Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

Scienze Farmacologiche e Tossicologiche
e dello sviluppo e del movimento

Ciclo XXVI

Settore Concorsuale di afferenza: 05/G1

Settore Scientifico disciplinare: BIO/14

TITOLO TESI:

CYP17A1 polymorphisms and clinical outcome of patients with metastatic castration-resistant prostate cancer treated with abiraterone

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Esame finale anno 2014

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I. Introduction

Prostate cancer is the most common noncutaneous cancer among men in Europe.[1] Carcinoma of the prostate is predominantly a tumor of older men: the median age at diagnosis is 72 years. It is an androgen dependent disease and inhibition of testosterone is a key element in the control of prostate tumor growth.

In Europe nearly 10 to 20% of patients present at diagnosis with metastatic disease and a significant rate of patients will develop metastases depite the primary treatment (surgery, radiotherapy and /or hormonal therapy).[1]

Androgen deprivation therapy (ADT) is the most effective treatment as initial treatment of advanced prostate cancer, but is inevitably characterized by progression after a median of 2-3 years with acquisition of a castration-resistant prostate cancer (CRPC) status.[1]

Patients with CRPC present frequently a rapid disease progression with an overall survival for symptomatic disease treated with chemotherapy ranging from 15 to 20 months.[2]

Abiraterone acetate, a pregnenolone derivative, is an oral selective and irreversible inhibitor of the enzyme CYP17 with dual 17-α hydroxylase and C17,20-lyase blocking activity, the result of which is decreased gonadal and extra-gonadal androgen synthesis. [3]

Abiraterone acetate has increased the overall survival of patients with metastatic CRPC.[4]

However, despite an initial response to treatment, all patients will develop resistance to the drug. To date, a number of predictive factors have been studied, but no information is available about the role of polymorphisms of CYP17A1 for out come prediction of abiraterone treatment in CRPC.

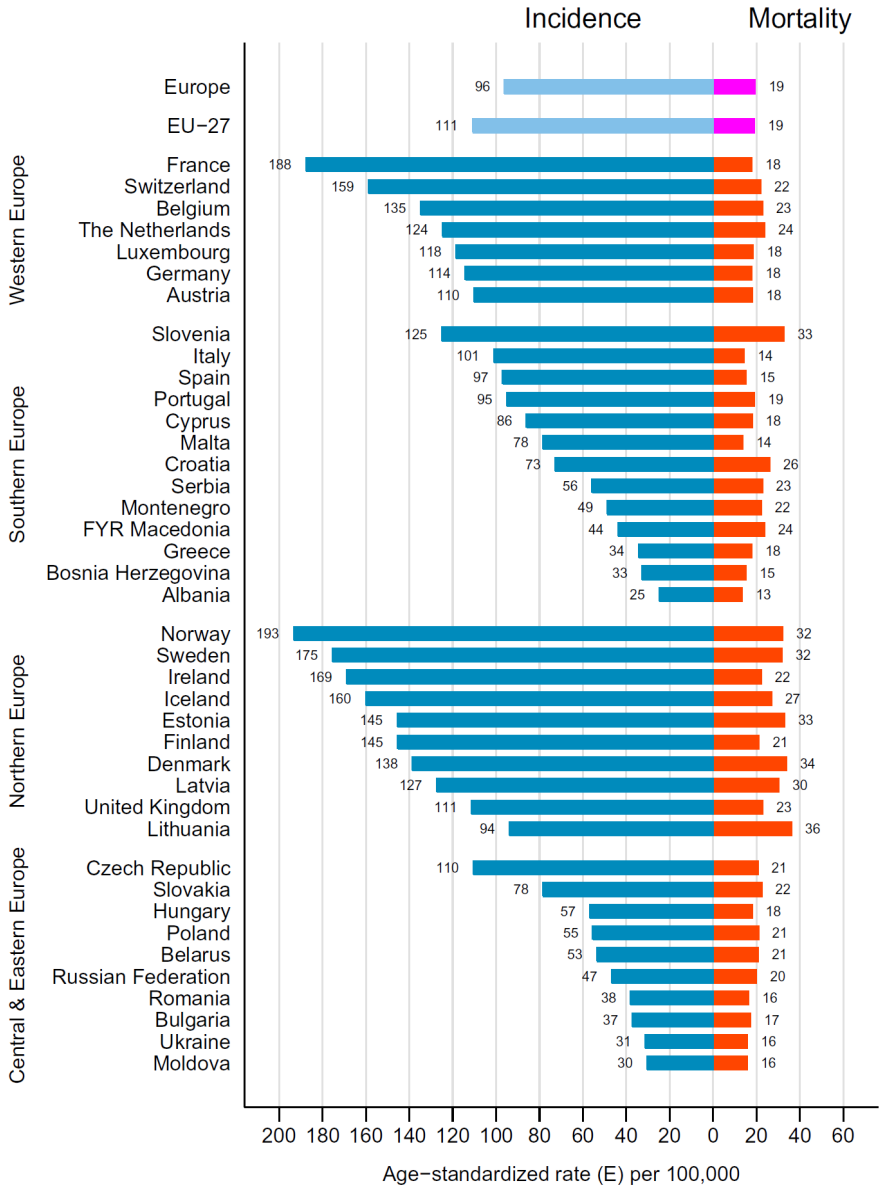
The first aim of this study was to establish the possible correlation between polymorphisms of CYP17A1 and the clinical outcome of patients with metastatic CRPC treated with abiraterone acetate after docetaxel.

II. Prostate cancer

Epidemiology

Prostate cancer is the third most common cancer diagnosed in Europe. Carcinoma of the prostate is characteristically a tumor of elderly patients with a the median age at diagnosis of 72 years.[5] It has emerged as the most frequent noncutaneous cancer in men in Europe.[6] A general increase in the incidence of prostate cancer has been reported in Europe, even if especially in Nothern and Western Europe, the rising trend of incidence is due in a large part to increase detection of latent disease following the large use of PSA as screening test.[7] Incidence rates of prostate cancer vary greatly with the highest rates estimated in Northern and Western European countries such as Norway and France and the lowest in Central and Eastern European countries – Republic of Moldova and Albania. In comparison with incidence, mortality rates vary much less, from the highest estimated rates in Lithuania or Denmark to the lowest in Malta or Albania (see Table in the next page).

Despite a significant morbidity and mortality to a lesser extent, the etiology of prostate cancer remains largely unknown. Indeed, the only well-established risk factors to date are age, ethnicity and a family history of prostate cancer. The rate of tumor growth varies from very slow to rapid, withsome patients who may have prolonged survival even after metastatization to distant sites. The 5-year relative survival rate for patients with local or regional disease is approximately 95-100%, whereas the 5-year relative survival rate for patients with metastatic disease is nearly 25-30.[8] The approach to treatment is influenced by age and coexisting medical problems. Side effects of various forms of treatment should be considered in selecting appropriate management.



Age-standardised incidence and mortality rates by area and country in Europe 2012: prostate cancer.

Diagnosis and Prognosis

A general increase in prostate cancer incidence has been reported, even if most European countries, especially in the highest resource countries in Northern and Western Europe. This rising trend in incidence is attributable to the increased detection of latent disease following the widespread availability of PSA test. Therefore the geographical variations in prostate cancer incidence rates largely reflect the prevalence of PSA testing and consequent biopsy, although other factors such as obesity and sedentary lifestyle may be risk factors for invasive disease.[7]

However, the issue of prostate cancer screening remains controversial. Randomized trials have achieved conflicting results.[9-11] Systematic literature reviews and meta-analyses have showed no clear evidence that screening with PSA decreases the risk of death from prostate cancer.[12,13]

Nearly 95% of primary prostate cancer is represented by adenocarcinoma, that is frequently multifocal and heterogeneous in patterns of differentiation.[14] A needle biopsy is the most common method used for the diagnosis of prostate cancer. The histologic grade of prostate adenocarcinoma is reported according to the Gleason score, which provides a useful information in determining prognosis. The Gleason score is calculated based on the dominant histologic grades, and is derived by adding the two most prevalent pattern grades, yielding a score ranging from 2 to 10.[15-17]

With respect to prostate cancer mortality, the rates are a better proxy of risk than incidence, revealing much less between-country variation than incidence, although they may be prone to variations in the quality of reporting of the underlying cause of death.[18] However, decreasing mortality trends have been observed in several European countries after the mid-90,s and the relative impact of the introduction of curative treatment versus early detection by PSA is still subject to much debate.

The survival of patients with prostate cancer is related to several factors, including the extension of the tumor, the histologic grade of tumor (Gleason score), patient age and comorbidities, the PSA level.[19-22] The tumor extension is determinant, when it is confined to the prostate gland, long-term prognosis is excellent, whereas if prostate cancer has spread to metastatic organs, the therapy will not cure it, and most of these patients will die of prostate cancer, even if, in this group of patients, indolent courses lasting for many years are observed.

Poorly differentiated tumors are more likely to have metastasized at diagnosis and are associated with a poorer prognosis. Any benefits of definitive local therapy with curative intent may take years to emerge. Therefore, therapy with curative intent is usually reserved for men with a sufficiently long life expectancy. The higher the level of PSA at baseline, the higher is the risk for distant disease and disease progression. However, it is an imprecise marker of risk.[23-27]

Several nomograms have been developed to predict the clinical outcome either prior to radical or after radical prostatectomy with intent to cure.[28-32] Preoperative nomograms are based on clinical stage, PSA level, Gleason score, and the number of positive and negative prostate biopsy cores.[29,30] Postoperative nomograms add pathologic findings, such as capsular invasion, surgical margins, seminal vesicle invasion, and lymph node involvement.[31,32]

The androgen receptor (AR)

The androgen receptor (AR) is a member of the super-family of the nuclear receptors, which works through a ligand-dependent transcription factors. Structurally, AR is constituted by 3 functional regions[33]:

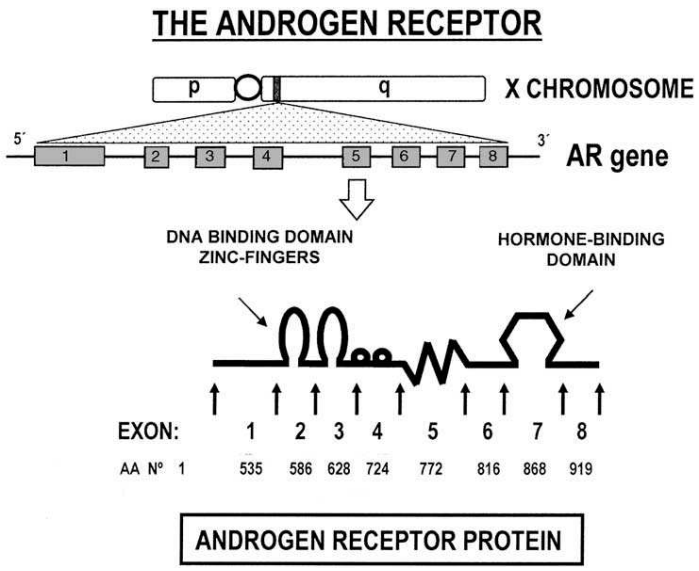
1 - N-terminal regulatory domain contains activation function 1 (AF-1) required for full ligand activated transcriptional activity activation function 5 (AF-5) is responsible for the constitutive activity (activity without bound ligand) dimerization surface

