STUDY PROTOCOL

PROTOCOL TITLE: Low-dose 500 mg abiraterone acetate in metastatic castration-resistant prostate cancer (mCRPC) and metastatic hormone-sensitive prostate cancer (mHSPC) patients: a Phase I proof-of-concept clinical study

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1. BACKGROUND AND RATIONALE

The Global Burden of Prostate Cancer and the Role of Abiraterone across the Continuum of Prostate Oncology

In 2018, statistics published by the International Agency for Research on Cancer ranked prostate cancer as the second most frequent cancer and the fifth leading cause of death among male malignancies worldwide. These findings parallel the considerable disease burden observed locally. While indolent, localized disease remains amenable to curative interventions via radiation or surgery, androgen deprivation therapy (ADT) targeted at disrupting androgen receptor (AR)-mediated signaling has become the standard of care in clinically advanced or disseminated disease. However, the initial response to ADT is often unsustainable and patients inevitably experience progression to castration resistant prostate cancer (CRPC) within 1-2 years, where reactivation of the androgen signaling axis occurs despite castrate levels (< 50 ng/dL) of circulating serum testosterone. Metastases (mCRPC) are evident in ≥84% of diagnosed cases and prognosis is poor in mCRPC, with median survival estimated at 9-13 months and a mortality statistic of >50% of patients within 3 years with standard docetaxel-based chemotherapies. Cytochrome P450 17A1 (CYP17A1) occupies a pivotal role in both adrenal and *de novo* intratumoural androgen biosynthesis, catalyzing sequential 17α-hydroxylation and C17,20-lyase reactions. Resultant product dehydroepiandrosterone (DHEA) is a critical steroidal precursor of downstream potent AR ligands, testosterone and dihydrotestosterone. The potential for augmented androgen ablation through exploiting CYP17A1 as a therapeutic target spurred the initial approval of abiraterone (administered as prodrug abiraterone acetate), a first in class CYP17A1 inhibitor for the therapeutic management of mCRPC.

The evidence in favour of abiraterone acetate treatment in mCRPC has since prompted its evaluation in the metastatic hormone sensitive prostate cancer (mHSPC) patient population. Specifically, the use of abiraterone acetate in this setting was studied in the STAMPEDE arm G and LATITUDE randomized controlled trials.[1,2] Considering evidence that clearly demonstrate overall survival benefits when abiraterone acetate was added to ADT versus ADT alone, combination systemic therapy with either docetaxel or abiraterone acetate is now standard of care for for men with mHSPC (especially those with high risk or high volume disease).

Challenges in Dosing Determination of Abiraterone Acetate – Was an Optimal Biologic Dose Defined?

Abiraterone acetate (AA) is currently indicated to be administered at a dose of 1000 mg daily either 1 h before or 2 h after food.[3] Given that no dose limiting toxicities (DLTs) were detected up to 2000 mg in a Phase I dose escalation trial, the current dose selection was justified based on a plateau in anticipated toxicities (i.e. upstream mineralocorticoid excess) observed at doses above 750 mg.[4] Implicit in this classical maximal tolerated dose (MTD) paradigm are the principles that 1) a linear dose-efficacy relationship exists and 2) toxicities are direct manifestations of exacerbated pharmacology.[5]

However, in a recent Phase II trial, comparisons of low-dose AA (LOW; 250 mg with a low-fat meal) versus standard dose AA (STD; 1000 mg fasting) demonstrated that despite systemic exposure and peak abiraterone concentrations being significantly higher in the STD group compared to the LOW group (**Figures 1A** and **1B**), the extent of dehydroepiandrosterone-sulfate (DHEA-S) suppression was similar (**Figure 1C**), verifying that the efficacy of CYP17A1 inhibition was preserved.[6]

