100. PSA declines from basline at week 12 are summarized in the table.
Of the 42 and 31 chemo-naive and post-chemotherapy pts across the three dose levels, 23 (55%) and 13 (42%) had a >50% decline in PSA at week 12 compared to baseline, respectively.

Table 1

Dose level	>50% Decline	>90% Decline
60 mg/day	41% (9/22)	9% (2/22)
150 mg/day	57% (13/23)	13% (3/23)
240 mg/day	50% (14/28)	29% (8/28)

Conclusions: MDV3100 is a novel AR antagonist in clinical investigation. The observed PSA responses are consistent with the inhibition of AR signaling. MDV3100 has been well-tolerated to date and appears to be a promising candidate for the treatment of CRPC. Pt recruitment and follow-up are continuing. The analyses of the associations among PSA, CTC, radiographic, and PET outcomes are ongoing and will be presented.

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Potent anticancer activity of panobinostat (LBH589) in models of hormone-refractory prostate cancer (HRPC): targeting the androgen receptor

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Background: Panobinostat (LBH589) is a highly potent pan-deacetylase (pan-DAC) inhibitor which has demonstrated preliminary clinical efficacy in hematologic and solid malignancies, including prostate cancer. Panobinostat inhibits the molecular chaperone heat shock protein 90, promoting degradation of client proteins such as the androgen receptor (AR) and human epidermal growth factor receptor type 2 (HER-2), which play critical roles in the growth and survival of prostate cancer. The anticancer effects of panobinostat were investigated in both *in vitro* and *in vivo* prostate cancer models, including effects on AR and HER-2 protein levels.
Materials and Methods: Cell lines of known AR status and different degrees of androgen dependence were treated with panobinostat. Effects on cell proliferation (IC_{50}) and viability (LD_{50}) were measured by MTS assay. Levels of target proteins were determined by immunoblotting. Mice bearing AR+, androgen-independent CWR22Rv1 prostate tumor xenografts were treated with panobinostat alone or in combination with docetaxel. Tumor growth inhibition and delay, and AR protein levels, were determined.
Results: Panobinostat inhibited growth of 10 prostate cancer cell lines (IC_{50} 0.9–22.4 nM) and induced potent cytotoxicity in AR+ prostate cancer cells (LD_{50} 20−81.9 nM). Interestingly, AR− cells were sensitive to the antiproliferative effect of panobinostat, but not to panobinostat-induced cell death (LD_{50} >1000 nM). Panobinostat treatment depleted AR and HER-2 in both androgen-dependent and -independent prostate cancer cells. In the hormone-refractory CWR22Rv1 tumor model, single-agent panobinostat induced prolonged tumor stasis, with concomitant depletion of AR from tumor tissues. The combination of panobinostat and standard of care agent docetaxel delayed tumor growth after cessation of treatment, and increased the time to study endpoint of 90 days or 2000 mm^3 tumor volume.
Conclusions: Panobinostat is a potent anticancer agent in both *in vivo* and *in vitro* models of prostate cancer. Panobinostat depletes AR and HER-2 in both AR+ androgen-dependent and -independent prostate cancer cells, and AR in an AR+ hormone-refractory prostate cancer xenograft model at clinically attainable levels. The combination of panobinostat with docetaxel *in vivo* results in enhanced anti-tumor effects and delay of tumor progression. These studies support the continued clinical investigation of panobinostat in HRPC.

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Effects of SOD2 silencing on androgen receptor function and gene regulation: implications for castration-resistant prostate cancer

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Background: Advanced prostate cancer is generally first treated with androgen deprivation therapy. However, tumors become resistant to and grow despite castrate levels of testosterone. Growth and proliferation of castration-resistant prostate cancer (CRPC) is mediated by gain-of-function changes in the androgen receptor (AR) and AR reactivation. Expression of manganese superoxide dismutase (SOD2), which regulates cellular reactive oxygen species, is markedly down-regulated in CRPC when compared to hormone responsive tumors.
Materials and Methods: Here, we knocked down SOD2 expression in AR-expressing LNCaP prostate cancer cells. We performed transcription factor DNA binding assays to determine changes in AR binding that occur with SOD2 knockdown. Furthermore, we performed DNA microarray analysis to identify gene expression changes induced in prostate cancer with SOD2 knockdown.
Results: Gene expression changes induced by SOD2 knockdown results in the up-regulation of genes which are also androgen responsive and 46% of genes up-regulated two-fold by the androgen ligand R1881 are also up-regulated to the same extent with SOD2 knockdown. The induction of many of these genes with SOD2 knockdown, such as VEGFA, is reversible with the antioxidant N-acetylcysteine (NAC), suggesting that this mechanism is directly linked to reactive oxygen species. Furthermore, an array for transcription factor DNA binding activity shows that SOD2 knockdown induces DNA binding by several transcription factors, including AR. SOD2 knockdown-induced AR activation was confirmed by electrophoretic mobility shift assay (EMSA) and was readily reversible with NAC.
Conclusions: These findings show that dysregulation of SOD2 induces AR activity in a reactive oxygen species-dependent manner, and suggests that there may be a role for antioxidant therapy in CRPC.

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The steroid sulfatase inhibitor BN83495 inhibits E1S-stimulated growth of DMBA-induced mammary tumour in rat

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Steroid sulfatase (STS) is a new target for the treatment of steroid hormone dependent diseases such as breast, prostate or endometrial cancer. In breast cancer, estrogens play a major role in the establishment of the disease and between one to two-thirds of tumours are estrogen receptor (ER) positive. Despite current hormonal treatments, improvement is still necessary to achieve better disease control and improve disease outcome. BN83495 is a non-steroidal, non estrogenic, potent, irreversible STS inhibitor that blocks both the formation of E1 from estrone sulfate and androstenediol from DHEAS. The ability of BN83495 to inhibit E1S-stimulated tumor growth in the rat was examined in a DMBA-induced mammary tumor model. Based on median tumor volume and the interquartile range at the end of the treatment period, BN83495 displayed the greatest antitumor activity compared to Tamoxifen or Fulvestrant. Addition of Fulvestrant or Tamoxifen to BN83495 did not improve the potent antitumor activity observed with BN83495 alone. Pharmacokinetic data of BN83945 and effects on estradiol levels are discussed. Altogether, these preclinical results have supported the entry of BN83945 into further clinical trials for estrogen receptor-positive breast cancer patients.

Metastasis and invasion

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Inhibition of CXCR-4 reduces breast cancer xenograft metastasis to multiple organs

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Regulation of metastasis occurs in part through chemokine secretion by distant organs/tissues. The chemokine CXCL12 is constitutively expressed in tissues where metastases develop, specifically lung, liver and bone. The primary receptor for CXCL12 is CXCR4. Expression of CXCR4 on breast cancer cells causes increased bone metastasis and poor overall survival in vivo. In addition, the CXCL12/CXCR4 pathway has been associated with increased migration and invasion of tumor cells in response to hypoxia and tumor angiogenesis through recruitment of bone marrow derived cells. It was hypothesized that treatment of mice with a CXCR4 antagonist would decrease the incidence of metastasis to bone and other organs in an in vivo model. The purpose of this study was to test the efficacy of CTCE-9908, a CXCR4 antagonist, as an antimetastatic agent for breast cancer. GFP-expressing MDA-MB-231 metastatic breast cancer cells were injected into the left cardiac ventricle or the tail vein of athymic mice. Mice were treated with 25 mg/kg CTCE-9908 daily beginning either the day previous to tumor cell injection or the day of tumor cell injection. After 6 or 8 weeks (intracardiac and tail vein injections, respectively),