2 - DNA binding domain (DBD)

3 - Ligand binding domain (LBD) containing activation function 2 (AF-2), responsible for agonist induced activity (activity in the presence of bound agonist)

4 - Hinge region - flexible region that connects the DBD with the LBD; along with the DBD, contains a ligand dependent nuclear localization signal

5 - C-terminal domain



Testosterone

The main androgens are testosterone and dihydrotestosterone (DHT). Testosterone is synthesized primarily in the testes and, to some extent, in the adrenal glands. In the circulation, about 45% of the total testosterone binds to sex hormone–binding globulin, about 50% binds loosely to albumin, and <4% is unbound.[34] Within the prostate, testosterone is converted irreversibly to 5α-dihydrotestosterone by the enzyme 5α-reductase type II, encoded by the SRD5A2 gene.[34] Although testosterone and 5α-dihydrotestosterone can bind the AR, AR has a higher affinity for 5α-dihydrotestosterone than for testosterone, and AR is more transcriptionally active when bound to 5α-dihydrotestosterone. The activity of the 5α-dihydrotestosterone–androgen receptor transcription factor complex is modulated by translocation to the cell nucleus and the binding of various androgen receptor coregulators, including coactivators and corepressors.[35] The 5α-dihydrotestosterone–androgen receptor–coregulator complex can translocate to the cell nucleus, where it activates transcription of genes with hormone-responsive elements in their promoters to induce androgen signaling. Thus, androgenic action in the prostate is determined by a multitude of factors, including concentration of AR and its coregulators as well as tissue levels of 5α-dihydrotestosterone.

Treatment of advanced disease

Among the men diagnosed annually with prostate cancer, approximately 10% to 20% present with metastatic disease. Currently, the standard of care for patients with newly diagnosed metastatic CRPC is androgen deprivation therapy (ADT), which consists of initiating a luteinizing hormone-releasing hormone (LHRH) agonist (medical castration) or in rare cases, orchiectomy (surgical castration) with or without concurrent anti-androgens.[36] The study SWOG S8894 reported that 77% of men newly diagnosed with metastatic prostate cancer lived less than 5 years and only approximately 7% of men treated with hormonal therapy were alive at or after 10 years. Several prognostic factors influence survival in M1 disease.[37] Median overall survival (OS) has been reported to range from 13 months up to 75 months depending on the presence of high-risk prognostic features such as high PSA concentration at diagnosis, high Gleason score, increased volume of metastatic disease as well as the presence of bony symptoms.[37] In this study, high-risk patients with shortest median OS will be selected based on parameters described previously. The high- isk prognostic factor of Gleason score ≥8 was selected based on data from the SWOG 9346 study which reported that it was a strong predictor for risk of death.[38] Baseline PSA alone was not considered a factor for selection of the high risk patient group because it was not as predictive of survival in univariate and multivariate models compared with Gleason score. In addition, baseline PSA alone did not show high association with post-baseline PSA decreases to below 4 ng/mL, a level that has been shown to have survival benefit.[37,38] The second and third high-risk prognostic factors are both related to high-volume disease (defined as 3 or more lesions by bone scan or involvement of viscera). A single-center study of 286 patients has reported OS was 3.1 years in those with high-volume disease compared with 7.8 years in those with low-volume disease.[37,38]

Because the reduction of testosterone to castrate levels has been shown to improve survival of prostate cancer patients, initiation of ADT is standard of care for patients with M1 disease. A large randomized study in 938 men as well as a systematic review in men with locally advanced or asymptomatic metastatic disease demonstrated improved overall survival in those treated early.[39,40] The same study also demonstrated that the risk of deferring treatment increases the risk of developing debilitating symptoms such as pathological fractures and spinal cord compressions.[40]

In recurrent prostate cancer, the selection of further treatment depends on the previous treatment, site of recurrence, coexistent illnesses, individual patient considerations.[41-42] Definitive radiation therapy can be given to patients with disease that fails only locally following prostatectomy.[43] Hormonal therapy is used to manage most relapsing patients with disseminated disease who initially received locoregional therapy with surgery or radiation therapy as well as for initially metastatic disease.[44]

III. Castration-Resistant Prostate Cancer (CRPC)

Definition

CRPC is defined as progressive disease despite ADT with serum testosterone <50 ng per deciliter. Despite the initial activity, ADT is not curative and after a median of 2-3 years patients progress to castration-resistance requiring further therapy including chemotherapy. Possible mechanisms of resistance to conventional ADT include not only the tumor growth independent from testosterone (androgen-independent prostate cancer), but also the persistence of androgen production despite medical or surgical castration resulting from adrenal sources of testosterone or the up-regulation of intratumor testosterone production.[45] Then, the cellular resistance to ADT is not necessarily a result of the acquisition of growth independence from testosterone, but rather that it might be a result of cellular acquisition of mechanisms to overcome castrate-levels of testosterone. Other mechanisms found to be associated with retained hormonal sensitivity include enhanced intracellular conversion of adrenal androgens to testosterone and dihydrotestosterone in prostate cancer cells, intratumoral androgen synthesis, increased expression of AR messenger rna (mrna), and ligand-independent aractivation.[46]

In parallel with the continuous progress in the biological characterization of in vitro and in vivo CRPC, clinical practice also supported the role of further hormonal treatment after the emergence of CRPC. Ketoconazole, an imidazole antifungal agent, suppresses the multistep process of adrenal and intratumoral steroidogenesis by inhibiting the 17,20-lyase and 17α-hydroxylase enzymatic activities of CYP19, desmolase, and 11β-hydroxylase. Formal clinical trials showed that ketoconazole was indeed able to elicit responses in some metastatic CRPC patients.[47] Despite the fact that the effect of ketoconazole was transitory of short duration, these studies provided clinical “proof-of-concept” to the retained hormonal sensitivity of prostate cancer cells in patients with CRPC.

Standard treatment

A number of agents have demonstrated activity in CRPC, but only a few have been tested for effectiveness in larger, randomized trials. Low-dose corticosteroids were shown to have some activity against prostate cancer with a beneficial effect on QOL.[48] Mitoxantrone, an anthracenedione, was also shown to have activity in prostate cancer, with moderate toxicity.[49] With encouraging results in Phase 2 trials involving docetaxel in CRPC,[50-53] two Phase 3 trials confirmed that docetaxel-based regimens, when used as first-line chemotherapy, were superior to mitoxantrone and prednisone.[2,54] The first Phase 3 trial, TAX 327, compared survival in patients with progressive metastatic CRPC treated with docetaxel or mitoxantrone.[2] One thousand and six patients were randomized to receive docetaxel 75 mg/M2 every 3 weeks, docetaxel 30 mg/M2 weekly for 5 out of 6 weeks, or mitoxantrone 12 mg/M2 every 3 weeks. All patients received daily prednisone. Overall survival was the primary endpoint. The overall survival rate was significantly higher (P=0.009) in the group given docetaxel every 3 weeks, but not in the group given docetaxel weekly, when compared with the group given mitoxantrone. The median duration of survival was 18.9 months in the group given docetaxel every 3 weeks compared to 16.5 months in the mitoxantrone group (P=0.002). Reduction in pain was significantly more frequent (P=0.01) and QOL was significantly improved (P=0.009) in the group given docetaxel every 3 weeks when compared to the group given mitoxantrone. Adverse events were more common in the groups that received docetaxel; however, the incidence of serious toxicity was low.

The second multicenter trial, conducted by the Southwest Oncology Group (SWOG 9916), compared the combination of docetaxel/estramustine to mitoxantrone/prednisone in 684 patients.[54] The median duration of survival was significantly improved with docetaxel/estramustine over that with mitoxantrone/prednisone (17.5 vs. 15.6 months, respectively P=0.01) and a superior median time to progression was also detected (6.3 vs. 3.2 months, respectively, P=0.0001) in patients treated with docetaxel/estramustine. There weresimilar rates of pain relief in both arms. However, grade 3/4 toxicities (febrile neutropenia, vomiting and cardiovascular events) were more frequent in the docetaxel/estramustine arm, most likely due to estramustine.

Based on the improved survival benefit from both of these studies, docetaxel and prednisone every 3 weeks was approved in 2004 for CRPC and has become the “standard of care”.

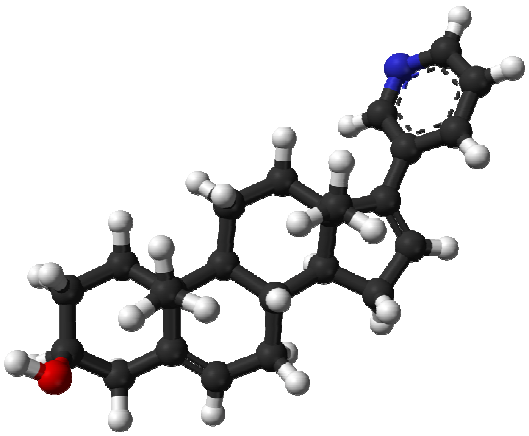
Until recently, cytotoxic chemotherapy had been the only therapy shown to improve overall survival for patients with CRPC.

Recently, five new agents with diverse mechanisms of action were approved by the FDA for the treatment of patients with CRPC (cabazitaxel, sipuleucel-T, denosumab, enzalutamide, and abiraterone acetate).

IV. Abiraterone Acetate

Mechanisms of Action

Abiraterone acetate is a prodrug of abiraterone, an irreversible inhibitor of 17α hydroxylase/C17, 20-lyase (cytochrome P450c17 [CYP17]), a key enzyme required for testosterone synthesis. This enzyme is found in the testes, adrenals, and prostate tumors.[55,56]



CYP17 catalyzes two sequential reactions: 1) the conversion of pregnenolone and progesterone to their 17α-hydroxy derivatives by 17α-hydroxylase activity and 2) the subsequent formation of dehydroepiandrosterone (DHEA) and androstenedione, respectively, by C17, 20 lyase activity. DHEA and androstenedione are androgens and are precursors of testosterone. Inhibition of CYP17 by abiraterone can also result in increased mineralocorticoid production by the adrenals.

Abiraterone works in CRPC, when such resistance could be due to persistent androgen signaling and to its de novo synthesis.[55,57,58] Low testosterone levels also have an effect on cancer progression, causing AR activation. Therefore, drugs using the androgen receptor pathway, such as abiraterone seems to be particularly effective for CRPC.[59]

Clinical pharmacology

Following administration of abiraterone acetate, the pharmacokinetics of abiraterone and abiraterone acetate have been studied in healthy subjects and in patients with metastatic CRPC. In vivo, abiraterone acetate is converted to abiraterone. In clinical studies, abiraterone acetate plasma concentrations were below detectable levels (< 0.2 ng/mL) in > 99% of the analyzed samples.

