

Supplementary Material – Populations and Methods

Analysis populations

Between December 2014 and December 2019, 518 patients were enrolled in the POSITIVE trial. At the time of the primary analysis conducted in 2022, 2 patients were excluded from the primary analysis population, 1 due to accidental enrollment, and another that withdrew shortly after enrollment without providing any baseline information[1]. A secondary endpoint analysis population included the 497 patients that provided post enrollment disease, pregnancy, and endocrine therapy status at least once. The secondary endpoint analysis population was the primary analysis population for analyses in this manuscript. Amongst the 497 patients 215 used Assisted reproductive Technologies (ART), while 282 did not. Supplemental Table 1 summarizes the number of patients in each analysis population of the translational fertility analysis. The analysis populations were as follows:

- Low ovarian reserve analysis population: 438 patients that had usable AMH sample at either month 3 or month 12.
- Premature ovarian insufficiency (POI) analysis population: 142 patients that had a usable sample of follicle stimulating hormone (FSH) collected at month 12.
- POI negative population: 127 POI analysis population patients that did not have POI.
- POI/Low ovarian reserve analysis population: 142 patients that were included in both LOR and POI analysis populations.
- Low ovarian reserve/no ART analysis population: 238 patients that were included in the low ovarian reserve analysis population and did not use ART.

- POI/FSH month 3 analysis population: 111 patients that were included in the POI analysis population and had a usable FSH sample at month 3.
- POI/AFC analysis population: 57 patients that were included in the POI analysis population and had a usable AFC sample at month 3.
- Low ovarian reserve/no art/FSH month 3 analysis population: 187 Low ovarian reserve analysis population patients that did not use ART and had a usable FSH sample at month 3.
- Ovulatory/no ART analysis population: 93 patients that did not use ART and had a usable progesterone sample at month 6.
- Prolactin (PRL) analysis population: 444 patients that had a PRL evaluation at month 3.
- Thyroid stimulating hormone (TSH) analysis population: 450 patients that had a TSH evaluation at month 3.

Statistical Methods

The secondary endpoint population of the POSITIVE trial consisted of 497 out of the 518 enrolled patients. Each specific analysis herein utilized data from patients who had usable samples for the required analysis. Samples of AMH, FSH, E2, and progesterone drawn during pregnancy were excluded from all analyses, as were measurements of endometrial thickness. FSH and E2 samples taken outside of the day 2-5 window of the menstrual cycle, except among those with amenorrhea, were also excluded from analysis.

Classification of low ovarian reserve (Yes/No), defined as AMH values $<0.5\text{ng/ml}$ [3-5], was based on serum AMH samples taken at month 3, or if unavailable, at month 12. The frequency

and percentage of low ovarian reserve were reported both overall and by POI. Factors associated with low ovarian reserve were evaluated using multivariable logistic regression and include the covariates: age at enrollment, ART use (Ovarian stimulation by FIVET/ICSI on trial, Embryo transfer, Other ART, and No ART), prior chemotherapy, prior ET, and duration prior ET. Odds ratio (OR) and 95% confidence intervals were reported.

The frequency and percentage of patients using ART was reported by AMH values (< 0.5 ng/ml, 0.5-1.5 ng/ml, > 1.5 ng/ml). A multivariable logistic regression model was used to assess the relationship between pregnancy (Yes/No) and covariates low ovarian reserve (Yes/No), prior chemotherapy, prior ET, duration prior ET, and ART. A second model replacing low ovarian reserve with continuous AMH was also evaluated. Odds ratio (OR) and 95% confidence intervals were reported. An interaction term for the relationship between ART (Yes/No) vs. low ovarian reserve (Yes/No) was evaluated. The interaction term was assessed for statistical significance; the p-value was reported.

The frequency and percentage of patients with POI (Yes/No), defined as FSH > 25 IU/L at month 12, was reported by patient and treatment characteristics including age at enrollment (<35, 35-39, and 40-42), prior chemotherapy (yes/no), and prior ET (OFS ± AI, SERM only, other). Duration of prior ET (months) was reported as median and interquartile range (IQR) by POI. Correlation between the FSH values at months 3 and 12 was estimated using Spearman's correlation. The distribution of 3-month FSH by POI was summarized using median and IQR. A univariable logistic regression model was used to estimate the odds ratio (OR) and 95% confidence intervals (CIs) of a 1 IU/L increase in 3-month FSH in relation to POI.

The distribution of Antral Follicular Count (AFC) was summarized as median and IQR. AFC count was assessed using a multivariable linear regression model that included both POI and low ovarian reserve as covariates. Covariate and 95% confidence intervals were reported.

Three-month FSH and E2 was assessed in patients that did not use ART. The distribution of 3-month FSH and E2 was summarized as median and IQR by pregnancy status. Pregnancy status was assessed using multivariable logistic regression and included covariates: age at enrollment, prior chemotherapy, prior ET, prior duration of ET, and 3-month FSH. Odds ratio (OR) and 95% confidence intervals were reported.

The frequency and percentage of patients experiencing oligomenorrhea amongst patients without POI was reported. Oligomenorrhea is defined as 120 consecutive days without menstruation and without pregnancy prior to the month 12 sample dates. The distribution of FSH and AMH at both 3- and 12-months was reported.

Ovulatory status was defined as progesterone levels $>3\text{ng/ml}$ in the luteal phase at month 6. The frequency and percentage of pregnancy was reported by ovulatory status. Pregnancy status was assessed using multivariable logistic regression including the following covariates: age at enrollment, prior chemotherapy, prior ET, duration ET, and ovulatory status. The frequency and percentage of ovulatory status was reported by ART use amongst all patients.

The percentage of patients with normal levels of PRL and TSH are reported. Pregnancy was assessed using multivariable logistic regression that included the following covariates: age at enrollment, prior chemotherapy, prior ET, duration of prior ET, use of ART, and PRL. A second separate model replaced PRL with TSH.

Hormonal Assays:

FSH, E2, progesterone and AMH were centrally measured. Serum was aliquoted after clotting and centrifugation and stored at -80 C until shipment on dry ice to the central laboratory (Biobank of Gynecology and Obstetrics, Research Laboratory on Human Reproduction, Brussels, Belgium) with continuous temperature control. Assays were performed in the Brussels Academic Hospital Laboratory using electrochemiluminescence immunoassay on the Cobas e 801 Analyzer (Roche Diagnosis, Mannheim, Germany). The limits of detection were 0.01 ng/ml for Elecsys AMH assay (Roche Diagnostics GmbH, Mannheim, Germany), 5pg/ml for Elecsys Estradiol III assay (Roche Diagnostics GmbH, Mannheim, Germany), 0.05 ng/ml for Elecsys Progesterone III assay (Roche Diagnostics GmbH, Mannheim, Germany) and 0.3 IU/L for Elecsys FSH assay (Roche Diagnostics GmbH, Mannheim, Germany). All repeatability and intermediate precision tests were <5%.

References

1. Partridge, A.H., et al., *Interrupting Endocrine Therapy to Attempt Pregnancy after Breast Cancer*. N Engl J Med, 2023. **388**(18): p. 1645-1656.