Title: RCT of Fixed vs Titrated Letrozole in Breast Cancer Patient Undergoing IVF
NCT01035099
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WCM IRB-Approval and Amendment History

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11/04/2008
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07/14/2010

Amendment-002
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09/21/2015

RESEARCH PLAN - RCT of Fixed vs Titrated Letrozole

Study Design (for example, hypothesis, research question, standard and experimental procedures, special or unusual equipment of procedures):

IVF is a process which involves a schedule of injectable medication to recruit several follicles, each containing an egg, to be retrieved under ultrasound guidance where they can be fertilized. Embryos are then selected to be transferred back into the patient's uterus or are cryopreserved and transferred at a later date.

Letrozole is a potent and highly selective third generation aromatase inhibitor that was developed in the early 1990's. Aromatase is an enzyme of the cytochrome P-450 superfamily and the product of the CYP19 gene, which catalyzes the reaction that converts androgenic substances to estrogens in many tissues, including the granulosa cells of ovarian follicles. Letrozole competitively inhibits the activity of the aromatase enzyme and has a half-life of approximately 48 hours. Because of its potent, sustained suppression in the plasma levels of estradiol, this drug has been recently found to be superior to tamoxifen in the treatment of advanced-stage post-menopausal breast cancer. Conventional ovarian stimulation often results in very high estrogen levels. Since the high estrogen levels are often unsafe for breast cancer patients, fixed dose aromatase inhibitor protocols with Letrozole were developed, to achieve effective ovarian stimulation with reduced estrogen levels to prevent tumor progression and short-term cancer recurrence.

A fixed dose of Letrozole has been used off-label as an ovulation induction agent in many centers to stimulate egg development for infertile couples and patients prior to undergoing chemotherapy for estrogen sensitive cancers.

The purpose of our study is to determine if titrated dose Letrozole in comparison to fixed dose Letrozole during gonadotropin stimulation in IVF in breast cancer patients results in lower estradiol levels and higher mature oocyte yield.

In patients who are scheduled to undergo treatment with IVF for fertility preservation due to breast cancer, we would like to prospectively randomize them to fixed dose vs. titrated dose of Letrozole. Due to time restraints for chemotherapy patients may present for ovarian stimulation prior to Day 2 of their menstrual cycle. If this were to occur, a gonadotropin-releasing hormone (GnRH) antagonist may be started to suppress the pituitary hormones for down regulation prior to starting stimulation medications. Eligible subjects will be fully informed

about the nature of the trial. Subjects could be enrolled for IVF treatment at any point after breast cancer diagnosis but before the initiation of chemotherapy, if chemotherapy has been prescribed by their oncologist. After obtaining written informed consent, the subjects will be screened based on inclusion/exclusion criteria, medical and infertility history, ovarian reserve (eg, antral follicle count, AMH, FSH) and estradiol, physical and gynecological examination, oncology clearance and the Center's standard screening evaluations for IVF patients. A thorough gynecologic and endocrinologic evaluation will be performed before the start of any treatment. Laboratory assessments will include CBC, chemistry and lipid profile (see attached history and physical form). Vital signs including blood pressure, pulse, and weight will be assessed at baseline (prior to start of stimulation medication), with preoperative evaluation, on day of egg retrieval, and with post retrieval visits.

Patients will have serum blood tests for bHCG, FSH, AMH, Estradiol, and LH and vaginal sonogram. Start of

stimulation medication will proceed only with documentation of a serum negative pregnancy test. Patients will only be eligible to participate in the study for one cycle of in vitro fertilization.
Patients who elect to participate in the study will be provided with gonadotropin medications.