Following oral administration of abiraterone acetate to patients with metastatic CRPC, the median time to reach maximum plasma abiraterone concentrations is 2 hours. Abiraterone accumulation is observed at steady-state, with a 2-fold higher exposure (steady-state AUC) compared to a single 1,000 mg dose of abiraterone acetate. At the dose of 1,000 mg daily in patients with metastatic CRPC, steady-state values (mean ± SD) of Cmax were 226 ± 178 ng/mL and of AUC were 1173 ± 690 ng.hr/mL. No major deviation from dose proportionality was observed in the dose range of 250 mg to 1,000 mg. However, the exposure was not significantly increased when the dose was doubled from 1,000 to 2,000 mg (8% increase in the mean AUC).

Systemic exposure of abiraterone is increased when abiraterone acetate is administered with food. Abiraterone Cmax and AUC0-∞ were approximately 7-and 5-fold higher, respectively, when abiraterone acetate was administered with a low-fat meal (7% fat, 300 calories) and approximately 17-and 10-fold higher, respectively, when abiraterone acetate was administered with a high-fat (57% fat, 825 calories) meal. Abiraterone is highly bound (> 99%) to the human plasma proteins, albumin and alpha-1 acid glycoprotein. The apparent steady-state volume of distribution (mean ± SD) is 19,669 ± 13,358 L. In vitro studies show that at clinically relevant concentrations, abiraterone acetate and abiraterone are not substrates of P-glycoprotein (P-gp) and that abiraterone acetate is an inhibitor of P-gp. No studies have been conducted with other transporter proteins.

Following oral administration of 14C-abiraterone acetate as capsules, abiraterone acetate is hydrolyzed to abiraterone (active metabolite). The conversion is likely through esterase activity (the esterases have not been identified) and is not CYP mediated. The two main circulating metabolites of abiraterone in human plasma are abiraterone sulphate (inactive) and N-oxide abiraterone sulphate (inactive), which account for about 43% of exposure each. CYP3A4 and SULT2A1 are the enzymes involved in the formation of N-oxide abiraterone sulphate and SULT2A1 is involved in the formation of abiraterone sulphate.

In patients with metastatic CRPC, the mean terminal half-life of abiraterone in plasma (mean ± SD) is 12 ± 5 hours. Following oral administration of 14C-abiraterone acetate, approximately 88% of the radioactive dose is recovered in feces and approximately 5% in urine. The major compounds present in feces are unchanged abiraterone acetate and abiraterone (approximately 55% and 22% of the administered dose, respectively).[60]

In vitro studies with human hepatic microsomes showed that abiraterone is a strong inhibitor of CYP1A2, CYP2D6 and CYP2C8 and a moderate inhibitor of CYP2C9, CYP2C19 and CYP3A4/5.

In an in vivo drug-drug interaction trial, the Cmax and AUC of dextromethorphan (CYP2D6 substrate) were increased 2.8- and 2.9-fold, respectively when dextromethorphan 30 mg was given with abiraterone acetate 1,000 mg daily (plus prednisone 5 mg twice daily). The AUC for dextrorphan, the active metabolite of dextromethorphan, increased approximately 1.3 fold.

In a clinical study to determine the effects of abiraterone acetate 1,000 mg daily (plus prednisone 5 mg twice daily) on a single 100 mg dose of the CYP1A2 substrate theophylline, no increase in systemic exposure of theophylline was observed.

Abiraterone is a substrate of CYP3A4, in vitro. In a clinical pharmacokinetic interaction study of healthy subjects pretreated with a strong CYP3A4 inducer (rifampin, 600 mg daily for 6 days) followed by a single dose of abiraterone acetate 1,000 mg, the mean plasma AUC∞ of abiraterone was decreased by 55% In a separate clinical pharmacokinetic interaction study of healthy subjects, co-administration of ketoconazole, a strong inhibitor of CYP3A4, had no clinically meaningful effect on the pharmacokinetics of abiraterone.[60]

Clinical Results

The study COU-AA-301 was a Phase 3, multinational, randomized, double-blind, placebo-controlled study of oral abiraterone acetate and oral prednisone in 1,195 subjects with mCRPC whose disease had progressed on or after 1 or 2 chemotherapy regimens, at least one of which contained docetaxel. The study conclusively demonstrated that further lowering testosterone concentrations below those achieved with standard therapy to suppress androgen production (LHRH agonists or orchiectomy) using CYP17 inhibition with abiraterone acetate improves survival in patients with mCRPC.[4] Study COU-AA-302 was a Phase 3, multinational, randomized, double-blind, placebo-controlled study of abiraterone acetate and oral prednisone in 1,088 asymptomatic or mildly symptomatic subjects with mCRPC who had not received chemotherapy. This study had co-primary endpoints of radiographic progression free survival (rPFS) and overall survival. Treatment with abiraterone acetate plus prednisone decreased the risk of radiographic progression or death by 57% compared with placebo plus prednisone (HR=0.425; p<0.0001).[61] There was a 25% decrease in the risk of death in the abiraterone acetate and prednisone group compared with the placebo plus prednisone group (HR=0.752; p=0.0097) when the Independent Data Monitoring Committee (IDMC) unanimously recommended unblinding the treatment and allowing subjects in the placebo group to receive abiraterone acetate. The median OS had not been reached for the abiraterone acetate group and was 27.2 months in the placebo group. The safety profile was similar, although the duration of treatment was longer, to that observed with abiraterone acetate plus prednisone in subjects in the post-docetaxel setting (COU-AA-301). In healthy subjects after single dose administration of 1,000 mg abiraterone acetate, there is a substantial food effect and absorption of abiraterone acetate increases greatly with increasing fat content of a meal (Study COU-AA-009). Compared to administration after an overnight fast, geometric mean maximum concentration (Cmax) and the area under the concentration-time curve (AUC) of abiraterone increased approximately 7-fold and 5-fold, respectively, when administered following a low-fat meal (estimated 2% of calories from fat) and increased by approximately 17-fold and 10-fold, respectively, when administered following a high-fat meal (estimated 56% of calories from fat). In the two large phase 3 randomized studies (COU-AA-301and COU-AA-302), treatment with abiraterone acetate and prednisone had an acceptable safety profile and resulted in a favorable benefit/risk ratio. The safety profile

of abiraterone acetate plus prednisone was distinct from that of cytotoxic agents. Adverse events usually did not interfere with administration of abiraterone acetate. In the combined dataset of safety for studies

COU-AA-301 and COU-AA-302, the most frequently reported adverse events were fatigue (43.8%), back pain (32.6%), nausea (28.4%), arthralgia (29.5%), constipation (26.1%), bone pain (24.2%), peripheral edema (26.0%), hot flush (20.6%) and diarrhea (20.5%). Most events were Grade 1 or 2 in severity. Abiraterone acetate may cause hypertension, hypokalemia, and fluid retention as a consequence of increased mineralocorticoid levels resulting from CYP17 inhibition. Co-administration of a corticosteroid suppresses adrenocorticotropic hormone (ACTH) drive resulting in a reduction in incidence and severity of these adverse reactions. Caution is required in treating patients whose underlying medical conditions might be compromised by increases in blood pressure, hypokalemia (eg, those on cardiac glycosides), or fluid retention (eg, those with heart failure), severe or unstable angina pectoris, recent myocardial infarction or ventricular arrhythmia, and those with severe renal impairment.

Predictive factors

Predictive biomarkers are factors related to the disease or the host that are associated with improvements in outcomes, e.g. survival, due to specific therapies. Such biomarkers have become of paramount importance in oncology to maximize the benefits of novel systemic agents while minimizing harm to individual patients and the costs to society. Given the number of newly approved and expensive systemic therapies, including novel hormonal therapies, like abiraterone, the role of predictive biomarkers is assumying an outstanding role.

A preliminary report showed a significant association between ERG rearrangements in therapy-naive tumors, CRPC, circulating tumor cells (CTC) and magnitude of PSA decline in CRPC patients treated with abiraterone acetate.[54] These data confirmed that CTC are malignant in origin and indicate that hormone-regulated expression of ERG persists in CRPC. More recently, in another study, the role of transmembrane protease, serine 2 (TMPRSS2)-v-ets erythroblastosis virus E26 oncogene homolog (ERG) fusion, an androgen-dependent growth factor, has been studied in CTC as a biomarker of sensitivity to abiraterone. Molecular profiles of CTC with an analytically valid assay identified the presence of the prostate cancer-specific TMPRSS2-ERG fusion but did not predict for response to AA treatment.[62]

From a clinical point of view, a composite score of baseline inflammatory markers as neutrophil/lymphocyte ratio and extent of metastatic spread has been recently associated with PSA response to abiraterone and OS.[63,64]

Predictive biomarkers are needed to give physicians a more rational sense of matching the right patient to the right therapy at a given time. There are currently no validated predictive biomarkers in CRPC patients including thsoe treated with abiraterone.

Biomarkers predictive of the efficacy of abiraterone are urgently needed in clinical practice to better address this treatment in patietns with CRPC.

V. Single Nucleotide Polymorphisms in Prostate Cancer

Prostate cancer is one of the most common leading causes of cancer death in men. Attributable to many genetic linkage and genome-wide association studies (GWAS) around the world, several high-penetrance genetic variants have been identified. Many polymorphisms in genes, have been recognized as important genetic factors that confer an increased risk of developing prostate cancer in many populations.

The CYP17A1 gene is located on chromosome 10q24.3 and encodes an enzyme that catalyzes key reactions in sex-steroid biosynthesis mediating 17α-hydrolase and 17,20-lyase activities.[65] The identification of somatic alterations in the specific target of abiraterone could help to select patients who will really benefit from this type of therapy. CYP17A1 intratumor overexpression has been detected in prostate cancer tissue biopsies from patients treated with abiraterone, suggesting that upregulation of the enzyme could play a key role in resistance to treatment.[66,67] CYP17A1 genotyping could represent a step in the right direction to define personalized treatment based on the use of abiraterone as it is known that genetic variants can cause changes in gene expression. However, despite its potential key role, there are few literature data regarding its genetic alterations and their potential application for prostate cancer prognosis. Carey and collaborators[68] identified a common a single base pair substitution, -34T>C (rs743572) in 5'-UTR CYP17A1, defining patients with homozygosis for the common allele as "*A1A1*", those with heterozygosis as "*A1A2*" (haplotype TC) and individuals with homozygosis for the variant allele as "*A2A2*". The authors hypothesized that this promoter variant has an effect on the level of the transcript. However, it is still not understood how this alteration affects the protein expression and, consequently, testosterone levels in serum.[69-71] The SNP rs743572 has also been correlated with the clinical outcome of patients who are resistant to hormone therapy, and men with the "*A2A2*" haplotype have a longer survival than those with the common allele.[72] Another important single nucleotide polymorphism would appear to be rs10883783, although few data are available on it. Wright and coworkers found that men with the minor variant allele A in rs10883783 showed a 56% lower risk of prostate cancer-specific mortality.[73]

V. Clinical and Pharmacology Study

Aims of the Study

Abiraterone acetate in combination with prednisone has been approved for the treatment of men with mCRPC who have received prior chemotherapy containing docetaxel. The efficacy and safety of abiraterone acetate (1,000 mg daily tablet dose) and prednisone (5 mg twice daily) therapy in patients with mCRPC is established by the results of Study COU-AA-301 and COU-AA-302, both Phase 3, multinational, randomized, double-blind, placebo-controlled studies. Study COU-AA-301 was the first Phase 3 study to demonstrate that further lowering testosterone concentrations below that achieved with ADT using CYP17 inhibition with abiraterone acetate improves survival in patients with mCRPC. The rationale for using abiraterone acetate in patients with high-risk prognostic factors in mHNPC is based on the positive results of Study COU-AA-3016 and COU-AA-30233 and the unmet medical need for alternative treatment options for these patients.