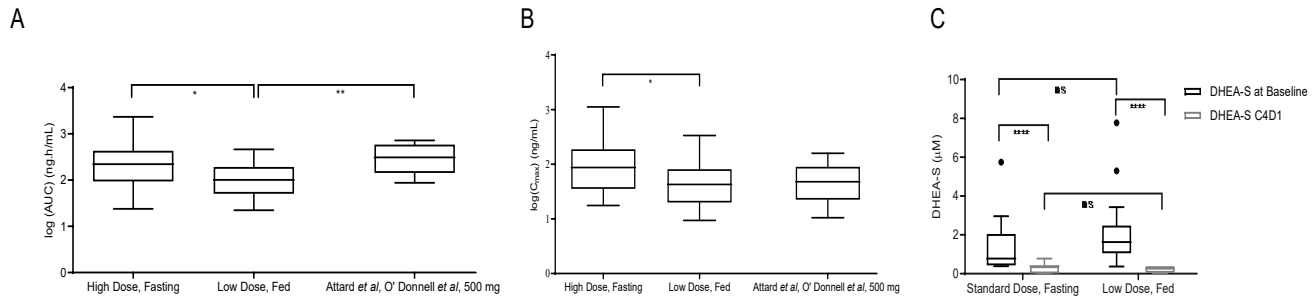

Figure 1. Investigating potential correlations between abiraterone drug levels and the extent of dehydroepiandrosterone-sulphate (DHEA-S) suppression. Results from the Phase II study *by Szmulewitz et al* demonstrated how (**A**) the area under the plasma concentration-time curve of abiraterone from 0-4 h (AUC_{0-4h}), and (B) peak plasma concentrations (C_{max}) remained significantly higher in the standard dose arm (1,000 mg abiraterone acetate - AA fasting, n = 20) compared to the low dose arm (250 mg AA, fed, n = 20) (two-sided p value for AUC0-4h = 0.0417 and two-sided p value for Cmax = 0.0474). Combined analyses of Phase I trials (*Attard et al* and *O' Donnell et al*, n= 10) in (**A**) and (**B**) revealed that administration of 500 mg of AA in a fasted state yielded AUC values that were greater than that measured in the low dose arm (two-sided p value = 0.0038) whereas C_{max} measurements were not significantly different. AUC and C_{max} data were log transformed and groups comparisons were performed using repeated-measures one-way ANOVA and Dunnett's multiple comparisons tests. (**C**) DHEA-S levels between standard dose and low dose treatment arms did not differ significantly prior to initiation of AA therapy. At the end of the 4th 28-day cycle (C4D1), DHEA-S levels were reduced to a similar extent regardless of treatment arm. Comparisons between treatment groups were performed using non-parametric Mann-Whitney U tests.

This observed pharmacokinetic-pharmacodynamic (PK-PD) uncoupling underscores how targeted therapeutics such as AA may achieve optimal antitumor activity at doses significantly lower than that required to elicit adverse outcomes. With the diminished utility of MTD-based strategies, rational dose selection for AA must instead be predicated on mechanistic and quantitative characterization of the relationships between target exposure, drug-target interactions and PD endpoints.[7,8]

Our *in vitro* and *in silico* analyses have demonstrated that abiraterone engages CYP17A1 via a two-step slow, tight binding mechanism, with a residence time of approximately 42 h within the CYP17A1 active site. Considering the estimated turnover of CYP17A1 and the temporal *in vivo* pharmacokinetics (PK) of abiraterone in relation to the lifetime of the drug-target complex, simulations demonstrate that even when the bulk of abiraterone has been eliminated systemically at the end of the dosing interval of 24 h, approximately constant apparent CYP17A1

occupancy would potentially be achieved *in vivo* (**Figure 2**).

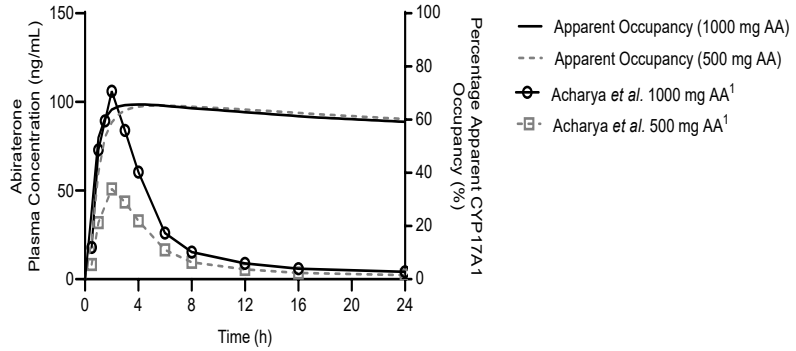

Figure 2. Simulated apparent CYP17A1 occupancy over time plots after a single in vivo dosing of 1000 mg or 500 mg of abiraterone acetate (AA). The clinical plasma concentration-time profiles of abiraterone are represented by open symbols while the solid and dashed lines indicate the predicted percentage apparent CYP17A1 occupancy over time.

Taken together, our novel findings would enable mechanistic rationalization of the clinically reported PK-PD disconnect, where equipotent reduction of downstream plasma DHEA-sulphate levels was achieved despite a lower systemic exposure of abiraterone, hence providing the impetus for re-evaluating the current dosing paradigm of AA.

1.1. General Introduction

Abiraterone (administered as prodrug abiraterone acetate) was initially approved for the treatment of metastatic castration resistant prostate cancer (mCRPC) by the US Food and Drug Administration (FDA) in 2011 as a novel hormonal ablative agent that inhibits cytochrome P450 17A1 (CYP17A1). CYP17A1 is a bifunctional enzyme that occupies a pivotal role in both adrenal and *de novo* steroidogenesis. With substantial evidence alluding to the reactivation of androgen receptor (AR)-mediated signalling as a key driver in disease progression despite castrate serum androgen levels, selective inhibition of CYP17A1 impairs androgen production, hence preventing continued maintenance of AR signalling that drives CRPC progression. Clinically, abiraterone in combination with prednisone has been shown to significantly increase overall survival and provide additional clinical benefits in both chemotherapy pretreated and chemotherapy naïve mCRPC patients.

In 2018, based on results from the LATITUDE[1] and STAMPEDE trial[2] where the addition of abiraterone plus prednisone to androgen deprivation therapy (ADT) was associated with longer overall survival and longer radiographic progression-free survival than ADT alone, FDA has also approved abiraterone in combination with prednisone for metastatic hormone-sensitive prostate cancer (mHSPC).