The starting dose of gonadotropins for both study groups would be determined by the patients' age and by antral follicle counts assessed by transvaginal ultrasound at the time of initial consultation. The HMG form of gonadotropin may be added to the FSH formulation, as needed. See below:
Age <35 , Antral follicle Count >15 gonadotropin dose 150 - 225 IU
Age <35, Antral follicle Count <15 gonadotropin dose 225 - 300 IU
Age 35-39, Antral follicle Count >10 gonadotropin dose 150 - 300 IU
Age 35-39, Antral follicle Count <10 gonadotropin dose 300 - 450 IU
Age>40 Independent of antral follicle count, gonadotropin dose 300 - 450 IU. All patients would also be given a daily medication to prevent them from ovulating (GnRH e.g. Ganirelix, Cetrotide), no later than stimulation day #7 and continuing until HCG administration.

Group#1-Fixed Letrozole Group: Provided their blood tests and sonograms were within normal limits, patients who are randomized to fixed dose Letrozole will start Letrozole 5mg daily (orally) and then gonadotropins 2 days later (Current Letrozole protocol at CRM). Patients will be monitored with blood tests for estradiol and LH and sonogram every 1-3 days. Adjustments to gonadotropin dosing will be made as per usual protocol for IVF. Letrozole dose will not change and will continue for up to two weeks after egg retrieval.

Group #2-Titrated Letrozole Group: Provided their blood tests and sonograms were within normal limits, patients who are randomized to the titrated dose of Letrozole, will start gonadotropins in the evening at the start of stimulation with injectable follicle stimulating hormone (FSH) and human menopausal gonadotropin (HMG). Oral Letrozole will be added to the stimulation in the following titrated regimen:

Serum Estradiol level <150 pg/ml- No Letrozole
Serum Estradiol Level 150-250 pg/ml- 2.5mg
Serum Estradiol Level 251-350 pg/ml- 5 mg
Serum Estradiol Level >350 pg/ml - 7.5 mg

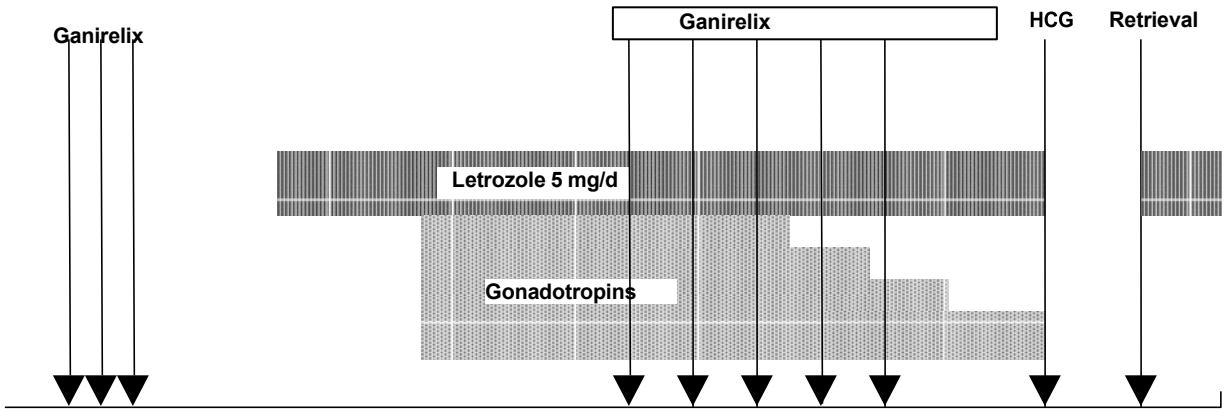
The maximal starting dose of Letrozole will be 5 mg, regardless of the initial estradiol level. The Letrozole dose may be reduced if the appropriate suppression of estradiol occurs. The maximal increase or decrease in Letrozole dose will be 2.5 mg/ day. Patients will be monitored with blood tests for estradiol and LH and sonograms starting on the second day of gonadotropin stimulation and every 1-3 days to monitor response and adjust medication dose as per our usual IVF protocol. Letrozole will be stopped for both groups on the day that HCG is given and resumed after egg retrieval. All patients will stay on the same dose of Letrozole that was required for their last day of ovarian stimulation for up to 2 weeks after stimulation to keep estradiol levels at a minimum. In addition, patients will have serum estradiol levels drawn weekly for 2 weeks after stimulation. Follow-up vital signs will be recorded along with CBC, liver function panel, and cholesterol panel 1-2 weeks after stimulation, if oncology treatment is not started immediately after retrieval. They will also be asked to return 6 months to 1 year after completion of chemotherapy for AMH and FSH levels to evaluate ovarian reserve, and then followed on a yearly basis by phone/mail questionnaire or in person with an annual gynecological examination.

