ABIRATERONE EXERTS A DIRECT ANABOLIC AND ANTI-RESORPTIVE ACTIVITY ON BONE MICROENVIRONMENT

Santini D.[1], Pantano F.[1], Iuliani M.[1], Fioramonti M.[1], Zoccoli A.[1], Ribelli G.[1], Vincenzi B.[1] and Tonini G.[1]

[1] Translational Oncology Laboratory, Medical Oncology, University Campus Bio-Medico of Rome, Rome, Italy

INTRODUCTION:

Abiraterone acetate (ABI) is associated not only with a significant survival advantage in both chemotherapy-naive and -treated patients with metastatic castration-resistant prostate cancer (mCRPC), but also with a reduced pain from skeletal metastases, a delay in time to development of Skeletal Related Events (SREs) and in radiological skeletal progression[1,2,3]. These bone benefits may be related to a direct effect on prostate cancer cells in bone or to a specific mechanism directed to bone microenvironment. To test this hypothesis we have designed an *in vitro* study aimed to evaluate a potential direct effect of ABI on human primary osteoclasts/osteoblasts (OCLs/OBLs) cultured with/without steroids.

METHODS:

Primary OCLs were differentiated from human monocytes; primary OBLs were obtained from human mesenchymal stem cells. OCL differentiation and activity were evaluated by TRAP and Bone Resorption assays; OBL differentiation was analyzed by ALP and Alizarin Red assays. Gene expression was performed by Real Time PCR using TaqMan® assays and protein analysis by Western Blot.

RESULTS:

Our results showed that non-cytotoxic doses of ABI (5μM/10μM) have a statistically significant inhibitory effect on OCL differentiation (P<0.001) and bone resorption activity (P<0.001) with and without steroids (fig 1).

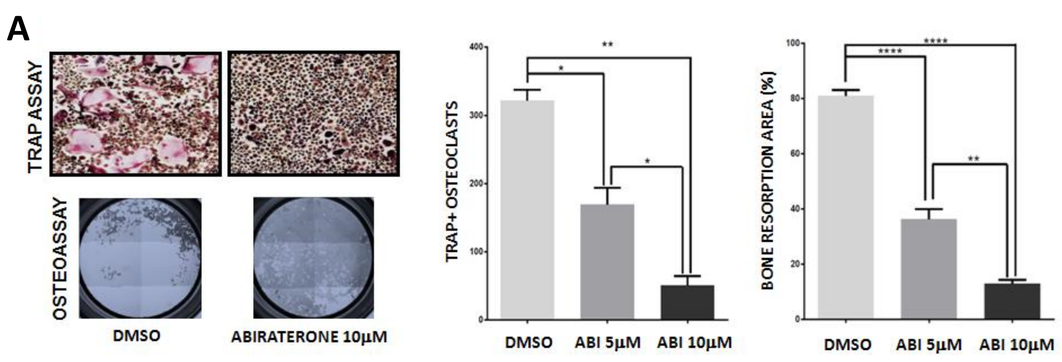


Figure 1: **Effect of abiraterone treatment on primary osteoclasts**. TRAP and Osteoassay in treated and untreated osteoclasts (DMSO) in presence (A) and absence (B) of steroids. * (P < 0.05) ** (P < 0.001) *** (P < 0.0001) **** (P < 0.00001).

Moreover we found that ABI down-modulated the expression of OCL marker genes: *TRAP* (tartrate-resistant acid phosphatase) (P<0.001), *CTSK* (cathepsin K) (P<0.001), *MMP9* (metalloproteinase-9) (P=0.001) (fig 2 A). The reduction of cathepsin K (CATH-K) levels was confirmed by Western Blot (fig 2 B).

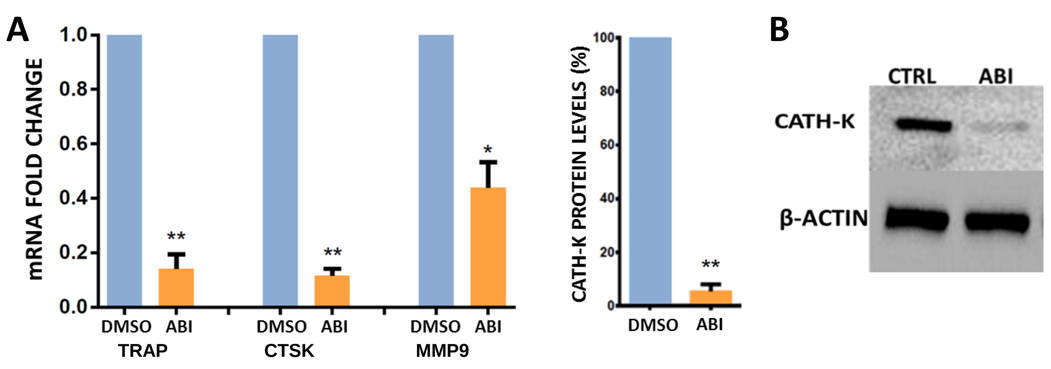
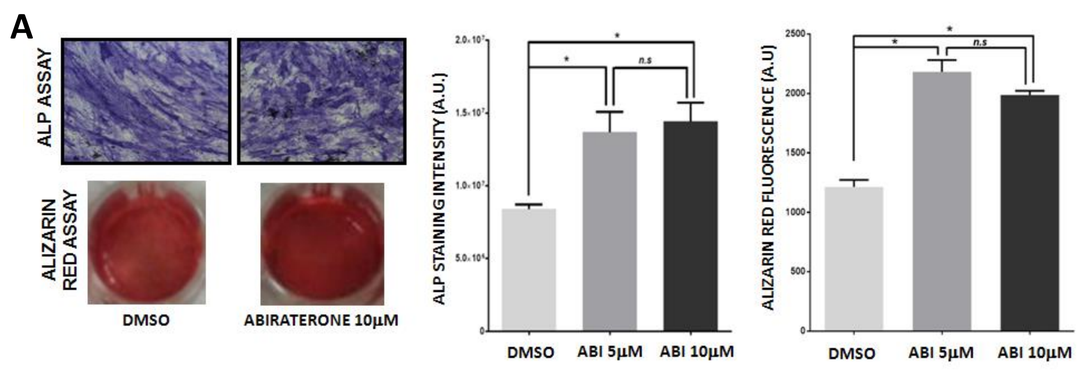


