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Name of Sponsor/Company: Ruprecht-Karls-Universität Heidelberg, Medizinische Fakultät, vertreten durch das Universitätsklinikum Heidelberg Germany		**Sponsor-Code of Study: Mesi-Strat-Woo AFmu-396_2018_ Cuno 517607**	*(For National Authority Use only)*
Name of (Finished) Product: Arimidex		**Name of Active Ingredient: Anastrazol**	
EudraCT-No.: 2018-000112-21	**Vorlage-No.: 4042889**	**IEC Antrags-No.: AFmo-396/2018**	

☒ **End of Trial Report** ☐ **Annual Safety Report**

SYNOPSIS STAND 23.1.2021

Title of Study:
Prospective window of opportunity trial - 3 weeks' neoadjuvant Anastrozole in postmenopausal women with estrogen receptor positive (ER+) breast cancer

Date of Approval / Vote:
BfArM: 3.9.2018 Ethics Committee: 25.09.2018

Amendments:
There were no amendments after study start. The study started and ended with protocol V1.2 dated 20.08.2018 (approved by CA on 03.09.2018 and by EC on 25.09.2018).

Investigators:
Principle Investigator:
Prof. Dr. Sarah Schott

Deputy Investigator:
Prof. Dr. Andreas Schneeweiss

Study Centre:
Universitätsfrauenklinik Heidelberg
Im Neuenheimer Feld 440
69120 Heidelberg, Germany

Publication (reference): none

Study period: **(date of first enrolment)** **(date of last completed)**	 20.12.2018 17.01.2020	**Phase of development:**	**Phase I/II**

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Objectives:

Primary

Comparison of kynurenine concentration before and after 21 days' treatment with Anastrozole.

Secondary

- Association between MESI networks & marker panels and biological/pathological responses;
- Percent change in Ki67 expression from baseline to the surgical tissue 3 weeks after the start of treatment with Anastrozole;
- Effect of Anastrozole on peripheral blood mononuclear cells (PBMC);
- Clinical response of the breast tumour to therapy as assessed by histopathology;
  response of the breast tumour as evaluated by ultrasound assessment before surgery and after 3 weeks of treatment with Anastrozole.

Methodology:

Monocentre, open label, non-controlled, non-randomised, prospective neoadjuvant trial, Phase I/II was taking place at the University Heidelberg. (European Economic Area)
In brief, at the beginning of the study blood, urine and tumor tissue from first diagnosed ER+ BC patients with indication for an adjuvant therapy with an aromatase inhibitor was collected. Then a neoadjuvant treatment with Anastrozol for 3 weeks was applied. Afterwards blood, urine and tumor tissue were collected once again and the patients underwent routine surgery. 2-3 weeks after surgery the trial ended with a last collection of blood and urine.

In Detail:

Study medication

- General Information

The study medication was received from the local pharmacy and stored as defined by the manufacturer instructions.

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- Characterization of the study medication

Proprietary name:	ARIMIDEX 1mg
International Nonproprietary Name (INN):	Anastrozole
ATC code, if officially registered:	L02BG03
Manufacturer:	AstraZeneca
Pharmaceutical formulation:	tablets
Mode of administration:	oral
Storage instructions:	No special storage instruction, room temperature

- Packaging and Labeling

There was no trial-specific labeling, because only drugs approved for human use in Germany were used and administered in unchanged form (according to §5 of GCP-V (8)). No individual packages for subjects were planned.

- Supplies and Drug Accountability

The investigator bought the study medication in the local pharmacy and ensured that the medication was stored safely and correctly in
accordance with manufacturer's instructions. The investigator documented the distribution and return of the trial medication to the patient with the date, recording the quantity distributed and used on the forms provided for this purpose as documented with a photo. The trial medication was handed out to the patient in portions of 30 tablets, which corresponded to the package size and suffices for therapy during at least 21 days. The site monitor periodically checked the photos of the trial medication blisters per patient held by the investigator to ensure the correct accountability of all trial medication used. It was assured that a final report of the drug accountability was prepared and maintained by the investigator.