All study patients will be provided with an emergency 24-hour phone number to reach a physician to report adverse reactions to medications, and these physicians will file adverse event report forms to the principal investigator and data safety monitoring board. In addition, they will be asked to report any adverse reactions to medications during follicular monitoring sonograms. All decisions regarding daily medication doses will be determined by the study principal investigator. Otherwise, the treatment of ovarian stimulation, egg retrieval, and cryopreservation is identical to non-study patients undergoing ovarian stimulation, oocyte retrieval, and embryo/oocyte cryopreservation.

After retrieval, follow-up instructions will be reviewed. Patients will be advised to refrain from embryo transfer for a minimum of 2 years after chemotherapy. Patients will not be permitted to undergo embryo transfer for a minimum of one year after completing chemotherapy and only after clearance for pregnancy has

been obtained from their medical oncologist. Patients will be followed for five years on an annual basis with a follow-up telephone call from one of the investigators inquiring about health status (see patient follow-up data sheet). A pregnancy registry will be created for any pregnancy occurring following enrollment in the study. Detailed pregnancy outcome will be collected between age 2-5 years (see baby follow-up data sheet attached).

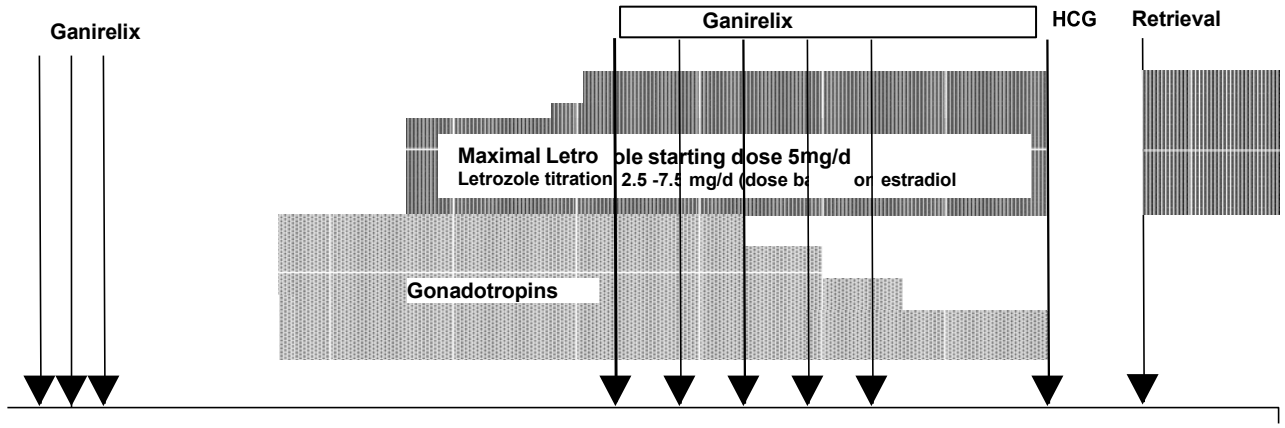
FIXED LETROZOLE PROTOCOL



cycle days 1 2 3 4 5 6 7 8 9 10 11 ……..

luteal phase menses

TITRATED LETROZOLE PROTOCOL



cycle days 1 2 3 4 5 6 7 8 9 10 11 ……..

luteal phase menses

A2. Rationale and justification for the study (for example, historical background, investigator's personal experience,

pertinent medical literature):