Since there is very little evidence of the correlation between CYP17A1 gene polymorphisms and clinical outcome with abiraterone therapy, we decided to evaluate different patient haplotypes and to verify their impact on treatment efficacy.

Patients and Methods

Case series and Study Design

Forty-eight CRPC patients with different clinical pathologic characteristics were recruited for the study (table I). Blood samples were collected from all patients in Paxgene blood DNA tubes before the start of treatment and stored at -80°C for a maximum of two years. Eligibility criteria comprised histological confirmation of adenocarcinoma of the prostate without neuroendocrine differentiation or small cell histology progressing on androgen deprivation. Patients were required to have received at least one but not more than two cytotoxic chemotherapy regimens for metastatic CRPC. At least one regimen should have contained docetaxel. Prior ketoconazole therapy was not permitted. Additional eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, adequate cardiac, renal, hepatic and bone marrow function, serum potassium level ≥ 3.5 mmol/L, and ongoing androgen deprivation with serum testosterone < 50 ng/dL. The protocol was approved by our Institutional Review Board. Written informed consent was obtained from all patients.

Treatment consisted of 28-day cycles of abiraterone acetate 1,000 mg taken daily on an empty stomach with prednisone 5 mg twice daily. Treatment continued until there was evidence of disease progression or unacceptable toxicity. Before starting treatment, patients underwent a baseline PSA blood test and a CT scan of the chest and abdomen. Patients were evaluated monthly for PSA response and toxicity. A CT scan was performed every 3 months during treatment with abiraterone. Disease progression was defined according to Prostate Cancer Working Group 2 (PCWG2) criteria.[74] Adverse events were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.

Genotyping

DNA was extracted from peripheral blood samples using the PreAnalytiX kit (Qiagen, Milan, Italy), according to the manufacturer's instructions. DNA was then quantified by spectrophotometry (NanoDrop® ND-1000, Celbio, Milan, Italy) and A260/A280 and A260/A230 ratios were determined to assess DNA quality.

Two SNPs (rs743572 and rs10883783) were genotyped by the ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) with fluorescence-based capillary electrophoresis system. Purified DNA was amplified for the CYP17A1 gene using the following primer sequences: rs743572 fw 5'-TTGGGCCAAAACAAATAAGC-3', rev 5'-GGGCTCCAGGAGAATCTTTC-3';rs10883783 fw 5'-CTATGGCAGGATGAGGGTGT-3', and rev 5'-TGAGTTTGCTGTGGACAAGG-3'. The two amplicons obtained were 208 bp and 248 bp long. PCR results were verified by agarose gel electrophoresis and sequenced using Big Dye Terminator 3.1 with the same PCR primers. The sequences were then analyzed with Sequencing Analysis Software.

The third polymorphism, rs17115100, was genotyped using commercial TaqMan SNP Genotyping assay (assay ID: 25597854_10). All DNA samples were analyzed in duplicate with TaqMan PCR Master Mix on a 7500 real-time PCR cycler, according to the manufacturer's instructions. The allele calls were identified by specific software. Two negative controls were added to each real-time experiment. The analysis was repeated if the difference between duplicate samples was greater than 1 cycle threshold.

Statistical Analysis

Progression-free survival was defined as the time from the starting date of abiraterone treatment to the first observed progression, relapse or death (whichever came first). Overall survival was defined as the time from the starting date of abiraterone treatment to the date of death from any cause. Patients who did not experience the outcome of interest were censored at the time of last follow up. Kaplan-Meier methods were used to estimate PFS and OS. The log-rank test and Wilcoxon test were calculated to compare the curves of the different patient haplotypes. Moreover, for all polymorphisms, differences in the allelic frequencies between our case series and the worldwide population were evaluated by the chi-square test. Allelic frequencies were determined by dbSNP short genetic variations.
A value of $p < 0.05$ was considered statistically significant. All p-values were two-sided. Data were analyzed using SAS 9.3 software (SAS Institute, Carry, NC).

Results

Forty-eight Caucasian patients with metastatic CRPC treated with abiraterone were genotyped for three polymorphisms in the CYP17A1 gene. Table 1 summarizes the clinical-pathological characteristics of these patients.

TABLE 1. Clinical-pathologic characteristics of CRPC patients

	No. cases (%)
Total patients	48
Median age, years (range), at the start of Abiraterone treatment	73.5 (57-87)
Gleason Score*	
6 - 7	21 (43.8)
8 - 9	26 (54.2)
ECOG performance status	
0 - 1	41 (85.4)
2	7 (14.6)
Site of disease	
Bone	37 (77.1)
Lymph node	25 (52.1)
Lung	6 (12.5)
Liver	5 (10.4)
No. of previous chemotherapeutic regimens	
1	21 (43.8)
≥ 2	27 (56.2)
Median baseline PSA (range)	35.5 (1-1501)

All samples were evaluable for both sequencing and TaqMan Genotyping assay. During the rs10883783 analysis another SNP, rs284849, was identified and included in the statistical evaluations. In our case series, the allelic frequencies were as follows: 37.5% for the minor allele G in rs743572; 23.96% for the minor allele A in rs10883783; 13.54% for the minor allele T in rs17115100; and 21.88% for the minor allele T rs284849. There were no statistically significant differences between these alleles and the allelic frequencies of the worldwide population (rs743572: p = 0.3688; rs10883783: p = 0.7194; rs17115100: p = 0.5344; rs284849: p = 0.0819) (Table 2).

TABLE 2. Allelic frequency for each polymorphism in our case series and in the general population

Allele	Allelic frequency		*p-value***
	Case series (%)	**Population (%)**	
rs743572			
A	60/96 (62.5)	1264/2184 (57.9)	0.369
G*	36/96 (37.5)	920/2184 (42.1)	
rs10883783			
T	73/96 (76.0)	1698/2188 (77.6)	0.719
A*	23/96 (24.0)	490/2188 (22.4)	
rs17115100			
G	83/96 (86.5)	1840/2188 (84.1)	0.534
T*	13/96 (13.5)	348/2188 (15.9)	
rs284849			
G	75/96 (78.1)	1855/2190 (84.7)	0.082
T*	21/96 (21.9)	335/2190 (15.3)	

*Less common allele; **Chi-square test

The CRPC patients treated with abiraterone had a median PFS and OS of 7.6 months (95% CI: 4.3-10.5) and 17.6 months (95% CI: 10.5-19.0), respectively (figure 1).

Figure 1. a) PFS - Kaplan Meier curves for all samples; b) OS - Kaplan Meier curves for all samples.

Figure 1 a) PFS - Kaplan Meier curves for all samples

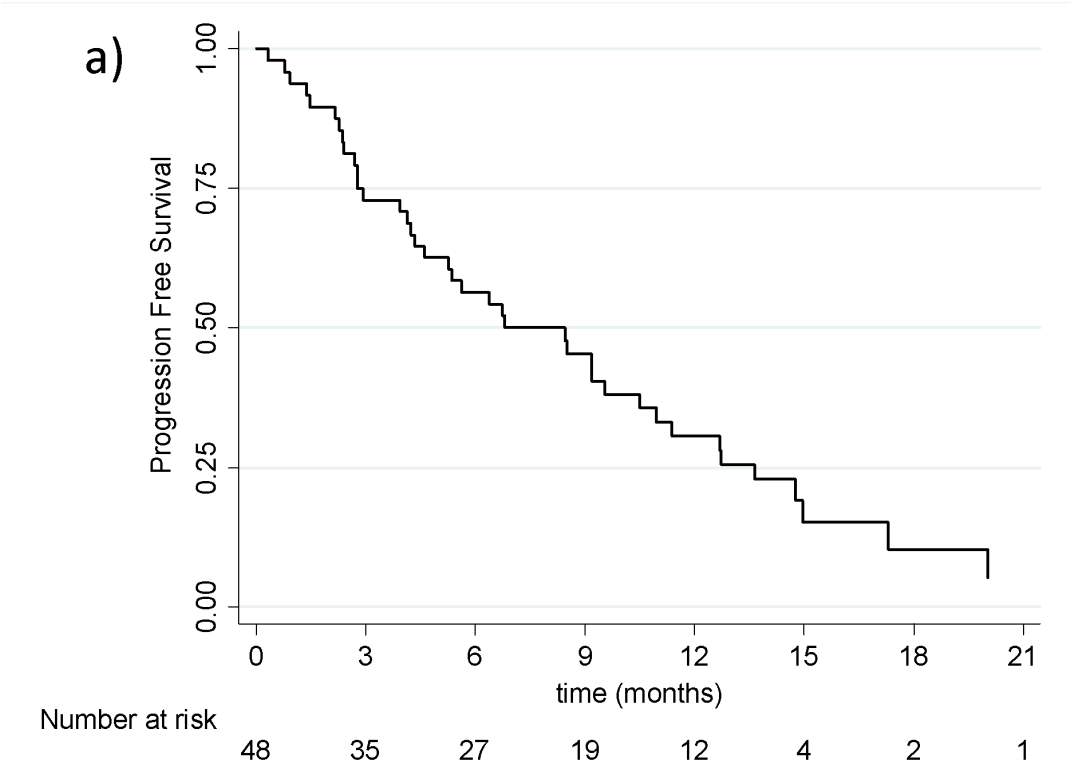
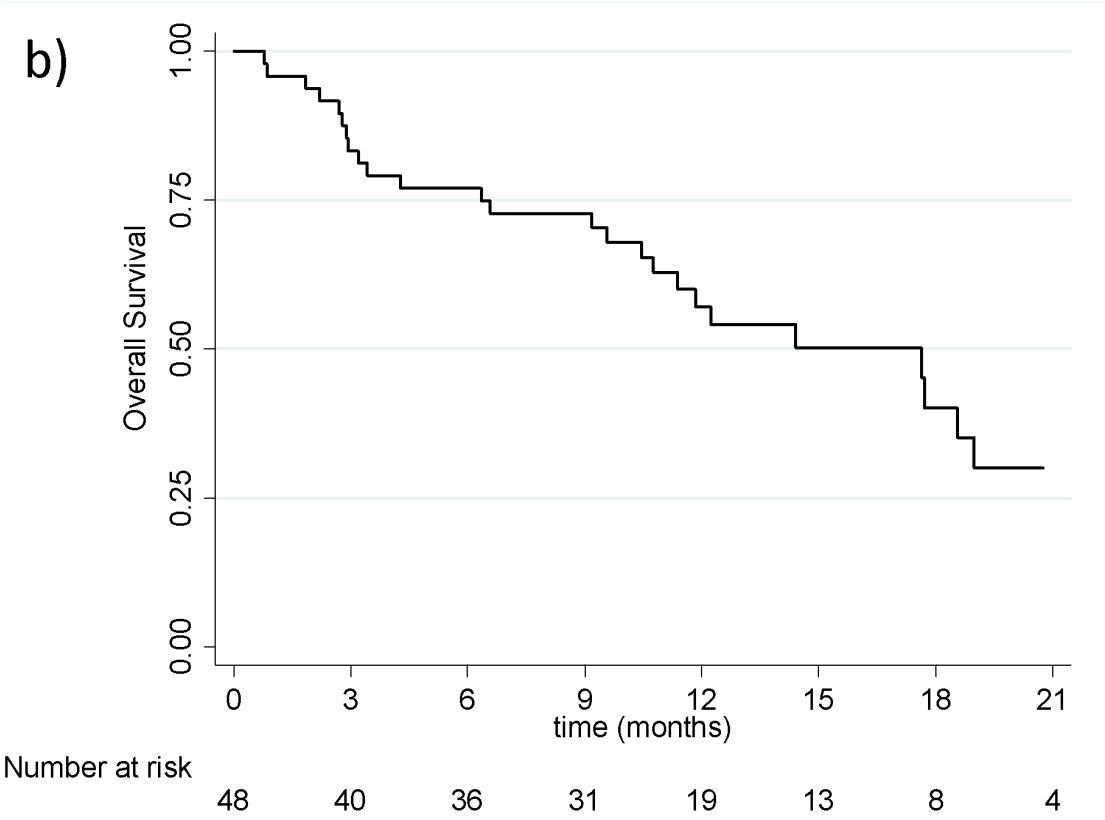


Figure 1 b) OS - Kaplan Meier curves for all samples.



