Amiloride in the management of the secondary mineralocorticoid excess syndrome induced by Abiraterone Acetate in Castration-Resistant Prostate Cancer (CRPC): *in vivo* and *in vitro* evidence

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Inhibition of CYP17A1 by Abiraterone Acetate (AA) causes a significant suppression of androgens and cortisol followed by a compensatory increase of ACTH [1]. In two phase III trials, the mineralcorticoid excess syndrome (SSME) due to the CYP17A1 block [2-4] was more frequently reported in AA/Prednisone-treated arm compared to the Prednisone arm. The SSME is usually controlled by increasing the dose of Prednisone or adding a mineralcorticoid receptor (MR) antagonist such as Spironolattone or Eplerenone [5-8]. However, all these drugs seem to be able to activate as well androgen receptors (ARs); in particular Spironolactone activates both *wild-type* ARs [9] and T877A-AR mutation, often observed in patients [10], while Eplerenone and Prednisolone (the active Prednisone metabolite) activates only the T877A-AR [10]. Thus, a new pharmacological approach is needed to treat AA-induced SSME. Drugs such as the sodium channel antagonist Amiloride [4] or the novel non-steroidal MR antagonist PF-03882845 [11] can be considered as alternatives.

***In vivo*: Case reports.** In our clinics, 5 CRPC patients in therapy with AA/Prednisone experienced a SSME, that was treated with Amiloride/Hydrochlorothiazide. The resolution of the acute event was observed and a suitable and long-lasting pressure control was obtained. Interstingly, in two chemotherapy *naive* patients, we reported an increase in the radiological PFS compared to the mean value in the COU -AA-302 trial [7].

***In vitro*: Cell line.** We investigated the ability of these drugs to modulate the prostate cancer cell proliferation, using the experimental *in vitro* model of LNCaP cell line, that expresses T877A-AR [12]. LNCaP cells were grown in a medium with charcoal-treated serum and concentration-response curves for each studied drug were performed to evaluate whether the cell proliferation rate was modified. Cells were treated for 3 days. Results obtained using the MTT assay demonstrated that LNCaP exposure to either Prednisolone, Spironolattone and Eplerenone induced a cell proliferation increase, while Amiloride and PF-03882845 did not modify it; rather, high concentrations of Amiloride (>1 μM), induced cell death. The AR-negative, androgen-insensitive PC3 cell line was used as internal negative control, indeed the studied drugs did not modify the cell proliferation rate.

We further investigate the ability of AA to directly interact with ARs: the concentration-response curves demonstrated that AA reduced the LNCaP cell proliferation, with the IC50 of 200 ± 12 nM. Applying the different combination of treatments, we observed that Prednisolone, Spironolactone and Eplerenone antagonized the AA-induced reduction of the cell proliferation rate in a concentration-dependent manner. An additive inhibitory effect at high (>1 μM) Amiloride concentrations was observed when LNCaP cells are treated with the combination AA + Amiloride. PF-03882845, in association with AA, did not significantly modified the AA-induced reduction of the cell proliferation rate.

Finally, LNCaP cells were treated with drug combinations used in clinic. Our results demonstrated that the combination AA (200nM) + Prednisolone (5μM) + Spironolactone (100nM) led to an increase in the cell proliferation that overcome the basal value. AA (200nM) + Prednisolone (5μM) + Eplerenone (100nM) association increased the proliferation compared to the basal that did not reach a statistically significant value. When Amiloride 5 μM was added to AA (200nM) + Prednisolone (5μM) we did not observe differences in the proliferation rate compared to AA alone, thus resulting in an overall reduction of cell proliferation.

Taken together, our results strongly suggest that Amiloride could represent a suitable alternative for the treatment of AA-induced SSME. Experiments are in course to highlight the direct mechanism of action of AA on ARs, as we demonstrated that LNCaP did not produce steroid hormones, both at the basal and after AA exposure.