Additionally, abiraterone was found to be a substrate of hepatic organic anion transporting polypeptides (OATP) 1B3 in our *in vitro* study and reported to show inhibitory effect on OATP1B1 as well[9]. Both of the transporters are responsible for the hepatic uptake of numerous drugs and endogenous compounds, which could act as the rate-limiting step for drug disposition and affect physiology, respectively. Coproporphyrin I and III (CP-I and CP-III) are endogenous porphyrin metabolites arising from heme synthesis and appear to be substrates for OATP1B1 and OATP1B3. Several *in vitro* and *in vivo* studies have demonstrated CP-I and CP-III as potential endogenous biomarkers for evaluation of the modulation in OATP1B1/1B3 functions[10]. Measuring the plasma levels of CP-I and CP-III provides unprecedented opportunities to investigate and establish (1) the hepatic OATP transporter functions in prostate cancer patients and (2) the potential *in vivo* inhibition of these transporters by abiraterone. The former knowledge will allow clinicians to better understand how extrahepatic cancer may affect the liver physiology. The latter knowledge will shed novel insights on potential drug-drug interactions between abiraterone and co-administered drugs that are OATP substrates such as the lipid lowering statins.

Rationale and Justification for the Study

1) Selection of an Alternative 500 mg Dose of Abiraterone Acetate for Investigation
Selection of a 500 mg dose of AA as an alternative to the current standard 1000 mg dose was **guided by both (1) pharmacokinetic and (2) pharmacodynamic considerations**.

1) As presented in **Figure 1C** (**Page 5**), the mean AUC ($AUC_{0\text{-}12h}$ and $AUC_{0\text{-}72h}$) associated with the 500 mg dose was expectedly greater than $AUC_{0\text{-}4h}$ associated with the low 250 mg dose (mean AUC ratio = 2.83, 95% CI = 1.42 to 5.62). Nevertheless, based on PK measurements made up to 72 h in a Phase I dose escalation trial,[11] the calculated $AUC_{0\text{-}4h}$ is approximately 50% of the total AUC across different doses. **Taken together, the systemic exposure of a 500 mg dose of AA (fasted) is likely to be similar to that of the 250 mg dose of AA (low-fat meal), which has been demonstrated to be non-inferior to the 1000 mg standard dose with respect to PSA metrics (change in PSA at 12 weeks).**[6]

2) A plateau in corticosterone concentrations at doses above 750 mg was employed to justify the selection of 1000 mg AA for subsequent Phase II and III evaluation.[4] We posit that this indirect endpoint inherently conflates the therapeutic and toxic effects of abiraterone, which was likely misguided considering recent *in vitro* evidence indicating the off target inhibitory effects of abiraterone on other steroid metabolizing enzymes (e.g. 3βHSD and CYP21A2) at clinically relevant concentrations, which are key enzymes mediating mineralocorticoid synthesis. [12,13]

   A **Phase 1 single dose study in castrate males** demonstrated that treatment with 500 mg of abiraterone acetate resulted in sustained suppression of the testosterone/androstenedione axis for Days 2 to 5 post-therapy.[14] Additionally, in a separate **Phase I dose escalation study** evaluating once-daily, continuous AA in three-patient cohorts, antitumor activity (i.e. testosterone suppression to undetectable levels (< 1

ng/dL) within 8 days) was also observed across all five doses (from 250 to 2,000 mg).[4] **In summary, single and multiple Phase I dose escalation studies have been performed and findings allude to the potential efficacy of the 500 mg dose of AA.**

2) Pharmacoeconomic Implications – Mitigating the Escalating Costs of Oncology Drug Treatment

The median duration of abiraterone acetate treatment for patients with mCRPC, based on clinical trials was 8 (post-chemotherapy) to 13.8 months (prechemotherapy) and for newly diagnosed high-risk mHSPC, 24 months.[3] Locally, abiraterone acetate alone has an approximate retail cost of $4800 per month. Consequently, the potential long treatment duration in conjunction with additional costs derived from background androgen deprivation therapy and bone-targeting treatments have created a quandary for oncologists aiming to strike a balance between high quality and high value care.

Hence, in 2019, recognizing the financial toxicities associated with abiraterone acetate treatment, the NCCN guidelines recommended that based on the findings from the randomized, Phase II, non-inferiority trial by Szmulewitz *et al*, abiraterone with prednisone can be given at 250 mg/day following a low-fat breakfast as an alternative to 1000 mg/day abiraterone fasting. We envision that such cost savings (~$2400 per month) could similarly be achieved with a reduction in the dose of abiraterone acetate, circumventing the high variability in oral bioavailability that often accompanies the food effect in drug PK.

Taken together, we hypothesize dose reduction of abiraterone acetate to 500 mg would achieve antitumor activity in mCRPC and mHPSC patients comparable to standard of care. Unfortunately, dose comparison studies of abiraterone acetate were not undertaken in Phase II evaluations, underscoring a critical dearth of data which could be utilized to show that a higher dose fails to result in significant improvements in efficacy outcomes. **To address the current research gap, we propose a pilot, open-label, Phase I study in mCRPC and mHSPC patients newly initiated on abiraterone acetate with the primary objective of determining the safety and pharmacodynamic efficacy of 500 mg daily abiraterone acetate, as quantified by changes in serum PSA.**

a. Rationale for the Study Purpose

b. Rationale for Study Population

PK-PD analyses using data from the Phase II trial by Szmulewitz *et al*[6] were used to support the investigation of the reduced 500 mg dose of abiraterone acetate in our proposed trial. Given that the Phase II trial was performed in patients with mCRPC, our study would similarly enrol mCRPC patients who are newly initiated on abiraterone acetate. Nevertheless, considering the expected protracted duration of abiraterone acetate therapy in mHSPC patients, the pharmacoeconomic implications of any potential dose reduction could be substantial, hence justifying the inclusion of mHSPC patients in this pilot trial.

c. Rationale for Study Design

This will be an open-label Phase I trial intended to assess the efficacy of a reduced 500 mg dose of abiraterone acetate.

2. HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

We hypothesize that dose reduction of abiraterone acetate to 500 mg once daily orally would achieve antitumor activity in metastatic castration-resistant prostate cancer (mCRPC) and metastatic hormone-sensitive prostate cancer (mHSPC) patients comparable to standard of care. Nevertheless, in the absence of data demonstrating the efficacy of the alternative 500 mg dose, an exploratory Phase I trial would first be performed.

2.2. Primary Objectives

As a preliminary Phase I trial, the primary objective of the study would be to evaluate the percentage change in prostate specific antigen (PSA) from baseline to 12 weeks. This is aligned with the criterion defined by the Prostate Cancer Clinical Trials Working Group (PCWG2). [15]

2.3. Secondary Objectives

Secondary objectives would be to (**1**) determine the proportion of patients achieving PSA response (≥ 50% reduction in PSA after 12 weeks of therapy), (**2**) evaluate the pharmacokinetics associated with the 500 mg dose of abiraterone acetate, (**3**) investigate the correlation between plasma exposure of abiraterone and CP-I or CP-III in order to support their utility as a biomarker of OATP1B1/1B3 function and (**4**) assess the pharmacodynamic effects of the reduced 500 mg dose on the maximal percentage change in serum androgens (dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEA-S), testosterone, androstenedione) from baseline.

2.4. Potential Risks and benefits:

a. End Points - Efficacy

There may not be direct benefits to the patients participating in the study. The proposed 500 mg dose of abiraterone acetate has not been tested in Phase II or Phase III trials and hence for patients enrolled, a potential risk would be reduced efficacy of treatment. However, if comparable efficacy is achieved at 500

mg, there would be anticipated cost savings to future patients.

b. End Points - Safety

Previous Phase I dose escalation trials investigating the 500 mg dose of abiraterone acetate have largely reported Grade 1 and 2 adverse events, which also include the anticipated toxicities relating to mineralocorticoid excess. Hence, no major safety concerns are expected.[4,16] There may be bruising, pain and discomfort while drawing blood samples.

3. STUDY POPULATION

3.1. List the number of subjects to be enrolled.

This study will be conducted in National University Hospital with a target enrolment of 10 patients. There are no restrictions based on ethnicity for subject recruitment.

3.2. Criteria for Recruitment

3.3. Inclusion Criteria

Eligible patients are required to meet the following inclusion criteria to participate in this study.

1. Histologically confirmed adenocarcinoma prostate
2. Age $\geq$21 years
3. Diagnosis of metastatic castration-resistant prostate cancer (mCRPC) (chemotherapy naïve and chemotherapy pre-treated patients) or metastatic hormone-sensitive prostate cancer (mHSPC)
4. For mCRPC patients, evidence of castration resistance is defined as disease progression despite a testosterone level <50ng/dL (or surgical castration)
5. Progressive disease was defined as either
    a. PSA progression according to Prostate Cancer Working Group (PCWG2) criteria[15]: PSA evidence for progressive prostate cancer consists of a minimum PSA level of at least 2 ng/ml, which has subsequently risen on at least 2 successive occasions, at least 1 week apart
    b. Radiographic progression according to RECIST 1.1 guidelines or
    c. 2 or more new lesions on bone scan
6. Newly initiated on abiraterone acetate therapy
7. Eastern Cooperative Oncology Group (ECOG) performance status grade of 0, 1 or 2.
8. Adequate hematologic, hepatic, and renal function would include:
   -hemoglobin ≥9.0 g/dL independent of transfusions
   -neutrophils ≥1.5 x 10^9/L
   -platelets ≥100 x 10^9/L
   -total bilirubin ≤1.5× upper limit of normal (ULN) [except for subjects with documented Gilbert's disease in which case total bilirubin not to exceed 10× ULN]
   -alanine (ALT) and aspartate (AST) aminotransferase ≤2.5X ULN
   -serum creatinine <1.5× ULN or calculated creatinine clearance ≥30 mL/min
   -serum potassium ≥3.5 mmol/L
9. Ability to provide informed consent

3.4. Exclusion Criteria

Recruited subjects who meet any of the following exclusion criteria at baseline will be excluded from participation.

1) Patients with prior use of enzalutamide or other potent androgen pathway targeted therapies
2) Concurrent therapy with strong inhibitors or inducers of CYP3A4 due to concerning possible drug-drug interactions with abiraterone.
3) Concurrent therapy with strong inhibitors or inducers of OATP transporters (e.g., rifampicin, cyclosporine) due to concerning possible effects on CP-I and CP-III.
4) NYHA class II, NYHA class III, or IV congestive heart failure (any symptomatic heart failure)
5) Uncontrolled hypertension (systolic blood pressure $\geqslant$ 160 mmHg or diastolic BP $\geqslant$ 95 mmHg). Subjects with a history of hypertension are allowed provided blood pressure is controlled by anti-hypertensive treatment
6) Patients who do not voluntarily consent to participate in the study

3.5. Withdrawal Criteria

Enrolled patients would be withdrawn if one or more of the following events occur:

1) PSA progression as defined by the Prostate Cancer Working Group 2 criteria[15] (a) No decline from baseline: ≥25% increase from the baseline value along with an increase in absolute value of ≥2 ng/mL after 12 weeks of treatment (b) Decline from baseline: first PSA increase that is ≥25% and ≥2 ng/mL above the nadir, and which is confirmed by a second value 3 or more weeks later (i.e. a confirmed rising trend)

2) Unacceptable toxicity is encountered

3) Patient withdraws consent

3.6. Subject Replacement

A significant number of patient dropouts is not expected before the 12-week endpoint.

