OBSERVATIONAL STUDY REPORT SYNOPSIS

FLABRA: Frontline approach for BRCA testing in OC treatment naïve population. A LATIN AMERICA Epidemiologic Study.

Milestones:	**Milestones**	**Date**
	Study Design	Aug-2016
	Final Protocol V1	18-Oct-2016
	First Subject In (FSI)	22-Dec-2016
	First Patient In (FPI)	26-Dec-2016
	Final Protocol V2	01-Mar-2017
	Final Protocol V3	13-Jul-2017
	Last Patient In (LPI)	31-May-2019
	Last Site Closed (LSC)	July-2020 (expected)
	Data Base Lock (DBL)	20-Aug-2019
	Analytic Datasets	17-Dec-2019
	Final Report	April-2020
	Final Manuscript	April-2020
Phase of development:	Not Applicable - Observational study	
Sponsor:	AstraZeneca	
Author:	Regional Coordination of the Study AstraZeneca - Argentina	

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

Study Centers:

The study was performed in 40 research centres distributed in multiple Latin American countries, including Argentina, Brazil, Colombia, Peru, Mexico and Panama.

Background/Rationale:

The majority of ovarian cancers are sporadic, but it is estimated that 17% of OC patients have hereditary mutations in Breast Cancer Susceptibility genes 1 or 2 (BRCA1 or BRCA2, respectively). Mutation carriers in BRCA 1 have a life time risk of 50-85% of developing breast

cancer, often of early onset; 15-45% risk of developing ovarian cancer, and it is associates with an increased risk of other cancers like prostate and colon. On the other hand, BRCA 2 mutation carriers have a 50-85% life-time risk of breast cancer, 6% of breast cancer in male carriers and a 10-20% risk of having ovarian cancer during their life-time. It is also associated with a greater risk for pancreatic, prostate and laryngeal cancers.

In ovarian cancer (OC) approximately 30% of BRCA1/2 mutation carriers do not have a family history and 25% of mutation carriers are older than 60 years of age. There is also no specific histologic type linked to this BRCA mutated OC, although high grade serous type is the most common with almost no association with mucinous type. BRCA mutated OC has distinct clinical characteristics like: better prognosis (increased overall survival compared to non BRCA mutated cancers), higher incidence of visceral metastasis, and increased sensibility to different chemotherapeutic agents platinum and non-platinum and more recently to DNA repair targeting agents like PARP inhibitors (Olaparib, rucaparib, niraparib).

In many areas of the world, the prevalence of BRCA mutations has not been well characterized, nor has the type of gBRCAm or sBRCAm been characterized. Latin American population is a paradigm of poly-ethnicity, with a mixture of native, Spanish, Italian, Portuguese and Jewish ancestries. In this sense, FLABRA is a cross-sectional, multi-centre, epidemiological observational study that was designed to evaluate the prevalence of BRCA mutations in newly diagnosed high grade serous ovarian cancer patients in Latin America. This study was designed in the context of SOLO 1 study conductions, understanding the need of having accurate information about prevalence of germline and somatic mutations in the frontline setting.

Objectives:

Primary Objective:

To estimate the prevalence of BRCA 1 and BRCA 2 mutations identified in tumor samples in newly diagnosed high grade serous ovarian cancer patients in LATAM population.

Secondary Objectives:

1. To estimate the prevalence of BRCA 1 and 2 mutations identified in tumor samples in newly diagnosed high grade serous ovarian cancer patients in LATAM ethnic sub-groups.

2. To estimate the prevalence of gBRCAm in newly diagnosed high grade serous ovarian cancer patients who have a BRCA mutation identified in the tumor sample, in LATAM population, and by ethnic sub-groups.

3. To collect information about stage at diagnosis, outcome of primary surgery, first line treatment indication.

Methods:

Study design:

FLABRA is a cross-sectional, multicenter, epidemiological observational study designed to evaluate the prevalence of BRCA mutations in newly diagnosed high grade serous ovarian cancer patients across understudied ethnic groups in Latin America. The study was performed between December, 2016 and August 2019, in 40 research centres distributed in multiple Latin American countries, including Argentina, Brazil, Colombia, Peru, Mexico and Panama.