- Administration of study medication

All patients who seemed suitable for study participation and took part in the screening received a screening number. At the end of the screening phase the eligibility of the patient was assessed finally. When the patient was included in the study (all inclusion criteria fit and none of the exclusion criteria), she was given a consecutive patient number. Patients withdrawn from the study retained their number. New patients were always allocated a new enrolment number.

- Dosage Schedule

The study medication was given only to patients who had consented to study participation. They received the standard treatment application of 1 mg. Maximum duration of treatment in the study: 21 days Maximum dose allowed per day: 1 mg

- Compliance

Trial medication was dispensed to the subjects by the investigators. Subjects were instructed to document the intake of study drug in a patient diary and to bring all (remaining) trial medication back to the trial site at every visit for drug accountability. Compliance was
recorded by counting the trial medication (tablets) returned prior to operation and it was documented by a photo of the blisters. The person responsible for the trial medication calculated the amount of used trial medication by counting the unused trial medication and
compared it with the documentation of administered medication.
The results were systematically documented on appropriate forms. Details were recorded in the eCRF.

- Prior and Concomitant Diseases

Relevant additional diseases present at the time of informed consent were regarded as concomitant diseases and documented on the appropriate pages of the electronic case report form (eCRF). Included conditions that were seasonal, cyclic, or intermittent (e.g. seasonal allergies; intermittent headache). Abnormalities which appeared for the first time or worsen (intensity, frequency) during the trial were adverse events (AEs) and documented on the appropriate pages of the eCRF.

- Prior and Concomitant Medication

The medication used by the patients was recorded in the clinical records and documented in the eCRF. There were no medications excluded.

- Description of trial visits

Study period for an individual patient consisted of a 3 weeks treatment period. Study period was up to 9 weeks for each patient.

- Screening Visit (V1)

The screening visit checked the inclusion/ exclusion criteria. Written informed consent was gained. Physical examination was performed and the medical history checked. Routine diagnostic examinations, for example sonography, were performed when breast cancer was suspected. If written informed consent was provided, it was checked if fresh frozen tissue was available and if tissue is stored in the local NCT Biobank. If no tissue was available an optional core biopsy was offered and the tissue was frozen for further analysis. Concomitant medication, including vitamins and dietary supplements, was recorded. Routine blood draw wasperformed as well as study blood. Furthermore, urine was checked for creatine and study urine was collected.

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- Visit (V2)

Before the therapy started blood and urine were collected for the study and routine checks and Vital signs checked. They got the study medication Anastrozole. Patients were instructed how to take the medication and how to document the intake of study drug and other medications, e.g. vitamins and dietary supplements, in a patient diary. Concomitant medication, including vitamins and dietary supplements, were recorded.

- Visit (V3)

The therapy ended and the intake of the medication was controlled by checking the blisters. The intake of medication is also documented by a photo of the blisters, which were returned by the patients to the study center. Study as well as the routine blood and urine were collected, Vital signs checked. Concomitant medication, including vitamins and dietary supplements, were recorded.

- Operation (V4)

One to three days before operation blood works for the operation as routine blood as well as study blood were collected. Furthermore, urine for study purposes and routine urine was collected. The operation sample received core cut biopsies to obtain fresh tissue as soon as the lumpectomy was performed and the tissue was outside the body before it was transferred for pathological assessment. Concomitant medication was recorded.

- End of Study Visit (EOS)

After operation the routine clinical follow up visit was performed 2-3 weeks after operation. Blood and urine (routine and study) was collected. Vital signs were checked. The final medical records were checked including the pathology record and the medical records from the hospital stay records and recorded in the eCRF. Concomitant medication was recorded.

- Planned treatment after study end

The treatment of patients was followed after study according to the current standard of care as outlined in generally accepted guidelines and in respect to the postoperative tumor board recommendation.

- Adverse events

Adverse events were continuously monitored with each visit.