Figure 2: **Gene and protein expression analyses**. *TRAP*, *CTSK* and *MMP9* mRNA levels (Real Time PCR) (A) and CATH-K protein levels (Western Blot) (B) in treated and untreated osteoclasts (DMSO) cultured with steroids.

Furthermore ABI strongly promoted OBL differentiation (P =0.02) and increased bone matrix deposition (P=0.014) in presence/absence of steroids (fig 3).



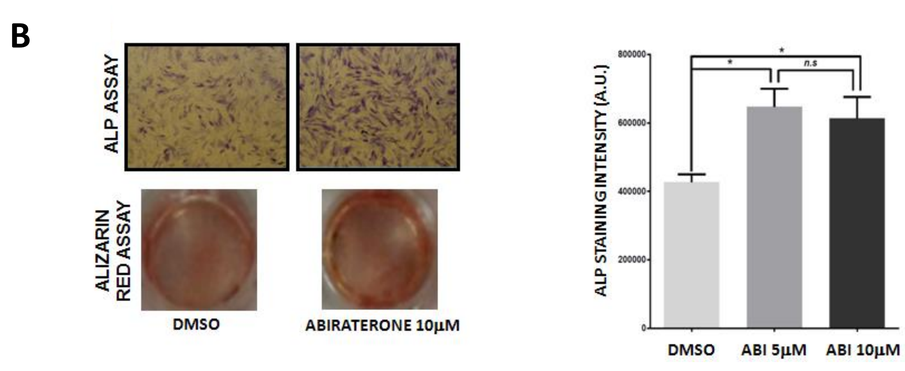


Figure 3: **Effect of abiraterone treatment on primary osteoblasts**. ALP and Alizarin Red assay in treated and untreated osteoblasts (DMSO) in presence (A) and absence (B) of steroids. * (P < 0.05).

Finally ABI showed a significant up-regulation of OBL specific genes *ALPL* (alkaline phosphatase) (P=0.015) and *BGLAP* (osteocalcin) (P=0.034) (fig 4 A). The increase of osteocalcin (OCN) expression was confirmed by Western Blot (fig 4 B).

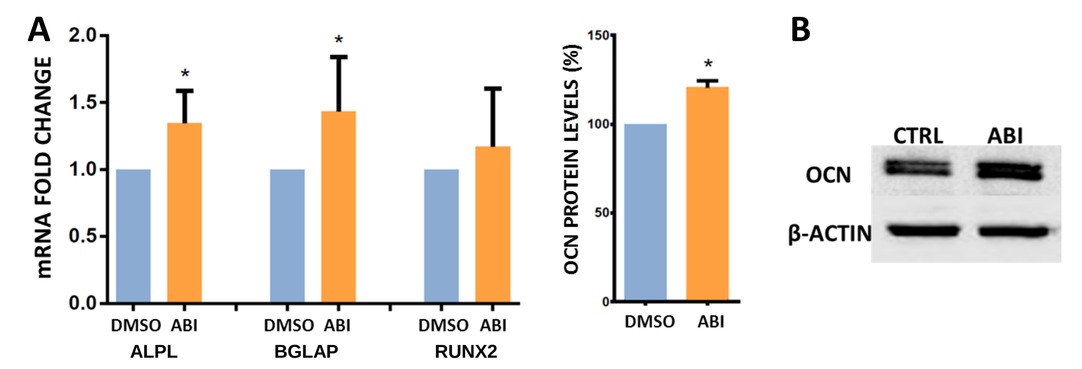


Figure 4: **Gene and protein expression analyses.** *ALPL, BGLAP, and RUNX2* mRNA levels (Real Time PCR) (A) and OCN protein levels (Western Blot) (B) in treated and untreated osteoblasts (DMSO) cultured with steroids. * (P < 0.05) ** (P < 0.001).

CONCLUSIONS:

Overall these findings represent the first evidence of a novel biological mechanism of ABI consisting in a direct bone anabolic and an anti-resorptive activity. These data may explain, together with the known antitumoral effect on CRPC cells, the high efficacy of ABI treatment in improving multiple skeletal disease specific clinical endpoints (time to SRE, bone rPFS, pain from bone metastases).

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The authors of this manuscript have no conflicts of interest to disclose.