The association between each CYP17A1 gene polymorphism and PFS and OS was evaluated (Figure 2 and 3).

Figure 2. PFS curves for rs743572 (a), rs10883783 (b), rs17115100 (c) and rs284849 (d).

Figure 2 a) PFS curves for rs743572. The solid and dotted lines represent the most and the less common haplotype, respectively.

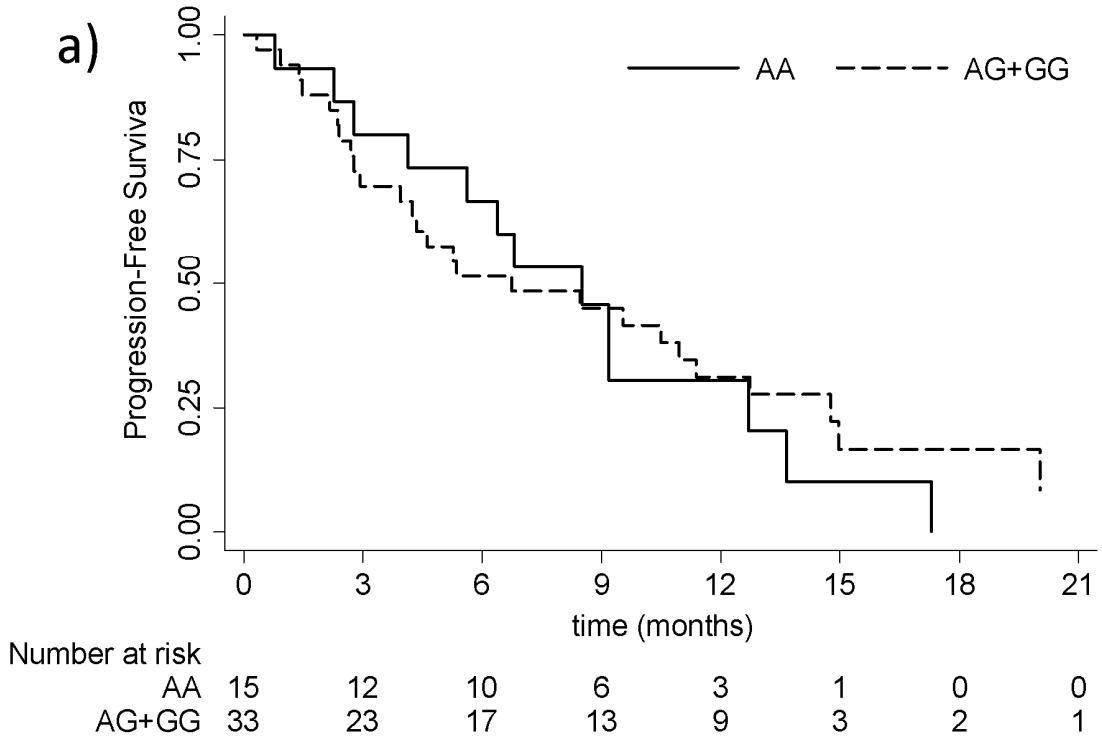


Figure 2 b) PFS curves for rs10883783. The solid and dotted lines represent the most and the less common haplotype, respectively.

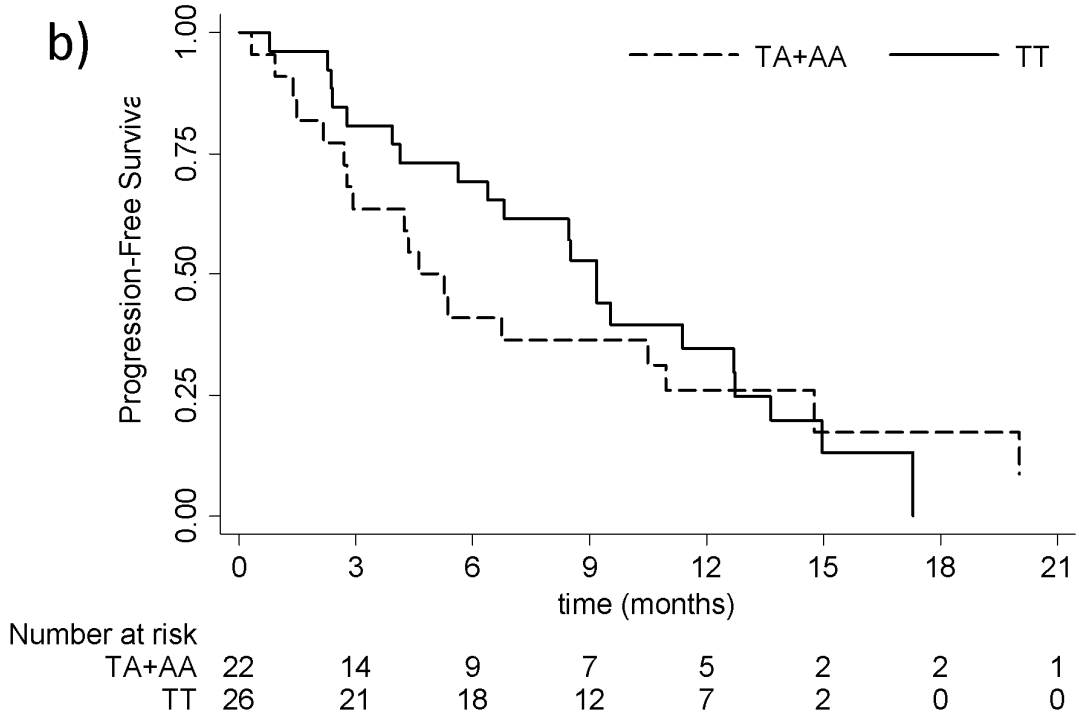


Figure 2 c) PFS curves for rs17115100. The solid and dotted lines represent the most and the less common haplotype, respectively.

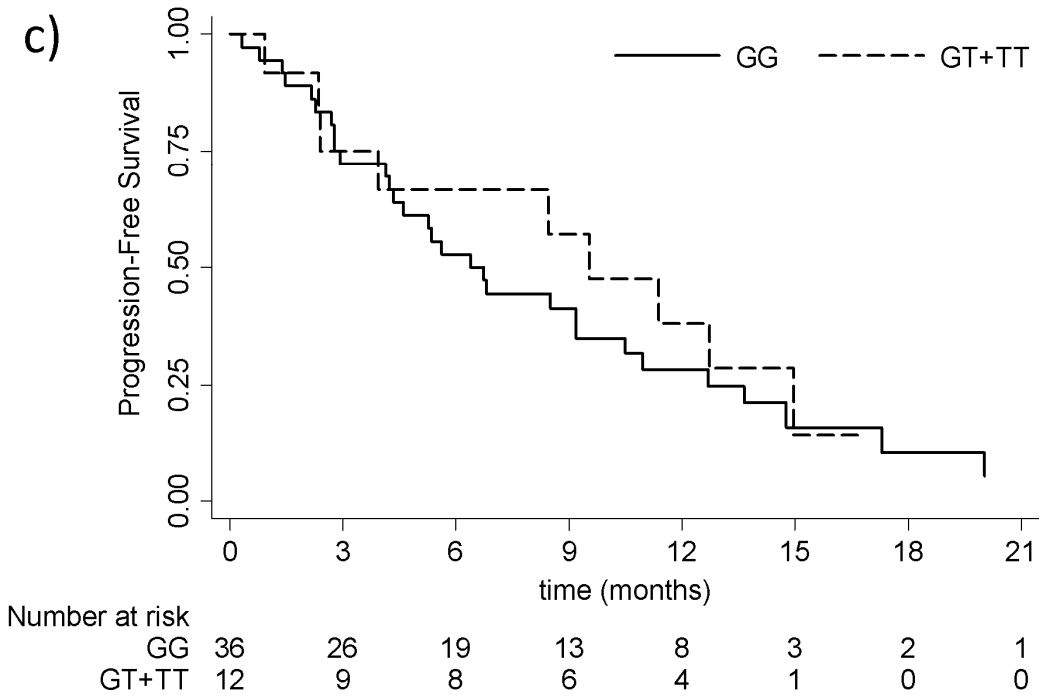


Figure 2 d) PFS curves for rs284849. The solid and dotted lines represent the most and the less common haplotype, respectively.

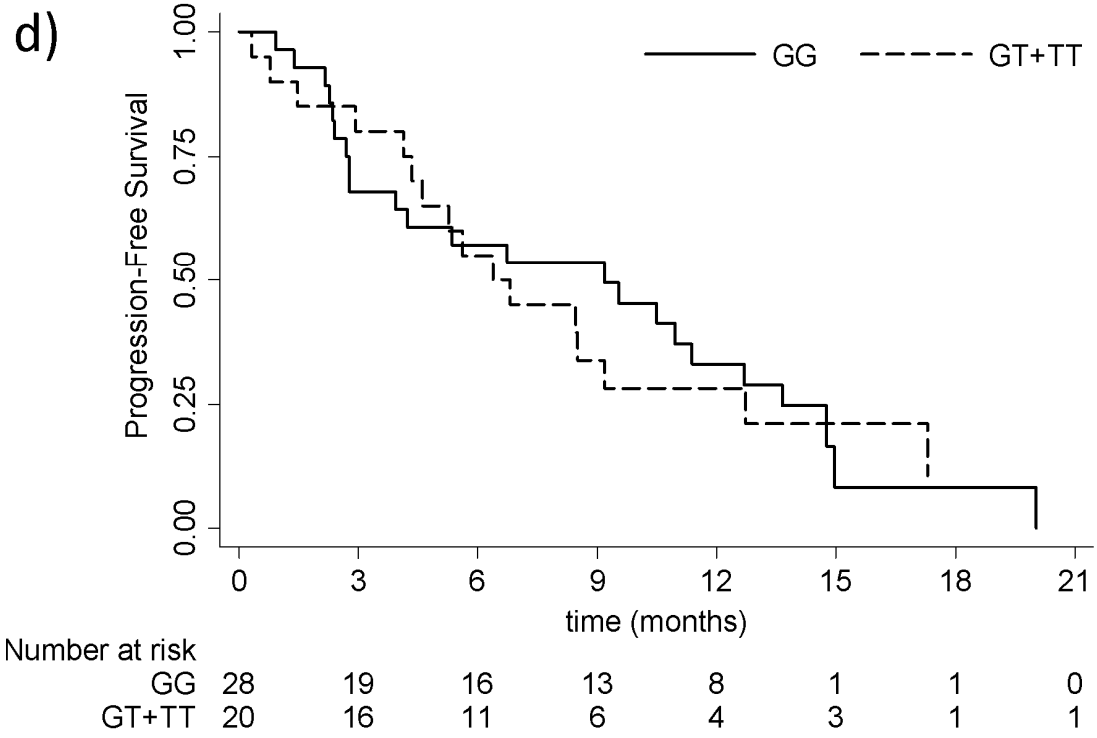


Figure 3. OS curves for rs743572 (a), rs10883783 (b), rs17115100 (c) and rs284849 (d).

