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Abiraterone is able to block AR activation induced by accumulating precursor hormones resulting from CYP17A1 inhibition

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Background & Aim

Although metastatic prostate cancer (PC) responds well to androgen ablation therapy, progression towards castration resistant PC (CRPC) occurs within 3 years. Despite low circulating levels of testosterone (T) in CRPC patients under hormonal therapy, the androgen receptor (AR) is still active, indicating its remaining role as target in the treatment of CPRC. We previously demonstrated that conversion of adrenal androgens into T, rather than intratumoral de novo steroidogenesis, is the major source of T in (CR)PC tumours [1]. Adrenal androgens are synthesized from progesterone (Prog) and pregnenolone (Preg) by the enzyme CYP17A1. Clinical trials have demonstrated that the CYP17A1 inhibitor Abiraterone (Abi) can increase survival in CRPC patients even after chemotherapy [2]. The blockade of CYP17A1, however, may lead to accumulation of precursor hormones that have the potential to activate the AR [3].

AIM: In this preclinical study, we tested if Abi is able to inhibit CRPC growth in vitro and whether resulting accumulation of precursor hormones can drive PC progression.

Materials & Methods

- Castration resistant clones were generated by long term culture of VCaP and DuCaP cell lines in steroid-stripped medium (DCC), with or without 1 μM bicalutamide or flutamide.
- Experiments with subset of AR-overexpressing CRPC clones were done in DCC with the addition of Preg, Prog, DHT and/or Abi.
- Cell proliferation was assessed by MTT-assay.
- Gene expression levels of AR target gene PSA was assessed by qRT-PCR.
- HEP3B cells with a GFP-tagged AR were used to study AR translocation under similar conditions.

Conclusion

High levels of Prog comparable to those found in patients during treatment with a CYP17A1 inhibitor can potentially activate AR-regulated cell growth in AR-overexpressing CRPC in vitro. Although treatment with Abi may lead to high precursor hormone levels in serum, its anti-androgenic properties may prove sufficient to block precursor hormone-induced AR activation.

References

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2. de Bono, J.S., et al., *Abiraterone and increased survival in metastatic prostate cancer.* N Engl J Med, 2011. 364(21): p. 1995-2005.
3. Attard, G., et al., *Clinical and biochemical consequences of CYP17A1 inhibition with abiraterone given with and without exogenous glucocorticoids in castrate men with advanced prostate cancer.* J Clin Endocrinol Metab, 2012. 97(2): p. 507-16.

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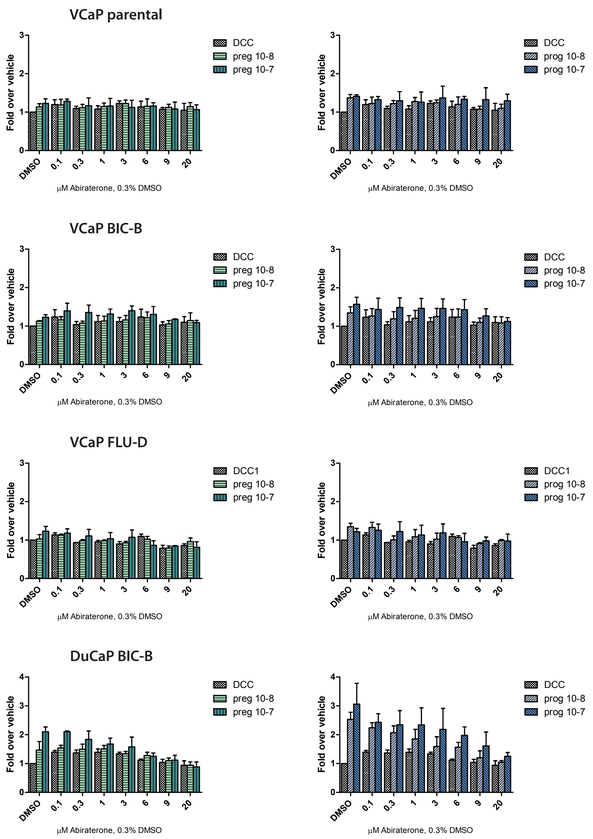
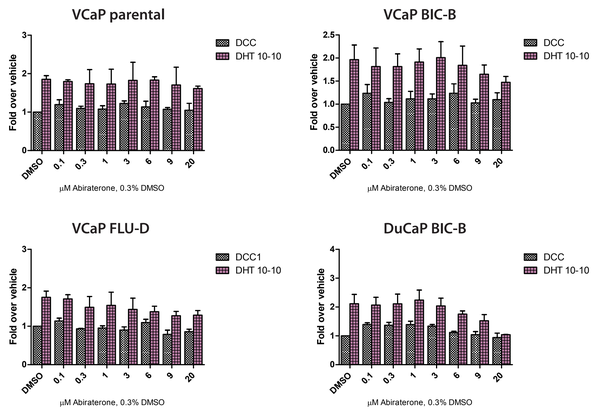
Results