Data Source(s):

During the screening period, data on patient characteristics was collected by review of the patient's medical records and by patient interview. The data that was extracted from the patient's medical records were: demographics data; past medical history; date of diagnosis; extent of disease, as determined by surgical and radiological reports, where applicable; pathological and histological characteristics of disease at diagnosis (histologic cell type and grading); stage of disease as defined by FIGO (Federation of Gynecology and Obstetrics); treatment history. On the other hand, the data that was collected by patient interview were: ethnicity (Native American, Afro-Caribbean, European, and its combinations, i.e. "Mestizos", "Mulatos", and "Zambos") as determined by self-reported ethnicity, and birth country of patient's grandparents, parents, and/or self, whichever generation is furthest removed from the patient; cancer family history. In addition, tumor samples (at least 5 10-µm fragments) from eligible research participants were ordered from the pathology laboratory and used for the BRCA mutation test. For the research participants in which a BRCA mutation was identified in the tumor, at the time of the return visit, an additional blood test was requested to investigate whether these research participants had a germline BRCA mutation.

During the Devolution Visit 1, patients received BRCA results and genetic counseling according to the local SOC (Standard Of Care). In addition, patients were interviewed by study personnel to obtain data on patient ethnicity and cancer family history. All other patient data was obtained through extraction from the patient's medical records. In case of non-mutated patients, this was the last visit of the study. But for patients who had *BRCA* mutation identified in tumor, a blood sample was used for germline BRCA testing. These patients had an additional visit to receive germline BRCA testing results. No other study-related visits were required.

Study Population:

A total number of 472 patients from 40 research centres across 6 different LATAM countries were enrolled. All patients met all inclusion criteria and none of the exclusion criteria mentioned below:

Inclusion criteria:

1. Be able and willing to sign the informed consent form (ICF);
2. Be ≥ 18 years of age;
3. Have histologically confirmed new diagnosis of Federation of Gynecology and Obstetrics (FIGO) stage III or IV high grade serous ovarian, primary peritoneal, or fallopian tube cancer made by one or more of the following:
    - standard staging laparotomy including bilateral salpingo-oophorectomy, omentectomy, and lymph node sampling and debulking and/or;

- surgical resection and radiographic evidence consistent with Stage III or IV ovarian cancer;
- paracentesis or biopsy with radiographic evidence consistent with Stage III or IV ovarian cancer (normal appearing pancreas, liver, and gastrointestinal tract).

4. Have availability of a minimum of twenty 10-μm sections of paraffin-embedded archived tumor tissue (preferred) or, if it is not possible, a paraffin-embebed block;
5. Have a diagnosis that is within 120 days of informed consent.

Exclusion criteria:

1. Have a diagnosis of any severe acute or chronic medical or psychiatric conditions that may increase the risk associated with study participation or may interfere with the interpretation of the study results and, in the judgement of the Investigator, would make the patient inappropriate for enrollment in this study;
2. Be a patient who, in the judgement of the Investigator, would be inappropriate for enrollment in this study.

Statistical Methods:

All statistical analyses were made by Shanghai GenomSeqCare Biotechnology Co. Ltd, using software R (https://www.r-project.org/).

Descriptive statistics were generated for all primary variables. The standard descriptive statistics for continuous variables included N, mean ± SD if normally distributed or median ± IQR if non-normally distributed, minimum and maximum. The standard descriptive statistics for categorical variables included N and percentage (%). T-test (for normally distributed variables) or Wilcoxon signed rank test (for non-normally distributed variables) was used to test the differences in means or medians of continuous variables between two groups. ANOVA (normality and variance homogeneity) or Kruskal-Wallis test (non-normality and variance homogeneity) was used to analyze the continuous variables' mean or medians differences among more than two groups. The percentages for concerned categorical variables, such as a given mutation type, was compared between two groups. Additionally, the heterogeneity among multiple groups was tested with Q statistic. Chi-square test was employed to test the correlation between two categorical variables. There were no specific hypotheses built into the protocol design. All tests were two-sided. Pvalue≤0.05 was considered statistically significant.