For several years in Europe and North America, aromatase inhibitors have been used to block estrogen production in the treatment of post-menopausal breast cancer. Researchers in the United States and Canada have used aromatase inhibitors in otherwise healthy women undergoing ovulation induction. By blocking estrogen production, many researchers have found that ovulation induction could be performed with significantly lower doses of fertility medications (Fertil Steril 75:305-9,2001; Fertil Steril 86(5):1428-1431; Fertil Steril 85:1774-7, 2006). Pregnancy outcome was similar to that of traditional stimulation protocols (Journal of the Society for Gynecologic Investigation 11(6):406-15,2004).

In 2005, an observational pilot study investigated 147 low responder patients with previously cancelled IVF cycles. 71 patients were treated with 2.5 mg Letrozole and FSH and 76 patients were similarly treated without Letrozole. Antagonist was added in both groups during the first 5 days of stimulation. The Letrozole group had more oocytes retrieved and higher implantation rates (Fertil Steril :84(1):82-7, 2005). In 2006, Verpoest et al. published a small randomized prospective pilot study investigating the effects of adding aromatase inhibitors to ovarian stimulation for IVF or ICSI on endocrine parameters, endometrial thickness and pregnancy rates. They showed that median estradiol concentrations were significantly lower in the Letrozole group vs. no Letrozole, whereas endometrial thickness was significantly greater in the Letrozole group.

In 2005, at our institution, a Letrozole protocol was applied to a population of women with breast cancer undergoing fertility preservation. 60 women with breast cancer, were prospectively followed after fertility preservation procedures with tamoxifen alone or in combination with low dose FSH, or Letrozole in combination with low dose FSH. After IVF, all resultant embryos were cryopreserved. The combination of low-dose FSH with tamoxifen or Letrozole resulted in higher embryo yield compared to tamoxifen alone. Recurrence rates did not seem to be increased. The Letrozole protocol resulted in lower peak estradiol levels. (J Clin Oncol 23:4347-4353, 2005).

In 2006, at out institution, 47 breast cancer patients were studied who received 5mg/d Letrozole and 150-300 IU FSH to cryopreserve embryos or oocytes. Age matched retrospective controls were selected from women who underwent IVF for tubal disease. Letrozole with FSH stimulation resulted in significantly lower peak estradiol levels and 44% reduction in gonadotropin requirement, compared to controls. Length of stimulation, number of embryos obtained, and fertilization rates were similar. The mean delay from surgery to cryopreservation without delay in chemotherapy was 39 days with 81% of patients completing their stimulation within the 8 weeks of surgery. (J Clin Endocrinol Metab 91:3885-3890, 2006)

At our institution, 215 women with breast cancer were prospectively evaluated for fertility preservation before adjuvant chemotherapy. Of those 79, elected to undergo controlled ovarian hyperstimulation with letrozole 5 mg x 5 days and gonadotropins for embryo and oocyte cryopreservation. The remaining 136 patients underwent no fertility-preservation procedures and served as controls. In the fertility preservation group the mean E2 was 406 ± 257 pg/ml. The hazard rate for recurrence and the survival rate after IVF was not compromised in comparison to the control group after 23 months. (J Clin Onc 26:2630-2635, 2008)

References:

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2. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J Clin Oncol. 2008 Jun 1;26(16):2630-5.

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6. Jee BC, Ku SY, Suh CS, Kim KC, Lee WD, Kim SH. Use of letrozole versus clomiphene citrate combined with gonadotropins in intrauterine insemination cycles: a pilot study. Fertil Steril. 2006 Jun;85(6):1774-7.

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8. Mitwally MF, Casper RF. Single-dose administration of an aromatase inhibitor for ovarian stimulation. Fertil Steril. 2005 Jan;83(1):229-31.