4. TRIAL SCHEDULE

5. STUDY DESIGN

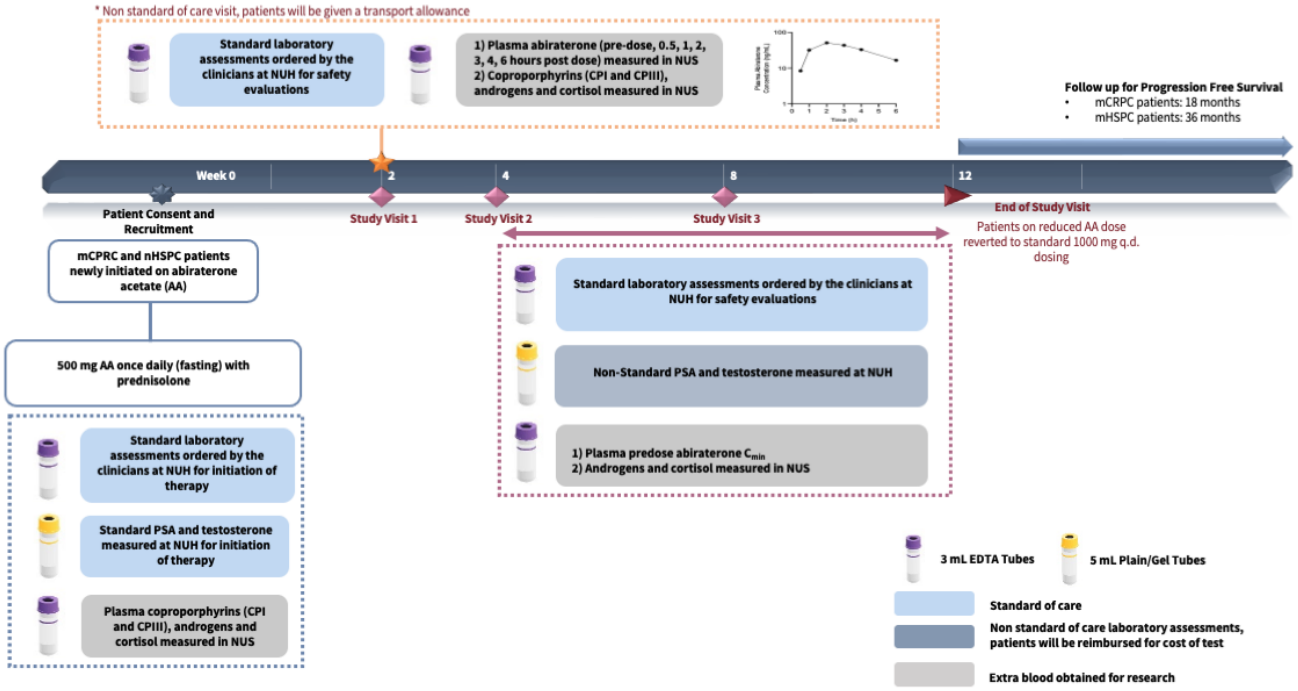

Figure 1. Phase I clinical study design to investigate a reduced dose (500 mg) of abiraterone acetate. Up to 10 mCPRC and mHSPC patients will be recruited and followed over 18 and 36 months respectively for mCRPC and mHSPC patients , with blood collection biweekly for the first month, followed by monthly thereafter for 12 weeks.

5.1. Summary of Study Design

This will be an open label, Phase I study conducted at the National University Hospital. Eligible mCRPC and mHSPC patients newly initiated on abiraterone acetate treatment will be recruited to receive a reduced 500 mg dose of abiraterone acetate plus prednisolone. The study treatment duration will span 12 weeks, after which patients being administered the reduced dose will be reverted to the standard 1000 mg dosing. Follow-up for mCRPC and mHSPC patients will last for 18 and 36 months, respectively, according to reported progression-free survival (PFS) data for respective patient cohort receiving 1000 mg abiraterone acetate. [19-21]

6. METHODS AND ASSESSMENTS

6.1. Randomisation and Blinding

NA

6.2. Contraception and Pregnancy Testing

NA

6.3. Study Visits and Procedures

On day 1 of initiation of abiraterone acetate, informed consent will be obtained by the delegated study personnel for patients who have had preliminary eligibility established based on **clinical assessment by the physician**. Upon enrolment, blood will be withdrawn for laboratory assessments (complete blood count, serum chemistries (including electrolytes, liver function tests)) to confirm eligibility as well as to obtain baseline PSA, plasma CP-I, CP-III, androgen (dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEA-S), testosterone, androstenedione) and plasma cortisol levels.

The following patient information will be collected and stored in the database using data entry forms (demographics, age, race, ECOG performance status, primary tumor data, diagnosis date, baseline staging, gleason grade, primary tumor treatment, systemic therapy administered, date of androgen ablation initiation, dates of anti-androgen therapy, and withdrawal, dates and types of other systemic therapy, dates and types of systemic radiotherapy and sites of metastatic disease).