All eligible patients who met the inclusion criteria and did not meet exclusion criterial were enrolled in full analysis set (FAS). FAS was summarized for the population overall for the primary and secondary objectives. Subgroup analyses were performed on variables such as age, ethnics and disease severity, etc. Prevalence range of BRCA mutations in women with high grade serous ovarian cancer was estimated for the Latin American population, and by country/clusters according to defined broad ethnic groups, based on self-reported ancestry information.

About the relationship analysis for secondary endpoints, machine learning techniques were used. Association rules, and other methods were used as well. Potential relationships suggested by machine learning were tested out using appropriate statistical methods. In the case of

categorical clinical parameters, Chi square test or Fisher test were used to discuss the relationships between gene mutation and clinical features. On the other hand, in the case of continuous clinical parameters, the samples were grouped by BRCA type. Then the difference between groups were tested. As the age is necessary when exploring the relationship above, the correlation coefficient test was used. After testing the relationships, an additional permutation (a simulation with the assumption that the identified relation is false) was used to exclude false positive.

The estimated enrolled patient number was 480, and 471 were included in the analysis.

Results:

Demographics and patient disposition

From January 2016 to April 2019, 472 patients with recent diagnosis of high-grade serous ovarian cancer were enrolled in this trial from 40 sites from six different countries in Latin America. Data from 471 patients is used for this analysis, following exclusion of one patient due to endometroid histology. Mean age at diagnosis was 57.8 years (standard deviation: 12.1). A total of 76% of patients presented with stage III disease, 60% had a family history of cancer and 7% had a personal history of cancer. Nine patients were excluded from the analysis because their samples did not meet the histologic criteria (one endometroid carcinoma and eight undifferentiated adenocarcinoma N.O.S). A further 52 were rejected due to poor DNA load (<20% tumor nuclei).

Prevalence of BRCA mutations in tumors

Out of 411 samples tested for tBRCAmut, the results from five were considered as inconclusive while 406 yielded a conclusive result. Of these conclusive results, 282 (69.4%) were tBRCAwt, 115 (28.3% were tBRCAmut pathogenic or likely pathogenic and nine (2.2%) were variants of uncertain significance. Further information on mutation type by country and by ethnicity, and family history analysis by tumor mutation status are shown in the supplementary materials.

Germline BRCA mutations

Of the 115 patients with pathogenic or likely pathogenic tBRCAmut, 110 were tested at the germline level to confirm the germline origin of the specific variant found (N=5 patients did not complete germline testing due to lack of interest or loss of follow-up). Of those, 77 had a gBRCAmut (19% of all evaluable samples) while in 33 mutations were not found at the germline level, so were identified as purely somatic (sBRCAmut) in origin. The number of patients enrolled per country was small, and the study did not have the power to robustly determine if there was a statistical difference in the prevalence of somatic versus germline BRCAmut between countries. Results by ethnicity are shown in the supplementary materials. There were no statistically significant differences in prevalence of germline mutations between patients with or without a family history of cancer in general; however, when considering specifically history of BRCA-related tumors (breast or ovarian cancer), significantly more patients with BRCA

mutations had related cancers (p=0.03).

Approach to genetic counseling

The results of the tumor analysis were discussed with the patient by their treating physician in 89% of cases, and by a trained genetic counselor in 11% of cases. For germline testing results, 18% were informed by a genetic counselor, with the majority of cases (82%) being discussed by a physician not skilled in genetics.

Treatment patterns

Surgical outcomes were reported in 342 patients; 123 (36%) had R0 as an outcome (all tumor residue removed), 53 (15.6%) had R1 (all macroscopic tumor removed, some microscopic residue present), and 166 (48.5%) had residual disease greater than 1cm. In terms of first-line treatment patterns, of 415 patients who received systemic treatment, 92% received platinum-based chemotherapy, while 18% received bevacizumab. Treatments stratified by setting and mutation status are shown in supplementary materials.

Conclusion:

This novel approach of starting testing with analysis of tumoral BRCA status not only enlarges the population who might benefit the most from PARP inhibitor maintenance but also means fewer patients require germline testing and eventually genetic counseling, a resource that seems to be very scant in Latin America. Therefore, this approach may result in a more cost-effective way to test and counsel our patients. Standardization of tBRCA testing technology and sample handling will improve and become the next way to test for BRCA mutation in recently diagnosed ovarian cancer patients in the next ten years.