STUDY REPORT SYNOPSIS

A multi-center, non-interventional, prospective cohort study for determination of prevalence and features of HRRm mCRPC

Milestones: Date of first subject - 23 October 2020
Date of last subject – 04 June 2021
Date of last subject last visit - 21 February 2023
Date of data base lock - 16 August 2023
Date of final report - 15 September 2023

Phase of development: Observational study

Sponsor: AstraZeneca

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This study was performed in compliance with Good Clinical Practice (GCP) and Good Pharmacoepidemiology Practice (GPP), including the archiving of essential documents.

The Investigator was perform the Observational Study in accordance with the regulations and guidelines governing medical practice and ethics in the country of the Observational Study and in accordance with currently acceptable techniques and know-how. The final protocol of the Observational Study, including the final version of the Subject Informed Consent Form, were approved and given a favourable opinion in writing by the Ethics Committee.

Background/rationale:

Prostate cancer is the second most common cancer in men, with 44,706 new cases diagnosed in Russia in 2019. In Russia in 2019, prostate cancer in 39.4% of patients was detected in stages III–IV, which requires combined and complex treatment, including hormone therapy. At the same time, mortality in the first year after the diagnosis was made was 7.3%[1]. Prostate cancer is the fifth leading cause of cancer death in men in Russia, with 13,007 deaths in 2018 [3].

Interpatient genomic heterogeneity in prostate cancer is well recognized. However, molecular stratification of prostate cancer to guide treatment selection based on predictive genomic biomarkers remains an unmet clinical need. Recent genomic studies have elucidated this interpatient heterogeneity, identifying multiple potentially actionable alterations which are now being evaluated in clinical trials. These studies have also described differences in the genomic landscape of the different clinical states of the disease (localized vs metastatic) [4–6]. Loss-of-function events in TP53, RB1, PTEN, and DNA damage repair (DDR) genes are more common in of metastatic castration-resistant prostate cancer (mCRPC) compared with nonmetastatic prostate cancer cohorts. Homologous recombination repair (HRR) is a "non-error prone" repair mechanism critical to restore potentially lethal deoxynucleic acid (DNA) double strand breaks (DSBs) and, therefore, provide a fundamental pathway associated with DDR [7].

A proportion of prostate tumours have loss-of-function mutations in candidate genes involved in HRR of DNA; this pathway is critical for DNA repair [8]. Bono et al. (2019) reported that HRR alteration was detected in 778 (27.9%) screened patients with a biomarker status reported [9]. DDR defects were found in 23% of mCRPC patients. Increasing evidence supports the fact that mutations that disrupt homologous recombination repair mutations (HRRm) have been shown to be associated with the aggressive clinical behavior of prostate cancer and cancer-specific mortality [10–12].

Determination of gene mutations involved in DNA repair by homologous recombination is also important for the possibility of prescribing targeted therapy with PARP inhibitors. Currently, one of these drugs is olaparib, which is registered in Russia and is recommended for monotherapy of mCRPC with germline or somatic mutations of genes involved in DNA repair by homologous recombination in patients with disease progression after therapy with new hormonal drugs [21, 22].

Molecular testing for detection of HRRm in patients with mCRPC is not widespread in Russia. Limited data is available on the population of patients with mCRPC and HRR gene mutations in Russia. These data will be significant in the development of molecular testing. Determination of the prevalence of HHRm in the population of mCRPC patients, as well as the possibility of using targeted therapy for these

types of mutations, will help to introduce the widespread use of HRRm testing in patients with mCRPC in Russia.

Objectives:

- To evaluate prevalence of HRRm in mCRPC patients;
- To define prevalence of different gene alterations associated with HRRm in patients with mCRPC;
- To describe differences in demographic, treatment pattern, clinical characteristics and outcomes between patients with HRRm and HRRwt mCRPC;
- To investigate concordance between the testing of tumor tissue and circulating tumor DNA (ctDNA) in plasma to identify deleterious alterations in 14 HRR genes in patients with metastatic castration-resistant prostate cancer (mCRPC) screened in the ADAM study

Study design:

This study was local, multi-center, prospective, cohort study to collect real world data related mCRPC patients, prevalence of HRRm and to assess possible influence of HRRm on treatment outcomes. Treatment assignment was done according to the current practice.