In DuCaP BIC-B, progesterone can induce growth despite adequate CYP17A1 blockade



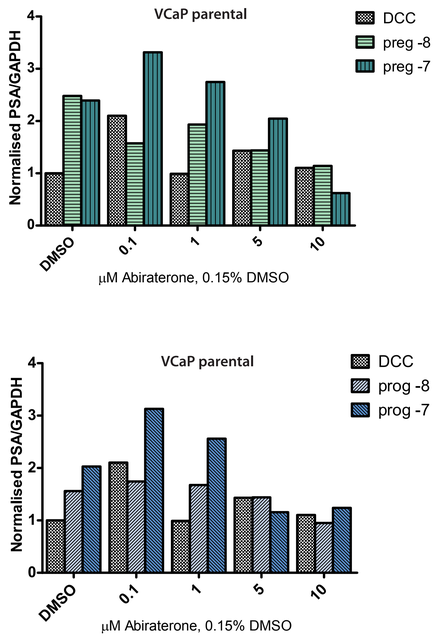
◄ **Left: CRPC proliferation in the presence of CYP17A1 substrate:** In VCaP parental and CRPC cells, 10 to 100 nM of preg and preg do not induce substantial growth. In DuCaP BIC-B, these precursors do induce growth, but this is not inhibited at Abi-concentrations that are known to adequately block CYP17A1 activity (0.1 μM).

DHT-induced growth is inhibited at higher concentrations of Abiraterone



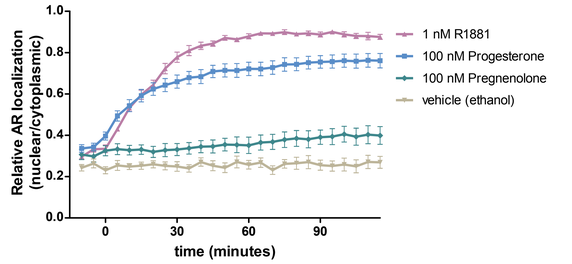
▲ **Above: High concentrations of Abi can inhibit DHT-induced CRPC growth.** In VCaP parental and CRPC cells, 0.1 nM DHT is optimal for stimulating cell growth (data not shown). At higher concentrations DHT-induced growth is partially blocked by Abi, which can not be explained by CYP17A1 inhibition. In DuCaP BIC-B, 20 μM Abi completely blocked DHT-induced cell growth.

Progesterone induces AR regulated gene expression which can be blocked by Abi



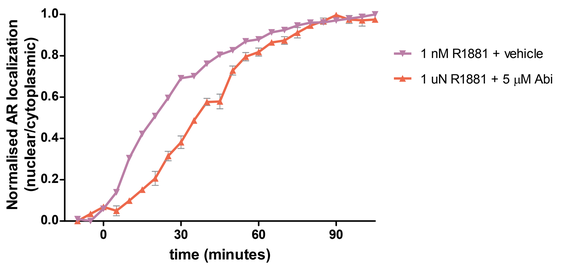
▲ **Above: high concentrations (100 nM) of preg and prog induce AR regulated gene expression in parental VCaP cells.** qRT-PCR for PSA reveals that Preg and Prog at high concentrations give a upregulation of AR-regulated genes. Blocking CYP17A1 at adequate levels of 0.1 and 1 μM do not inhibit this activity, but higher concentrations of Abi blocked expression of PSA.

Precursor hormones induce AR translocation



▲ **Above: high concentrations (100 nM) of progesterone induce AR translocation.** HEP3B cells with a GFP-tagged AR showed AR translocation after treatment with 100nM progesterone. Optimal stimulus with 1 nM R1881 was used a a positive control. Ethanol (vehicle control) showed AR translocation to the nucleus, 100 nM pregnenolone showed only minor AR-translocation.

Abi inhibits AR translocation



▲ **Above: 5 μM Abiraterone slows down AR-translocation.** In HEP3B cells with an GFP-tagged AR, R1881-induced translocation of the AR to the nucleus is slowed down after overnight treatment with Abiraterone.