Upon successful registration by the clinical trial coordinator, patients would be initiated on 500 mg once daily (two 250 mg tablets), plus prednisolone 5 mg twice daily orally for mCRPC and 5mg once daily orally for mHSPC.

a. Study Visits and Procedures

All study visits are **part of routine treatment**, with no additional research-related visits. On study visits will occur **biweekly for a month**, then **monthly up to week 12** (**Figure 1**).

For each visit, the following full set of blood tests will need to be obtained (**Figure 1**):

Standard of care laboratory assessments include complete blood count, serum chemistries (including renal and liver function tests) ordered by the clinicians.

Non-standard of care laboratory assessments would include monthly PSA (by NUH) as well as plasma androgen and cortisol levels, which will be measured in the following manner.

1) PSA and testosterone in NUH
2) Plasma dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEA-S), testosterone, androstenedione and cortisol in NUS
3) Pharmacokinetic measurements would also be performed to facilitate downstream pharmacokinetic-pharmacodynamic correlations. Blood samples would be collected for drug pharmacokinetic (abiraterone) analysis after 2 weeks of therapy (Study Visit 1: before and 0.5, 1, 2, 3, 4 and 6 hours after dosing) and monthly for the first 3 months (before dosing). Blood samples obtained during Study Visit 1 would also be utilized for plasma CP-I and CP-III analysis. To ensure accurate determination of plasma abiraterone C_{min} levels, study visits will be scheduled in the morning prior to abiraterone acetate administration. Blood samples will be collected according to the following schedule for PK studies.

1. **Week 0, Patient Consent and Recruitment**: before ingestion of first abiraterone acetate dose
2. **Week 2, Study Visit 1**: before ingestion of abiraterone acetate, 0.5, 1, 2, 3, 4, 6 hours after dosing
3. **Week 4, Study Visit 2**: before ingestion of abiraterone acetate dose
4. **Week 8, Study Visit 3**: before ingestion of abiraterone acetate dose
5. **Week 12, Study Visit 4/End of Study Visit:** before ingestion of abiraterone acetate dose

Blood Samples for PSA, Plasma Androgens, Plasma Cortisol, Plasma CP-I, CP-III and Pharmacokinetic Analysis: For these samples, approximately 7-10 mL (in total) of blood will be collected into one plain (yellow top) vacutainer tube (for PSA and testosterone measurement at NUH) and one sodium EDTA (lavender top-for plasma) vacutainer tube, for analysis at NUS) (**Figure 1**).

Blood samples for analysis at NUS are processed soon after collection (within 1 hour). Tubes will be

centrifuged (2500 rpm, 20 min, 4ºC) and plasma immediately separated and transferred as two aliquots into storage Eppendorf tubes, Samples will be stored at -80°C until liquid chromatography tandem mass spectrometry (LC/MS/MS) batch analysis.

Plasma Androgen and Cortisol Quantification: Each calibrator, quality control and patient plasma sample was taken through the following sample preparation steps as described by Dong *et al*. [17] Androgens are extracted from human plasma using a liquid–liquid extraction procedure. 200 µL of plasma with spiked analytes is placed in a glass tube, and 2 µL of 2 µg/mL internal standard (^{13}C testosterone and estrone sulfate) working solution is added. After addition of 1 mL MTBE, the samples are vortexed for 1 min. The samples are then centrifuged for 5 min at 4000 rpm at 4°C. The organic layer is transferred to another tube and evaporated to dryness under nitrogen at 40°C. The dried extract is reconstituted with 100 µL of methanol, where 50 µL is subjected to LC/MS/MS analysis for DHEA-S and cortisol using the AB Sciex QTRAP 5500 coupled to the Agilent 1290 UPLC system. The remaining 50 µL aliquot is subjected to oxime derivatization before LC/MS/MS analysis for androgens (testosterone, DHEA and androstenedione).

Plasma Abiraterone Quantification: Each calibrator, quality control and patient serum sample is taken through the following sample preparation steps as described by Alyamani *et al*. [18] Abiraterone is extracted from human plasma using a liquid–liquid extraction procedure. 100 µL of plasma with spiked analytes is placed in a 1.5 mL Eppendorf tube, and 20 µL of 1.25 µg/mL internal standard (d4-abiraterone) working solution is added. The samples are vortexed for 30 s. After addition of 1 mL MTBE, the samples are vortexed for 1 min. The samples are then centrifuged for 5 min at 4000 rpm at 4°C. The organic layer is transferred to another tube and evaporated to dryness under nitrogen at 40°C. The dried extract is then reconstituted with 50 µL of methanol for LC/MS/MS analysis using the AB Sciex QTRAP 5500 coupled to the Agilent 1290 UPLC system.