Number of Volunteers (planned and analysed):

Planned: 100 screened and 50 to be enrolled

Enrolled at study termination: 25.02.2020: n = 22

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Diagnosis and main criteria for inclusion:
C50.9, Breast Cancer Frist diagnose

- Ability of subject to understand character and individual consequences of clinical trial
- Postmenopausal women; postmenopausal status is defined either by:
  - Prior bilateral oophorectomy
  - Amenorrhea for 12 or more months
- A current first diagnosis of estrogen receptor (ER)+ breast cancer
- Breast cancers with a tumour size >/= cT1c.
- Not legally incapacitated
- Written informed consent (must be available before enrolment in the trial)
- Only women that receive an aromatase inhibitor as their standard treatment after surgery

Test product (IMP being tested), trade name, MA holder, MA number, dose and mode of administration, batch number(s):
Anastrozole, ARIMIDEX 1 mg, tablet (AstraZeneca) per os, once daily for 21 day.
Two batch numbers were used: NW899; PG355;
CAS number 120511-73-1;
EV Substance Code SUB05502MIG

Reference therapy (IMP used a comparator), trade name, MA holder, MA number dose and mode of administration: Not applicable

Duration of treatment: 21 days

Criteria for evaluation: (efficacy, safety)

Efficacy

Multi-omics measurements were planned to explore *metabolic and signalling (MESI)* networks in tissue, PBMC, and biological fluids upon Anastrozole treatment. The tissue as well as the blood and urine samples were to be analysed at the level of DNA, RNA, protein, and metabolites. DNA was planned to be analysed by sequencing and methylation assays. RNA expression was intended to be examined using RNASeq or microarrays, and protein expression was intended to be examined by proteomics. Furthermore, ER, CDK4/6, PARP and Her2 signalling assessment was planned. For proteogenomics, de novo RNASeq data were to be used to prepare a sample-specific protein sequence database containing sample-specific protein forms such as single amino acid variants, differentially spliced isoforms, insertions, deletions, and translocations.

Metabolites were planned to be measured by mass spectrometry (MS), high performance liquid chromatography (HPLC), or nuclear magnetic resonance (NMR). Additional metabolic pathways were planned depending on additional results from the MESI-STRAT consortium but were not performed. The results of the MESI marker panels measured in study blood and urine samples, i.e. signalling activity and metabolites from Multi-OMICS measurements, were to be determined after completion of the clinical activities. Due to early study termination, these analyses were not performed.

Ki67 expression

Ki67 was assessed in the tissue samples by immunohistochemistry. Tumour tissue collected through a core biopsy at baseline (outside of this study but available in the NCT tissue bank) and after 21 days of Anastrozole (visit 4, routine surgery) were used to determine Ki67 expression. Ki67 expression is defined as the percent of cells staining positive by validated and routine pathological assay.

Analysis of PBMC

PMBCs were isolated directly from the blood and analysed directly or frozen until further analysis. The PBMC were to be cultivated and analysed by MULTI-OMICS analyses (see above). In addition, flow cytometry and functional assays such as proliferation assays, migration assays, mixed leukocyte reactions, and tumour cell killing assays were also planned.

Evaluation by routine histopathology

Routine histopathological assessment of the tissue after 3 weeks of Anastrozole was performed and compared to baseline.

Assessment of response by imaging

Ultrasound was assessed routinely prior to operation. Sonographic response (assessed at routine examination; the study was planned to be evaluated independently as the percentage of participants with CR, PR, PD, and SD). A responder is defined as any participant who exhibits a CR or PR.

CR (complete response) is the disappearance of all target lesions.

PR (partial response) is a 30% decrease in the sum of target lesion diameters, taking the sum of baseline diameters as the reference.

PD (progressive disease) is a 20% increase in the sum of target lesion diameters taking the smallest sum and the appearance of 1 or more new lesions as the reference.

SD (stable disease) no change of target lesions

Safety:

Anastrozole is a standard of care therapy for postmenopausal women. For ethical reasons, only women who would receive an aromatase inhibitor after surgery were approached to be included in the trial. Therefore, only the sequence of aromatase inhibitor application will be altered, as Anastrozole is

given for 3 weeks before routine surgery as opposed to its regular adjuvant application. Anastrozole is well known and approved by German regulatory authorities for the treatment of postmenopausal ER+ BC patients. All relevant safety information is available and can be found in the Summary of Product Characteristics (SmPC).

Adverse Events: Adverse events were asked about at each contact between the study team and subject. Furthermore, all relevant findings from physical examinations, vital signs, and clinical chemistry were documented as adverse events. Adverse events were reported with subject ID, start and end date, description, grading, seriousness, relationship, action taken, and outcome.