9. Mitwally MF, Casper RF. Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. Fertil Steril. 2001 Feb;75(2):305-9.

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11. Oktay K, Hourvitz A, Sahin G, Oktem O, Safro B, Cil A, Bang H. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. J Clin Endocrinol Metab. 2006 Oct;91(10):3885-90.

12. Verpoest WM, Kolibianakis E, Papanikolaou E, Smitz J, Van Steirteghem A, Devroey P. Aromatase inhibitors in ovarian stimulation for IVF/ICSI: a pilot study. Reprod Biomed Online. 2006 Aug;13(2):166-72.

A3. Primary study endpoint:

Mature oocyte yield

A4. Primary objective:

The purpose of our study is to determine if titrated dose Letrozole in comparison to fixed dose Letrozole during gonadotropin stimulation in IVF in breast cancer patients results in 50% increased mature oocyte yield

A5. Secondary objectives:

Peak Serum Estradiol Level (pg/ml)
FSH ng/ml on Cycle Day 2
AMH ng/ml on Cycle Day 2
Antral follicle count on Cycle Day 2
Total days of stimulation medications
Total medication dose
Adverse events from medications
Total number of oocytes
Number of mature oocytes
Number of follicles on day of HCG
Number of fertilized (2PN) oocytes (if applicable)
Number of good quality embryos (if applicable)
Cycle cancellation rate
1 year follow-up breast cancer recurrence rate
2 year follow-up breast cancer recurrence rate
5 year follow-up breast cancer recurrence rate

A6. Inclusion/Exclusion criteria:

Inclusion Criteria:

Female breast cancer patient with breast cancer diagnosis undergoing chemotherapy desiring fertility preservation with oocyte or embryo cryopreservation

Healthy subject according to documented medical history and physical examination who has been diagnosed with breast cancer (estrogen and progesterone receptor positive and/or negative)

Age less that 45 years at time of informed consent

Verbal or written clearance from medical or surgical oncologist to undergo controlled ovarian hyperstimulation-IVF.

Delay to surgery or chemotherapy treatment will not jeopardize cancer treatment outcome

Ovarian stimulation will not affect cancer treatment plan

Transvaginal ultrasound scan (US) within one month of starting stimulation with no clinically significant pelvic mass

Serum FSH level (Day 2-4) less than 25, since our center has documented pregnancies with FSH up to 27miu/ml

Negative pregnancy test prior to beginning Letrozole or gonadotropin therapy

Willing and able to comply with the protocol

Voluntary provision of written informed consent, prior to any study-related procedure that was not part of

normal medical care, with the understanding that the subject can withdraw consent at any time without prejudice to her future medical care

Willingness to provide follow-up information on herself and babies born as part of this study

Exclusion Criteria:
Patients not medically cleared by their oncologist

Patients with stage IV breast cancer based on the poor prognosis, general health of the patient, and higher uncertainty with delaying chemotherapy

Any clinically relevant abnormal laboratory value (FSH >25 miu/ml, renal function, (greater than two times normal value), hepatic function (greater than two times normal value), blood biochemistry, hematology (elevated white blood count greater than 1.5 times the normal value, hemoglobin <10mg/dL, thrombocytopenia), abnormal cholesterol profile (total cholesterol ≥ 300mg/dL, abnormal LDL greater than 2 times normal value,) based on a fasting sample during the screening phase

Contraindications for the use of gonadotropins (i.e. Tumors other than breast cancer, pregnancy, lactation, undiagnosed vaginal bleeding)

Recent or current medical conditions where the patient is not medically stable to undergo stimulation or egg retrieval, HIV infection, diabetes, cardiovascular disease, gastrointestinal, hepatic disease, undiagnosed pelvic mass, renal or pulmonary disease

History or presence of alcohol or drug abuse within 12 months of signing consent

History of severe allergic or anaphylactic reactions or hypersensitivity to any of the concomitant medication prescribed as part of the treatment regimen in this protocol

Administration of investigational drugs within three months prior to signing the informed consent

Use of insulin sensitizing agents at least one month prior to signing informed consent