Figure 3 a) OS curves for rs743572. The solid and dotted lines represent the most and the less common haplotype, respectively.

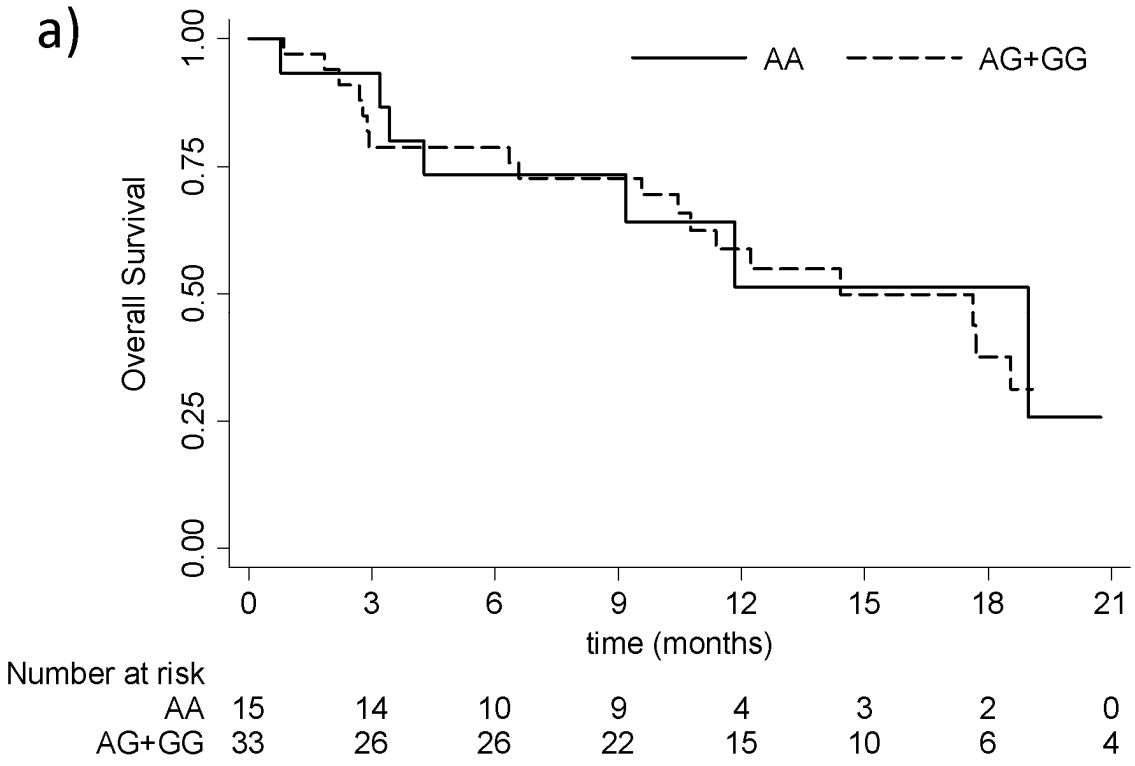


Figure 3 b) OS curves for rs10883783. The solid and dotted lines represent the most and the less common haplotype, respectively.

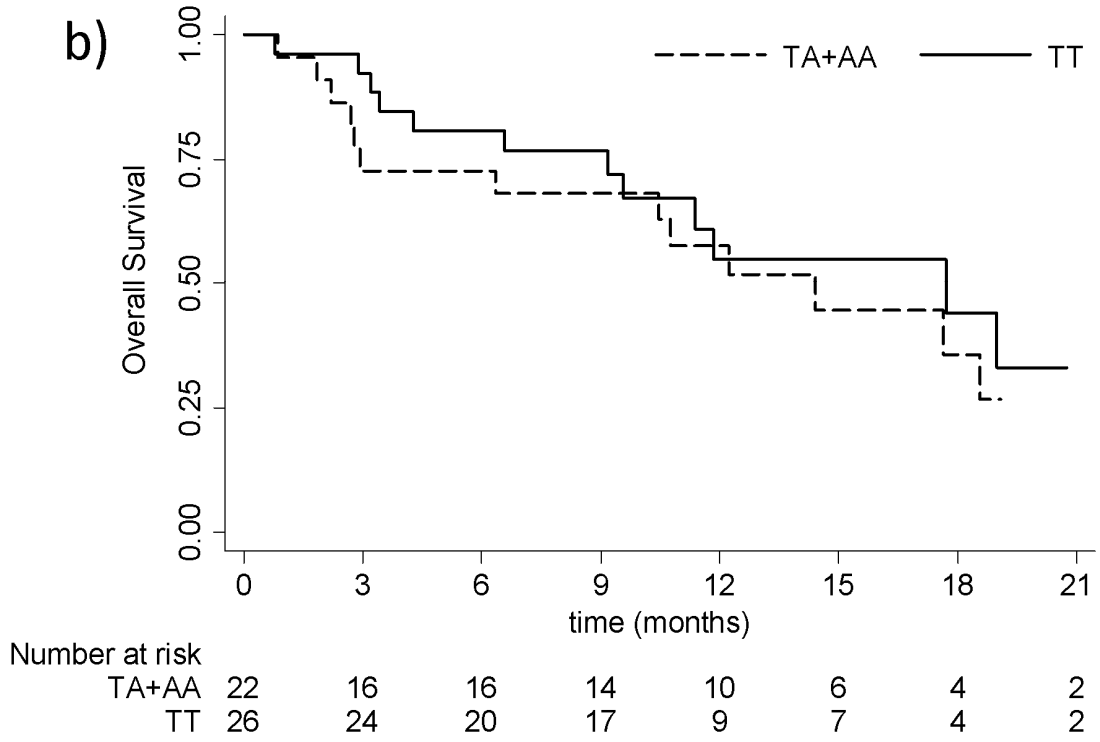


Figure 3 c) OS curves for rs17115100. The solid and dotted lines represent the most and the less common haplotype, respectively.

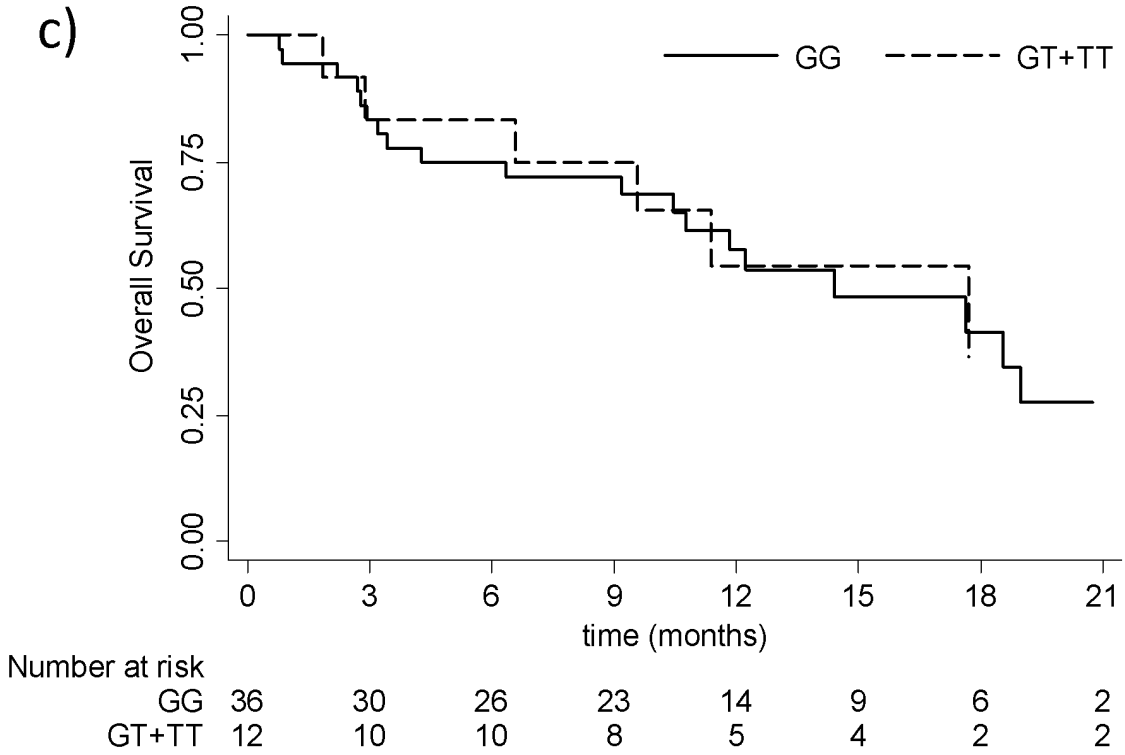
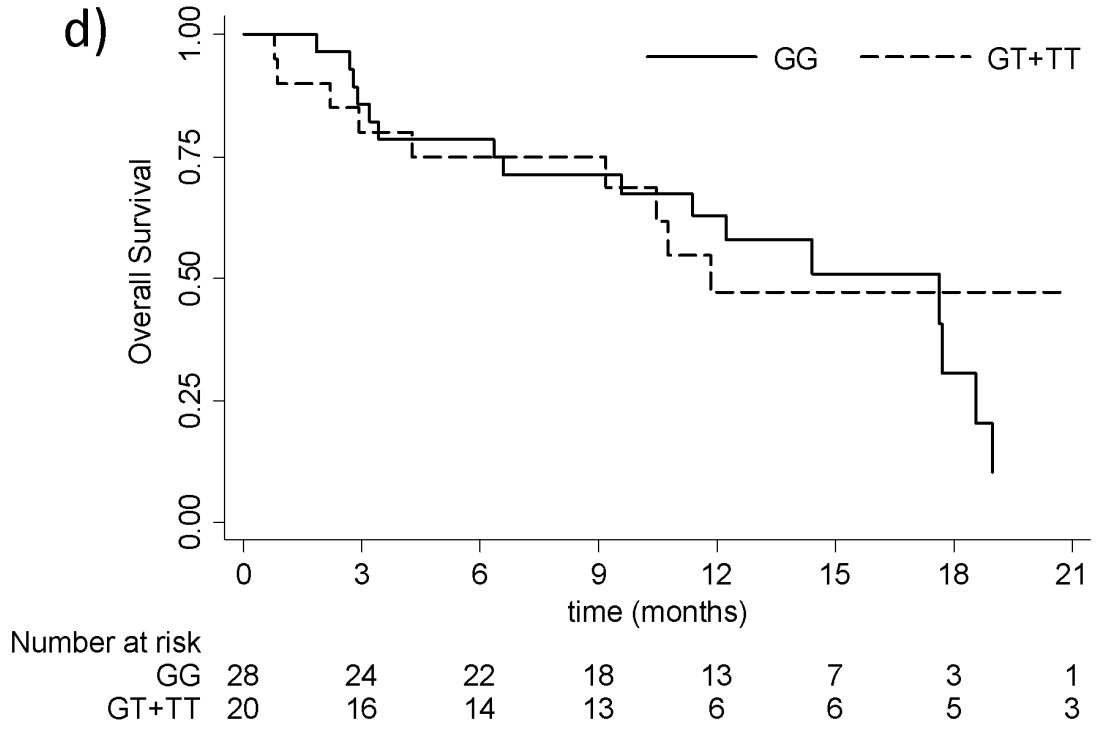


Figure 3 d) OS curves for rs284849. The solid and dotted lines represent the most and the less common haplotype, respectively.