Plasma CP-I and CP-III Quantification: Each calibrator, quality control and patient serum sample is taken through the following sample preparation steps as described by Kalluri *et al*. [10], CP-I and CP-III are extracted from human plasma using a liquid–liquid extraction procedure with ethyl acetate. 100 µL of plasma with spiked analytes is placed in a 1.5 mL Eppendorf tube, and 20 µL of 1 ng/mL internal standard (CP-I-$^{15}N_4$ or other suitable chemicals) working solution is added. The samples are vortexed for 1 min and then centrifuged for 5 min at 4000 rpm at 4°C. The organic layer is transferred to another tube and evaporated to dryness under nitrogen at 40°C. The dried extract is then reconstituted with 50 µL of methanol for LC/MS/MS analysis using the AB Sciex QTRAP 5500 coupled to the Agilent 1290 UPLC system.

b. Final Study Visit:

At the final study visit on week 12, patients should be instructed to revert to the standard dosing of 1000 mg abiraterone acetate once daily. Blood collection procedures and assessments will correspond to that performed during the prior study visits 2-3.

c. Post Study Follow up and Procedures

All the prescribed follow-up visits are routine clinical visits for patients on abiraterone acetate therapy. Although response is not the primary endpoint of this study, patients will be assessed for progression-free survival (PFS), which is defined as the duration of time from start of treatment to time of progression (by either PSA or RECIST) or death, whichever occurs first. mCRPC and mHSPC study patients will be followed for 18 and 36 months, respectively, according to reported PFS data for respective patient cohort receiving 1000 mg abiraterone acetate. [19-21]

d. Discontinuation Visit and Procedures

For patients who voluntarily withdraw from the study, blood collection procedures and assessments during the discontinuation visit will correspond to that performed during study visits 2-4. Further clinical management of the patients will be determined by physician.

7. TRIAL MATERIALS

7.1. Trial Product (s)

Therapy consists of abiraterone acetate 500 mg once daily (two 250 mg tablets), plus prednisolone 5 mg twice daily orally for mCRPC and 5mg once daily orally for mHSPC.

Abiraterone acetate will be taken first thing in the morning after an overnight fast of a minimum 8 hours. It will be taken at least 2 hours prior to any food intake. The co-administered dose of prednisolone is aligned with the dosing guidance provided in the product insert, but the protocol allows for steroid dose alteration or steroid switching based on the clinician's discretion.

7.2. Storage and Drug Accountability

Treatment will be administered on an outpatient basis continuously. Study drug is commercially available and will be procured by the patients through their pharmacy.

8. SAFETY MEASUREMENTS

8.1. Definitions

Define terms e.g. what would be regarded as UPIRTSO events, Serious adverse events etc.
Include details of the protocol specific reporting, procedures, including the individual responsible for each step (e.g. the Investigator, the medical monitor, etc.), how decisions will be made regarding determining relatedness and grading severity, how reports will be distributed and what follow up are required. Include specific details of reporting procedures for:

- Deaths and life threatening events
- other SAEs
- Other adverse events

8.2. Collecting, Recording and Reporting of "Unanticipated Problems Involving Risk to Subjects or Others" – UPIRTSO events to the NHG Domain Specific Review Boards (DSRB)

UPIRTSO events refers to problems, in general, to include any incident, experience, or outcome (including adverse events) that meets ALL of the following criteria:

1. **Unexpected**
   In terms of nature, severity or frequency of the problem as described in the study documentation (eg: Protocol, Consent documents etc).

2. **Related or possibly related to participation in the research**
   Possibly related means there is a reasonable possibility that the problem may have been caused by the

procedures involved in the research; and

3. **Risk of harm**
   Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Reporting Timeline for UPIRTSO Events to the NHG DSRB.

1. Urgent Reporting: All problems involving local deaths, whether related or not, should be reported immediately – within 24 hours after first knowledge by the NHG investigator.

2. Expedited Reporting: All other problems must be reported as soon as possible but not later than 7 calendar days after first knowledge by the NHG investigator.

8.3. Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to the Health Science Authority (HSA)

1. **For Industry sponsored Trials**
   All SAEs will be reported to HSA according to the HSA Guidance for Industry "Safety Reporting Requirements for Clinical Drug Trials."

2. **For Principal Investigator initiated Trials**
   All SAEs that are unexpected and related to the study drug must be reported to HSA.

"A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that:

- Results in death.
- Is life-threatening (immediate risk of death).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in congenital anomaly/birth defect.
- Is a Medically important event.

Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes, the important medical event should be reported as serious."

All SAEs that are unexpected and related to the study drug will be reported. The investigator is responsible for informing HSA no later than 15 calendar days after first knowledge that the case qualifies for expedited reporting. Follow-information will be actively sought and submitted as it becomes available. For fatal or life-threatening cases, HSA will be notified as soon as possible but no later than 7 calendar days after first knowledge that a case qualifies, followed by a complete report within 8 additional calendar days.

8.4. Safety Monitoring Plan

Safety Measurements

Standard disinfection and procedure for cubital blood collection. Any adverse event will be reported to ethics committee (NHG Domain Specific Review Board).
Collecting, Recording and Reporting of "Unanticipated problems involving Risk to Subjects or others"-UPIRTSO events to the NHG Domain Specific Review Boards (DSRB)

Adverse event will be reported by clinical study coordinator and study investigator. Study investigator will determine the grade and severity of the event and whether it needs to be reported. Study coordinator will assist the PI and study investigators draft UPRITSO in ROAM for PI to review and submit.

8.5. Complaint Handling –

Contact details of PI and DSRB are stated in informed consent form. Recruited participants can call the numbers if they need. DSRB will take necessary action following their rules and regulations. PI will discuss the complaint with study team if he receives any complaints regarding study procedures. He will then follow NUH policy to resolve any issues pertaining to the study.

9. DATA ANALYSIS

9.1. Data Quality Assurance

Data integrity will be reviewed by study team quarterly to ensure that study data is authentic, accurate and complete, and if the data correlates with data collection forms.