Data source:

For testing archival samples (formalin fixed and paraffin embedded [FFPE]) from primary tumor or metastatic lesion were used [1]. 14 HRR genes (BRCA1, BRCA2, ATM, BRIP1, BARD1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D and RAD54L) were analyzed using NGS (Next Generation Sequencing) in dedicated central laboratory facilities. Choice of laboratory for each center was made based on logistical proximity. Each NGS laboratory determined and reported clinical significance of alterations found using database search or other predictors to classify variants as deleterious or suspected deleterious. VUS was reported separately. Benign variants were not reported in this study.

[1] The order of preference of source of samples: radical prostatectomy, transurethral resection of the prostate, excisional biopsy, incisional biopsy, needle biopsy.

During routine disease assessment including blood tests, additional blood samples were taken for circulating tumor DNA analysis. 14 HRR genes (BRCA1, BRCA2, ATM, BRIP1, BARD1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D and RAD54L) were analyzed using NGS (Next Generation Sequencing) in dedicated central laboratory.

All clinical and demographic data for patients with finished therapy was collected in prospective or retrospective (where applicable) manner during the study visits. The second visit took place when the disease progression or death occurs or in a year after the first visit whichever happens first. The second visit might occur earlier or later then one year from the first visit – it was depend on the date of the planned routine patient visit. If a patient past second visit and remained under medical supervision in a clinic by the time this amendment was approved, blood samples might be taken to evaluate circulating tumor DNA under routine evaluation of disease progression, which includes blood sampling, on condition that such patient has provided signed informed consent form. Data was entered in the eCRF. The site investigator was responsible for ensuring that all required data was collected and entered into the eCRF with the involvement of clinical research organization.

Study population:

Study population was consist of mCRPC patients with available medical history and FFPE specimen of archival tissue from primary tumor or metastatic lesion and who received at least one line of therapy for mCRPC or currently was receiving this therapy. There were no limits of the count of therapy lines. It was estimated that approximately 300 patients were enrolled in the first stage. After interim analysis total number of the patients could be increased, depending on the number of NGS failures. It was estimated that approximately 30 sites in total could participate in the study. The were 333 patinets screened and 329 patients included in 20 sites in the study. There were 2 cohorts of patients: HRRm and homologous recombination repair wild-type (HRRwt) to reveal possible differences in treatment pattern, demographic and clinical characteristics and outcomes in mCRPC patients with and without HRRm.

Inclusion criteria:

- Male 18 years age or older;
- Provision of written informed consent;
- Histologically confirmed diagnosis of prostate cancer;

- Documented evidence of metastatic castration resistant prostate cancer (mCRPC);
- Patients who were on the first line therapy or already received one line of therapy due to mCRPC previously(no limits of the count of therapy lines);
- Availability of archival FFPE tissue from primary prostate tumor or metastatic lesion;
- Availability of medical history (e.g. out-patient medical records or disease histories for hospitalized patients)

Withdrawal criteria:

- Patients participating in clinical studies

Statistical Analysis:

One interim analysis was performed after all subjects were included in the study. Final analysis was performed after all subjects passed through all the planned follow-up visits.

This study was not formally powered to test any hypothesis or identify any significant difference in subgroups or cohorts, thus, all data was presented descriptively, both in general sample and by cohort using the following descriptive statistics:

- for quantitative data: number of valid observations, mean, standard deviation, median, interquartile range, minimum and maximum.
- for qualitative (categorical) data: number of valid observations, number of observations with the corresponding category (absolute frequency) and % (relative frequency).

Given the observational nature of the study and the presence of possible confounders, there was a need to account for multiple factors when describing the differences in demographics, treatment patterns, clinical characteristics and outcomes between patients with HRRm and HRRwt mCRPC.

In the absence of significant factors (influencing) of the logistic regression in relation to the assessment of mutations presence, comparisons between groups were additionally carried out for all variables included in the logistic regression model. Comparison of patient cohorts (HRRm and HRRwt) was performed using Fisher's exact test/$\chi 2$ test or Student's t-test/Wilcoxon test, depending on the data type and distribution.