For the rs743572 polymorphism, median PFS for individuals with the AA haplotype was 8.5 (95% CI: 2.8-12.7) vs. 6.7 months (95% CI: 4.0-11.4) in those with the less common allele (haplotype AG+GG). The median OS was 19 months (95% CI: 3.4-…) for AA haplotype patients and 14.4 (95% CI: 10.5-21.9) in individuals with the AG+GG haplotype. No statistically significant differences were found in either PFS (log-rank test p = 0.6543; Wilcoxon test p = 0.8134) or OS (log-rank test p = 0.9763; Wilcoxon test p = 0.9896) curves.

The PFS Kaplan-Meier curve for rs10883783 showed a positive trend for individuals with the most common TT haplotype, who lived around 4 months longer than patients with the TA+AA haplotype: 9.2 vs. 4.9 months, respectively (95% CI: 5.6-12.7 vs. 2.7-11 and log-rank test p = 0.66; Wilcoxon test p = 0.1903). This difference was confirmed in the OS curve: median OS of 17.7 months (95% CI: 9.2-21.9) for the TT haplotypes vs. 14 months (95% CI: 2.9-…) for the TA+AA haplotype (log-rank test p = 0.6798; Wilcoxon test p = 0.4754).

For the polymorphism rs17115100, a median PFS of 9.5 months (95% CI: 2.4-15) was observed for GT+TT haplotype patients vs. 6.6 months (95% CI: 4.2-10.5) for those with the most common GG haplotype (log-rank test p = 0.5465; Wilcoxon test p = 0.4858). The OS curve for this polymorphism showed a similar difference in the median values: 17.7 (95% CI: 2.9-…) for the GT+TT haplotype vs. 14.4 (95% CI: 10.5-19) for the most common GG haplotype (log-rank test p = 0.9381; Wilcoxon test p = 0.8373).

The median PFS in individuals with the most common GG haplotype for the rs284849 polymorphism was 9.2 months (95% CI: 2.8-12.7) vs. 6.6 (95% CI: 4.1-9.2) in GT+TT patients (log-rank test p = 0.9841; Wilcoxon test p = 0.8470). The median OS for this polymorphism was 17.6 (95% CI: 9.6-18.6) in patients with the GG haplotype and 11.8 (95% CI: 4.3-…) in those with the GT+TT haplotype (log-rank test p = 0.5989; Wilcoxon test p = 0.8540).

We also evaluated the relation between each polymorphism and PFS probability 6 months after starting abiraterone treatment (figure 4).

Figure 4. Progression-free survival (PFS) six months after the start of treatment: rs743572 (a), rs10883783 (b), rs17115100 (c) and rs284849 (d).

Figure 4 a) Progression-free survival (PFS) six months after the start of treatment: rs743572. The solid and dotted lines represent the most and the less common haplotype, respectively.

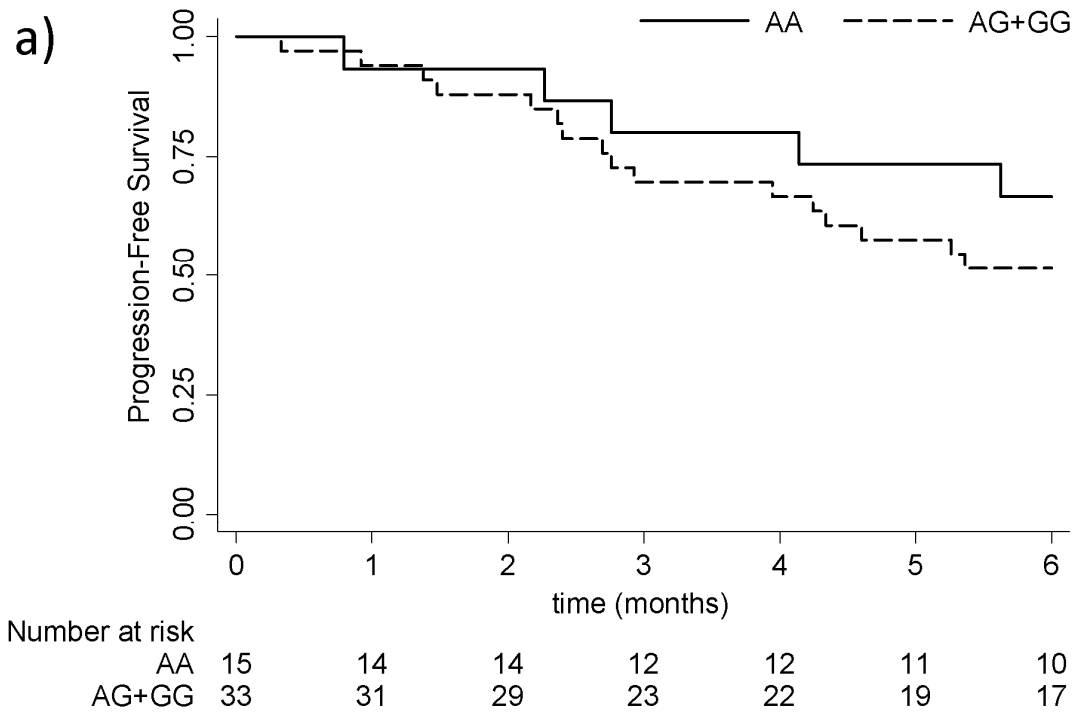


Figure 4 b) Progression-free survival (PFS) six months after the start of treatment: rs10883783. The solid and dotted lines represent the most and the less common haplotype, respectively.

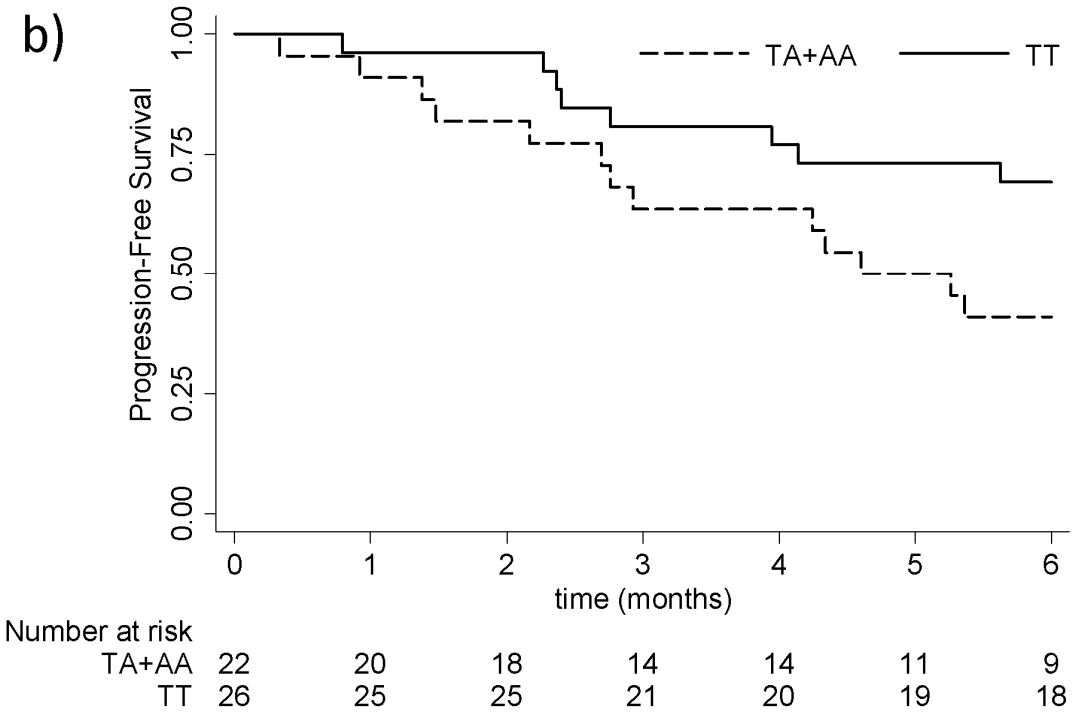


Figure 4 c) Progression-free survival (PFS) six months after the start of treatment: rs17115100. The solid and dotted lines represent the most and the less common haplotype, respectively.

