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Dual activity of abiraterone: CYP17 mediated androgen ablation and direct AR inhibition

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Background & Aim

Androgen ablation is the primary therapy for metastatic prostate cancer (PC). However, within 3 years progression towards castration resistant PC (CRPC) will occur. Despite low serum levels of testosterone (T) in patients under hormonal therapy, the androgen receptor (AR) remains active, indicating its remaining role in CPRC growth regulation. Previously, we demonstrated that conversion of adrenal androgens into T, rather than intratumoral de novo steroidogenesis, is the major source for AR activation in CRPC patients. AA are synthesized from precursor hormones, progesterone (Prog) and pregnenolone (Preg) by the enzyme CYP17A1. The CYP17A1 inhibitor Abiraterone (Abi) has shown to increase survival in CRPC patients even post-docetaxel. Blocking CYP17A1, however, may lead to the accumulation of precursor hormones such as Preg and Prog that have the potential to activate (over expressed) AR.

In this preclinical study, we tested if Abi is able to block growth of CRPC cell lines and if resulting accumulation of Preg and Prog can sustain cell growth.

Materials & Methods

- CRPC clones were generated by long term culture of VCaP and DuCaP cell lines in steroid-stripped medium (DCC), with or without anti-androgens.
- Experiments using a 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.
- mRNA levels of AR target gene (PSA) was assessed by RT-PCR.
- To study AR translocation HEP3B cells with GFP-tagged AR were used.

Conclusions

- **Progesterone at 100 nM can activate AR driven cell growth in AR overexpressing CRPC *in vitro*.**
- **The direct anti-androgen activity of abi at exposures achieved in patients may counter the precursor hormone levels induced by its CYP17 inhibitory activity.**
- **Abiraterone may interfere with (CR)PC growth at two levels: CYP17 inhibition as well as direct AR inhibition.**

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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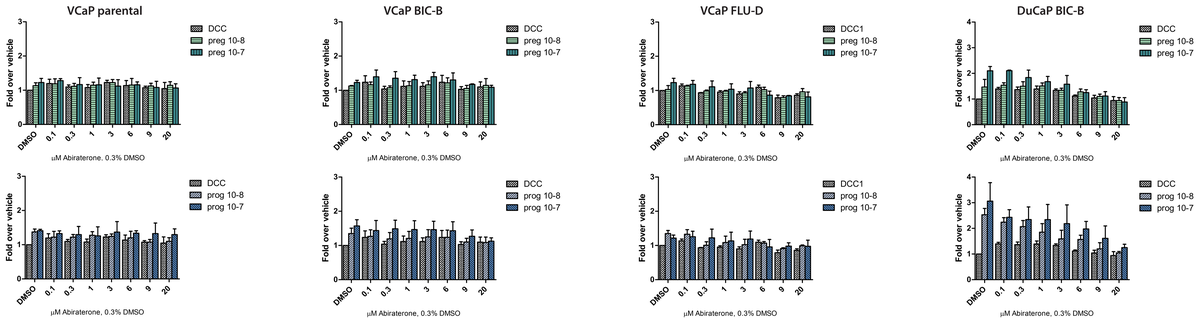
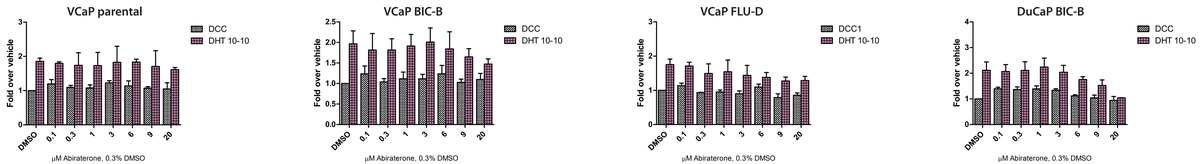
Results

Figure 1. In DuCaP CRPC cell line BIC-B, but not VCaP or VCaP CRPC cell lines, androgen precursors can induce cell growth despite adequate CYP17A1 blockade



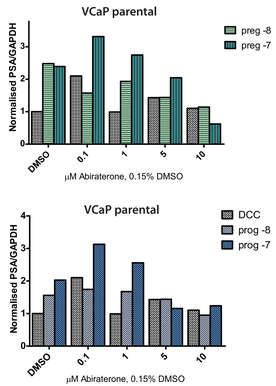
◀ Figure 1: **CRPC proliferation in the presence of CYP17A1 substrate:** In VCaP parental and CRPC cells, 10 to 100 nM of preg and preg do not induce substantial growth. In DuCaP BIC-B, these precursors do induce growth, but this is not inhibited at Abi-concentrations that are known to adequately block CYP17A1 activity (0.1 µM).

Figure 2. Higher levels of Abiraterone inhibit DHT-induced growth in both DuCaP and VCaP CRPC cell lines



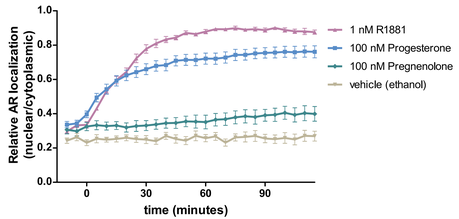
◀ Figure 2: **High concentrations of Abi can inhibit DHT-induced CRPC growth.** In VCaP parental and CRPC cells, 0.1 nM DHT is optimal for stimulating cell growth (data not shown). At higher concentrations DHT-induced growth is partially blocked by Abi, which cannot be explained by CYP17A1 inhibition. In DuCaP BIC-B, 20 µM Abi completely blocked DHT-induced cell growth.

Figure 3. Precursors induce AR regulated gene expression which can be blocked by Abi



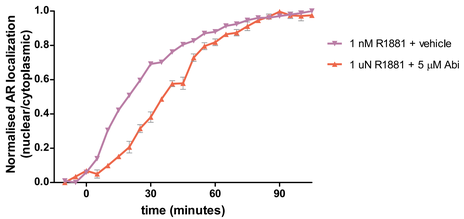
▲ **Figure 3: high concentrations (100 nM) of preg and prog induce AR regulated gene expression in parental VCaP cells.** qRT-PCR for PSA reveals that Preg and Prog at high concentrations give a upregulation of AR-regulated genes. Blocking CYP17A1 at adequate levels of 0.1 and 1 µM did not inhibit this activity, but higher concentrations of Abi blocked expression of PSA.

Figure 4. Precursor hormones induce AR translocation



◀ Figure 4: **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.

Figure 5. Abiraterone slows down AR translocation



◀ **Figure 5: 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.