Vital signs: Vital signs (pulse rate, systolic and diastolic blood pressure, and body temperature) determined on predefined study days were documented as numerical values in the appropriate eCRF sections. Furthermore, vital signs were to be recorded at any time, if medically imperative for clarification of clinical signs and symptoms. Pathological and clinically relevant findings were to be documented as adverse events/ serious adverse events.

Clinical chemistry: The following parameters were determined on the predefined study days:

Clinical chemistry: total protein, albumin, glucose, creatinine, urea, bilirubin, ASAT, ALAT, GGT, LDH, AP, CRP.

Haematology: leukocytes, neutrophils, eosinophils, basophiles, lymphocytes, monocytes, erythrocytes, thrombocytes, haematocrit, haemoglobin.

Clotting: aPTT, INR.

Urine: Protein-creatinine ratio, creatinine

After collection, the samples were immediately delivered to the central laboratory for examination. All parameters were documented in the appropriate eCRF sections. Pathological and clinically relevant findings were documented as adverse events/ serious adverse events.

Statistical methods:

The clinical results are of a descriptive nature only. The analysis of mutiomics were not performed.

Summary – Conclusions:

Premature closure of the clinical trial on 25.02.2020. The clinical management stopped the recruitment and the continuation of the clinical trial due to organisational matters.

In total, 22 women with primary estrogen receptor positive breast cancer participated in this study between December 2019 and February 2020. The mean age was 70 (range 56-83 years). 5 women did not finish the study either due to withdrawal (n=3), exclusion criteria (n=1), or early study termination (n=1),

Efficacy Results:

Multiomics / Analysis of PBMC

The efficacy measures regarding the mutiomics analysis were not performed due to earlier study termination. Further, no analysis of PBMC was performed. The marker-set of MESI-STRAT has not yet been defined.

Ki67 expression

The pre- and postoperative Ki67 measurements were routinely taken among n=3 women. The results of the analysed specimens include Ki67 immunohistochemistry staining of the core cut biopsy sample as well as the surgical tissue given in percentages. In two women, the Ki67 expression remained the same and in one the Ki67 staining changed from 30% to 40%. All other surgical samples were not investigated routinely by immunohistochemistry in a clinical work up. Additional study-specific staining of available surgical tumour specimens was not performed due to early study termination.

Evaluation by routine histopathology

All surgical samples were histologically assessed as part of standard of care. Complete pathological response was not noted.

Assessment of response by imaging

Ultrasound imaging was performed at screening and prior to operation to assess the size and response of the breast cancer. In general, the average tumour size pre and post treatment was 19.8 mm (8-41 mm). At screening the average size was 19.9 mm; after three weeks of treatment, the average size was 19.7 mm. Overall 10 tumours decreased in size (range 0 to 9 mm) and five tumours increased in size (1 to 13 mm), two remained stable in diameter, 5 women did not finish the study.

8 women showed a partial response (PR) with a 30% decrease in the sum of the diameters of the targeted lesion on imaging, two showed progressive disease (PD) with a 20% increase in the sum of the diameters of the targeted lesion, and seven stable disease (SD) as evaluated using the RECIST criteria.

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Safety Results:

1.3 Intake of trial medication

Number of days from first to last intake	*If premature end: reason*	*Number of patients*
22 days		3
21 days		14
5 days	Intolerable adverse events	1
No intake	Patient's request to withdraw	3
No intake	closure study	1

Twelve women reported adverse events during the trail. These included backpain, cold (2x), depression, diarrhoea (2x), fatigue (2x), feeling of thoracic pressure, headache (2x), hot flush (4x), insomnia, loss of appetite, nausea (2x), pain, rash/acne, sensory disturbance (2x), stomatitis, vaginal dryness, and vomiting.
The women with intolerable adverse events showed headaches, vomiting, nausea, stomatitis, and rash/acne. The causal relationship was probable, and therefore the drug was withdrawn and treatment was stopped after the intake of 5 tablets. All symptoms resolved.

Conclusion:
Premature closure of the clinical trial on 25.02.2020 did not allow conclusions regarding the assessable endpoints. Therefore, the results are of a descriptive nature only. A larger study cohort is needed to answer the questions. There are several multicentre studies underway with similar approaches.

Date of report: 19th January 2021