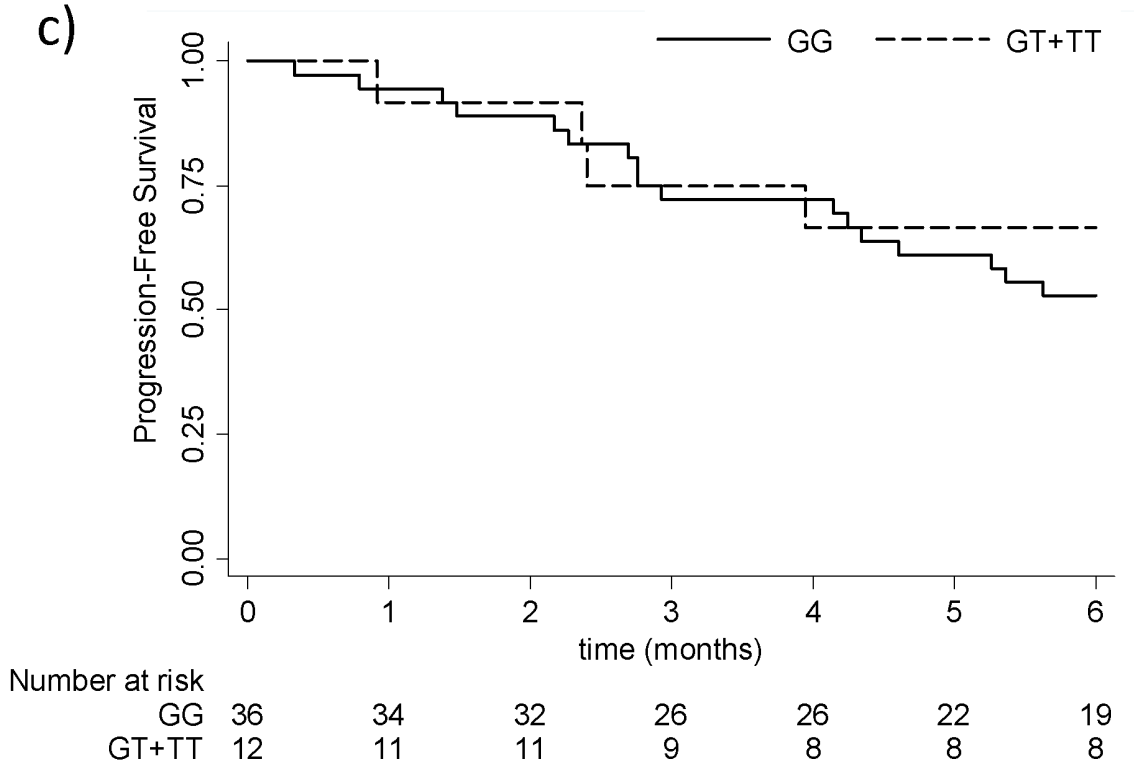
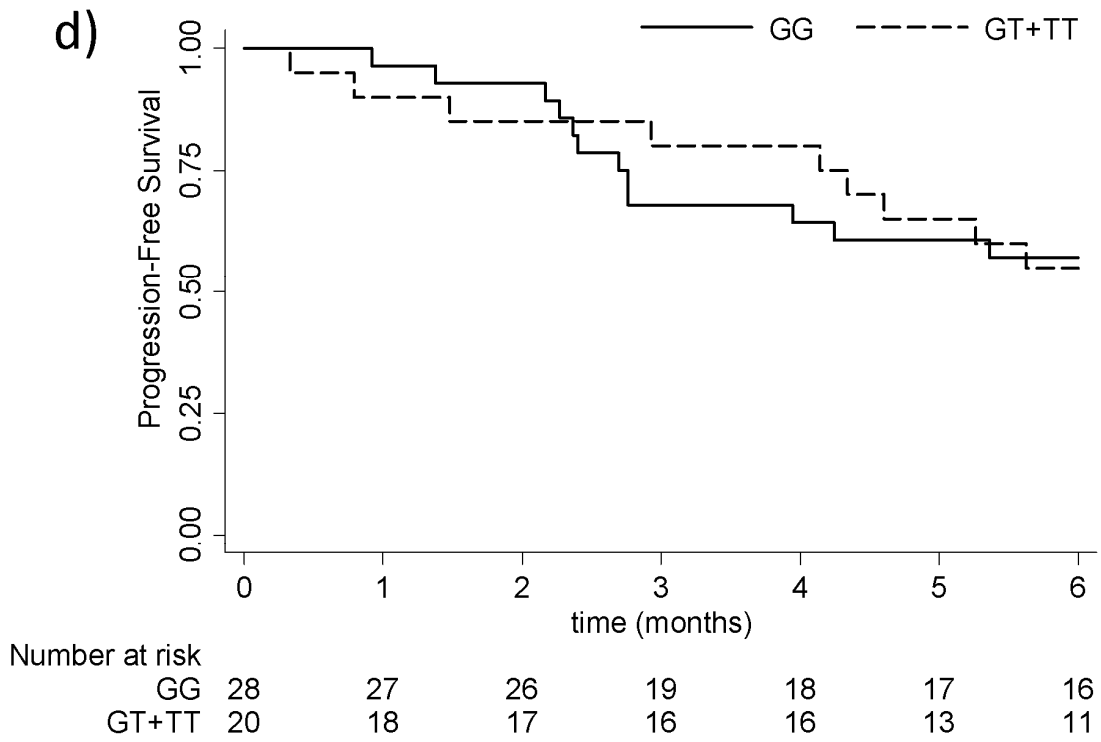


Figure 4 d) Progression-free survival (PFS) six months after the start of treatment: rs284849. The solid and dotted lines represent the most and the less common haplotype, respectively.



The polymorphism rs10883783 was associated with a PFS probability of 41% (95% CI: 21-60%) in AT+AA haplotype patients vs. 69% (95% CI: 48-83%) in those with the common allele, showing a trend towards statistical significance (log-rank test p = 0.0534; Wilcoxon test p = 0.0639). Instead, in other polymorphisms there was no evidence of difference: patients with the AA haplotype for the rs743572 polymorphism showed a PFS probability of 67% (95% CI: 38-85%) vs. 52% (95% CI: 34-67%) for individuals with the AG+GG haplotype (log-rank test p = 0.335; Wilcoxon test p = 0.3469); patients with the less common GT+TT haplotype for the rs17115100 polymorphism had a 6-month PFS probability of 67% (95% CI: 34-86%) vs. 53% (95% CI: 35-67%) in GG haplotype patients (log-rank test p = 0.5064; Wilcoxon test p = 0.6014), in those with GG or GT+TT haplotypes for the rs284849 polymorphism the PFS probability was similar: 57% (95% CI: 37-73%) vs. 55% (95% CI: 31-73%), respectively (log-rank test p = 0.9851; Wilcoxon test p = 0.8904).

In order to verify how the polymorphisms could affect the CYP17A1 protein expression, we also performed immunohistochemical analyses on a small case series of samples but we didn’t find any significant correlation between a specific SNP and the expression of the enzyme (data not shown).

Discussion

Abiraterone is a new hormonal agent blocking androgen production in the testes, adrenal glands, and tumor cells by inhibiting Cytochrome P450, family 17, subfamily A, polypeptide 1 (CYP17A1). A Phase III study demonstrated that abiraterone is well tolerated and prolongs overall survival by 4 months relative to placebo in CRPC patients previously treated with taxanes.[4] These results indicate that AR signaling continues to play a critical role in the setting of castration-resistant disease.

Nongonadal sources of testosterone include the adrenal glands and prostate cancer cells through intracrine production, both of which contribute to disease progression despite castrate levels of testosterone.[75-77] Androgen-deprivation therapy with orchiectomy or LHRH analogs reduces testicular androgen production without affecting adrenal or intracrine androgen synthesis.[78] In castration-resistant disease, extragonadal synthesis produces tumor androgen levels exceeding those in the prostates of eugonadal men that are sufficient to activate AR signaling.[79,80] Androgens, such as androstenedione and dehydroepiandrosterone sulfate (DHEAS), are AR agonists that may affect disease progression.[81] These androgens and testosterone have been the target of therapeutic trials with corticosteroids and ketoconazole.[82,83] Higher androstenedione levels were associated with PSA decline in ketoconazole plus hydrocortisone–treated patients.[84] Higher baseline serum testosterone, and precursors DHEAS and androstenedione, may be prognostic by identifying mCRPC patients with tumors that may be more dependent on androgens for growth regardless of the source. Although treatment with abiraterone can significantly delay progression of disease and improve OS, there is nearly universal development of therapeutic resistance and disease progression. Thus, there is a continued need for improved therapy for patients with metastatic CRPC.

The genotyping of genes involved in CRPC carcinogenesis, cancer progression and drug metabolism could help us to better understand the behavior of CRPC and, consequently, patient response to therapy.[85,86] CYP17A1 is a key regulatory enzyme in the steroidogenic pathway. The lack of CYP17 results in impaired synthesis of cortisol, androgen and estrogen, as well as mineralocorticoid overproduction.[87,88]

The aim of the present paper was to evaluate the impact of some polymorphisms in the CYP17A1 gene on response to treatment with abiraterone and patient outcome. Our analysis of four selected SNPs (rs10883783, rs17115100, rs284849, rs743572) revealed an

association between rs10883783 and PFS, with a trend towards statistical significance. Genetic variations may affect expression levels of CYP17A1 and, consequently, may modulate response to treatment. Although it has been shown that advanced prostate cancer expresses higher levels of CYP17A1 than those of the primary tumor, the mechanisms or gene modifications responsible for this expression modulation are still unknown.[89] A single nucleotide variation in a gene may exert an effect on its expression level in different ways, *e.g.* by altering the splicing process.[90,91] One of the polymorphisms we analyzed (rs743572) is located at the 5'-UTR CYP17A1 gene and can lead to promoter activity alteration. The others are intronic and may be involved in splicing mechanisms. Using a splicing motif predictor tool (Human Splicing Finder http://www.umd.be/HSF), rs10883783 would seem to be located in a branch point motif and may thus be involved in a variation in consensus sequences required for correct splicing.

Abiraterone showed impressive results, substantially increasing the PFS and OS of CRPC patients pretreated with docetaxel,[4] but, almost one third of these patients showed disease progression during the first few months of therapy, resulting in the need to identify markers predictive of patient outcome. Our findings indicate the potential role of the SNP rs10883783 as a predictive marker for abiraterone in CRPC. Patients with the less common allele A for this polymorphism showed a shorter time to progression than those with the common haplotype. To further understand the role of rs10883783, we also evaluated PFS at 6 months after the start of abiraterone treatment. The PFS curve highlighted a difference approaching statistical significance between patient haplotypes (log-rank test $p = 0.0534$), suggesting that rs10883783 could predict patient outcome, even if larger patient population is probably needed to have firm conclusions.

This is the first study to highlight the role of the CYP17A1 gene polymorphism in prognosis and/or in predicting response to new hormone therapies in CRPC patients. Specific tumor-related characteristics such as epigenetic modifications and genetic alterations are known to predict clinical outcome in patients treated with abiraterone.[54] Thus, it would be interesting to focus on CYP17A1 gene polymorphisms in groups of CRPC patients subdivided on the basis of tumor-related characteristics in blood and/or tumor tissue. Abiraterone is currently being investigated in combination with other drugs including hormone therapies and inhibitors of the PI3K-AKT-mTOR signaling pathways,[92] making the identification of biomarkers increasingly important. Finally, we only analyzed four CYP17A1 gene SNPs, but full-gene genotyping could undoubtedly help to identify other important alterations.

Conclusion

The genetic characterization of CYP17A1 could facilitate our understanding of patient response/resistance to abiraterone therapy. In our case series of 48 treated patients, rs10883783 only was identified as a possible predictive marker, results showing a trend toward statistical significance. Further analysis of this polymorphism is needed in larger series of patients to confirm our findings.

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ACKNOWLEDGMENTS

Prof Giorgio Cantelli Forti, *Department of Pharmacology. University of Bologna, Bologna,* to give me the opportunity to work on the Pharmacology and Toxicology of new drugs in urological tumors and to support me in this work.

Dr Dino Amadori *Department of Medical Oncology, IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola* to give me the opportunity to work on the predictive factors of new drugs in urological tumors in my Institution and to support this study.

Dr Samanta Salvi, Dr Valentina Casadio and Dr Wainer Zoli *Biosciences Laboratory, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.) - IRCCS Meldola* to collaborate in the conception of this study and the analysis of samples and itnerpretation of data.

Dr Salvatore Luca Burgio, Dr Lorena Rossi, Dr Cecilia Menna, Dr Emanuela Bianchi, Dr Vincenza Conteduca, *Department of Medical Oncology, IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola* to collaborate in the design of this study and the sample collection

Dr Elisa Carretta, *Unit of Biostatistics and Clinical Trials, IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola* to support me in the statistical analysis and figure preparation