Any patient who is not a candidate for IVF

A7. Treatment plan: (include amount and frequency of samples of blood and other bodily fluids in chart below)

Bodily Fluid	**Amount**	**Frequency**	**Total**
Blood	5cc	12-14	60-70cc
Urine	20cc	1	20 cc
Other_____			

A8. Statistical considerations (e.g. justification for sample size or "n"):

Sample size:
The primary aim of the study is to determine if titrated dose Letrozole in comparison to fixed dose Letrozole during gonadotropin stimulation in IVF in breast cancer patients results in a 50% increased mature oocyte yield. With a sample size of 25 patients per group, the study will have 80% power (alpha=0.05) to detect a difference in the mean number of mature oocytes of 4.3 between the titrated dose group and the fixed dose group. This is based on an expected mature oocyte yield of 8.7±4.8 in the fixed dose group and an expected 50% increase in the mature oocyte yield in the titrated dose group (e.g., [(13.0-8.7)/8.7 = 50% increase]).

Analysis Plan:
The mean and median mature oocyte yield will initially be compared between the titrated and fixed dose groups by the two-group t-test and Wilcoxon rank-sum test, respectively, in an intent-to-treat analysis. Stratification by age group at the time of randomization will be taken into account by performing analysis of covariance (ANCOVA) for the primary endpoint (i.e., ooctye yield), using the stratification factor (age group) as a covariate in the ANCOVA model (in addition to the dose group variable) (this will also be an intent-to-treat analysis). The ANCOVA procedure recognizes the reduction in variability in the titrated-versus-fixed dose comparison that is attained by use of stratification (by age group) at the time of randomization. This reduction in variability will increase the power of the study (>80%) to detect the hypothesized difference and will potentially allow for the detection of smaller differences with the stated sample size of 50 patients.

Secondary continuous endpoints will initially be compared by the t-test or Wilcoxon rank-sum test, as appropriate, and secondary categorical endpoints will be compared by the chi-square test or Fisher's exact test, as appropriate. Similarly, to account for stratification at randomization, ANCOVA and multivariate logistic regression will be performed for the secondary endpoints, as appropriate, by using age group as a covariate in the multivariate models. One-year and 2-year recurrence-free survival will be preliminary assessed by the Kaplan-Meier method and the log-rank test will be employed for comparing recurrence-free survival between the titrated and fixed dose groups.

Subjects who discontinue the study prior to egg retrieval will be still be considered in the primary intent-to-treat analysis by assigning a value of zero eggs retrieved for the primary endpoint (i.e., oocyte yield). This will conservatively underestimate any observed titrated-versus-fixed dose effect but will preserve the benefit of randomization. A secondary analysis will be performed to assess the between dose group effect after these patients have been removed, but this will only be for descriptive purposes.

All p-values will be two-sided with statistical significance evaluated at the 0.05 alpha level and 95% confidence intervals will be calculated to assess the precision of the obtained estimates. All analyses will be performed in SAS Version 9.1 (SAS Institute, Inc., Cary, NC).

Randomization:
A series of randomized blocks of four will be generated for the study. This will provide assurance that after four patients are enrolled, there will be two patients assigned to the titrated dose letrozole group and two patients assigned to the fixed dose letrozole group. The blocked randomization will be stratified according to age group: ≤32 years, 33-36 years, and ≥37 years. This process of blocked randomization and stratification ensures an equal distribution between the two study arms of patients in the specified age groups. The study research coordinator will have access to the computer generated randomization file and will make assignments accordingly.

A9. Relative importance/value of the trial, considering "standard" therapy and published/competing trials. Please
specify what the current standard of care is.
This trial will investigate if a superior and safer controlled ovarian hyperstimulation protocol with Letrozole exists for breast cancer patients for fertility preservation and cryopreservation of oocytes or embryos. As these patients cannot delay chemotherapy, any improvement in oocyte yield would be a significant benefit.

WCM IRB-approved approval and amendments History

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