9.2. Data Entry and Storage

Documents with patient identifiers like Informed Consent Forms (ICF) and Data Collection Forms (DCF) will be stored in designated locked cabinet in research coordinator room at NUH that is accessible to authorized study personnel only

Electronic data will be stored on in a secured computer that is password-protected. The databases will not contain subject identifiers and the data linking subject identifiers and the subject identification codes will be stored separately.

10. SAMPLE SIZE AND STATISTICAL METHODS

10.1. Determination of Sample Size

The statistical design of Phase I dose escalation studies typically adheres to a Fibonacci 3 + 3 dose escalation scheme to define the maximal tolerated dose (MTD). Subsequently, to better characterize the toxicity profile or identify early signs of efficacy, dose expansion cohorts have increasingly been utilized, where additional patients will be treated at MTD. Simulations have demonstrated how on average; 20 to 35 patients are sufficient to estimate safety and efficacy in expansion cohorts with a homogeneous group of patients.

Given that design of this Phase I trial aims to mimic that of a dose expansion cohort, where the previously tested 500 mg dose of abiraterone acetate is further evaluated for potential efficacy, **10 patients** would first be recruited and monitored for androgen and PSA suppression on the 500 mg dose of AA. An interim analysis would be performed at 12 weeks by the Data Safety Monitoring Board (DSMB), during which safety and efficacy of the proposed dose reduction would be assessed. Subsequently, if significant and positive results are observed on interim analysis, recommendations would then be provided to continue with trial expansion to a target enrolment of **24 subjects**.

10.2. Statistical and Analytical Plans

a. General Considerations

Mean plasma levels between the two treatment arms will be compared using a two-sample t test. To assess the efficacy of the reduced 500 mg dose of abiraterone acetate relative to the standard 1000 mg dose, the

percentage change in serum PSA from baseline to 12 weeks measured in this trial would be compared against historical data. PSA changes at 12 weeks reported in patient cohorts being administered the 1000 mg dose would be pooled from both Phase I[4,16] and Phase II trials and weighted by sample size. A similar approach would be utilized to assess the pharmacodynamic effects of dose reduction on androgen production (dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEA-S), testosterone, androstenedione). Descriptive statistics will be employed to note the maximal percentage change from baseline, as well as the mean, median, and range of percentage change within each dosing group. Group comparisons will subsequently be performed using two-sample t tests or nonparametric, Wilcoxon rank-sum tests.

The proportion of patients achieving PSA decline of ≥50% from baseline after 12 weeks would also be compared against historical data using chi-square or Fisher's exact tests.

For pharmacokinetic data of abiraterone obtained from study visit 1, noncompartmental pharmacokinetic analyses would be performed using WinNonlin (Scientific Consultant, Apex, NC) software. Estimated parameters would include the peak plasma concentrations of abiraterone (C_{max}), time to attain the Cmax (T_{max}), terminal half-life ($T_{1/2}$), total body apparent clearance (CL/F), apparent volume of distribution (V_d), and area under the curve (AUC) from the time of dosing to the last measurable concentration ($AUC_{0\text{-}last}$) and extrapolated to infinity ($AUC_{0\text{-}inf}$). The plasma concentrations of CP-I and CP-III are plotted along the same time-scale and correlated with the plasma concentration of abiraterone versus time profile temporally.

b. Safety Analyses

Safety analysis will be conducted on the full analysis set. All adverse events (AEs) occurring on study will be listed by subject in a data listing. The type of AEs, severity, and incidence rates will be presented in all treated subjects. Comparison of the frequency of adverse events (for example, percentage of patients with worst toxicity grade 2 or higher) will be conducted using chi-square or Fisher exact tests.

c. Interim Analyses

For the initial cohort of 10 patients, interim analyses would be performed by the Data Safety Monitoring Board (DSMB) at 12 weeks to evaluate both safety and efficacy of the reduced 500 dose of AA.

11. ETHICAL CONSIDERATIONS

11.1. Informed Consent

Informed consent will be obtained before performing any study-related procedures. Explanation about the study will be done, by Principal Investigator or Co-investigator who is qualified practitioner and delegated by the PI to obtain informed consent. The consent process will be done by the investigator(s) should the subject decide to participate in the study. Only study team members who have been delegated by the PI can obtain consent from the participants. This will be documented in the Study Responsibility Log. The PI will ensure that the study staff who are delegated to obtain consent have received proper training (e.g. CITI, SGGCP, PCR course) and appropriately qualified to adequately answer questions from potential participants. Where medical opinion is required, a medically trained staff will conduct the informed consent discussion.

Participants will not be approached just prior to a surgical procedure or under sedation. The participant will be given sufficient time to decide whether or not to participate in the research and have the option of further discussing with their family members before making the decision.

Participants will be approached in a quiet and conducive environment where there is privacy and no intrusion. Consent process will be conducted in the respective interview rooms at NUH.

11.2. IRB review

DSRB application number 2020/00258 has been approved. .

11.3. Confidentiality of Data and Patient Records

Documents with patient identifiers like Informed Consent Forms (ICF) and Data Collection Forms (DCF) will be stored in designated locked cabinet in Medical Center (Research Coordinator Room) that are accessible to authorized study personnel only.

Electronic data will be stored on in a secured computer that is password-protected. The databases will not contain subject identifiers and the data linking subject identifiers and the subject identification codes will be stored separately.

12. PUBLICATIONS

Principal Investigator will work with study team in publishing the study data.

13. RETENTION OF TRIAL DOCUMENTS

All study files and source documents will be stored for 6 years according to NUH guidelines.

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