Also Cox regression tables that described information about time to death of the patient, the ratio of risks (Hazard Ratio, HR) of death in patient cohorts were presented. The model included various factors (age, medical history, etc.). At the same time, for each factor included in the model, a point estimate for HR and a 95% confidence interval for HR were given. Time to outcome (death) was calculated from the start of therapy for mCRPC. To assess the survival function, the method of constructing Kaplan-Meier curves was applied in relation to overall survival and progression-free survival within the first and second lines of therapy, where the HRRm status (presence or absence of mutations associated with HRRm) of the patient acts as a condition. The result of the evaluation is pictures of the graphs, which was given along with the Cox regression tables. In the course of this method, a data censoring procedure was carried out, which amended the initial data used to construct the curves, taking into account subjects who dropped out of the study ahead of time and subjects for whom the indicated event did not occur.

Factors taken into account when constructing regressions are:

- HRRm status (only for Cox regression);
- Age;
- The time interval from the initial diagnosis of prostate cancer to the diagnosis of mCRPC;
- The stage of the primary disease;
- The sum of points on the Gleason scale;
- The presence of metastases in the visceral organs;
- The presence of metastases in the lymph nodes;
- 1-year overall survival after starting therapy for mCRPC;
- 2-year overall survival after starting therapy for mCRPC;
- Progression-free survival of the 1st line of therapy for mCRPC;
- Progression-free survival of the 2nd line of therapy for mCRPC;
- Availability of Taxane group drugs as part of the 1st line of therapy for mCRPC;

- Presence of the following single drugs in first-line therapy for mCRPC: enzalutamide, abiraterone, olaparib;
- The presence of progression during the 1st line of therapy;
- Name of the gene with the HRR mutation (BRCA1, BRCA 2, ATM) (for Cox regression only).

Overall, statistical goal of the study was to adequately describe the data on therapy durations, treatment patterns and other aspects of real-world evidence in patients with mCRPC.

Results: The analysis of the primary endpoint demonstrated that the number of patients with a positive mutation status in the study sample was 59 (17.93%) patients.
The analysis of the additional efficacy endpoints demonstrated the following results:

- Of the 66 samples analyzed: 59 samples were concordant (57 patients with a negative tumor tissue and plasma mutation status and 2 patients with a positive tumor tissue and plasma mutation status) and 7 samples were discordant.
- In 4 patients, the positive mutation status in tumor tissue samples changed to a negative status in blood plasma; in 3 patients, the negative mutation status in tumor tissue samples changed to a positive status in blood plasma.
- Thus, taking into account the validation work and the resolution of discordant test results, 59 (17.93%) patients had a positive mutation status, of which 56 (17.02%) patients had a positive mutation status in tumor tissue samples and 5 (7.58%) patients had a positive mutation status. mutations in blood plasma.

When analyzing the relationship between the presence of mutations and various factors in patients with mCRPC, a statistically significant difference was revealed in the factor of median progression-free survival after first-line therapy for mCRPC. In the HRRwt cohort it was 20.80 months compared to the HRRm cohort 12.8 months.
For other factors (age, family history of cancer, stage of the disease, Gleason score, time to diagnosis of mCRPC, presence of metastases to visceral organs at the time of diagnosis of mCRPC, presence of lymph node metastases at the time diagnosis of mCRPC, 1-year overall survival after starting therapy for mCRPC, 19-month overall survival after starting therapy for mCRPC, 2-year overall survival after starting therapy for mCRPC, median progression-free survival after the second-line therapy for mCRPC) no statistically significant differences were found.

When analyzing the influence of various factors on the occurrence of death in patients with mCRPC, it was revealed that progression-free survival after 1 and 2 lines by 1 month statistically significantly reduces the risk of death.

When analyzing the safety profile it was revealed that in total, 12 AEs were registered in the study in 9 (2.74%) patients, of which there were no mild AEs; AEs of moderate severity were reported in 5 (1.52%) patients (6 AEs in total); AEs of severe severity - in 5 (1.52%) patients (total 6 AEs); and 3 (0.91%) patients had fatal AEs.

A total of 6 SAEs were reported in 3 (0.91%) patients.

3 SAEs with a fatal outcome were registered: 2 AEs - Coronavirus infection COVID-19 and 1 AE - Acute cerebrovascular accident.

Conclusion: Based on the data analysis, we can say that molecular testing to identify HRRm in patients with mCRPC for the purpose of using targeted therapy should be introduced in Russia.

Publications: None