Protocol Title

A 3D bioprinted hormone-producing model for *BRCA* mutated patients after Risk Reducing Surgery: the DISC-OVARY trial

(Version 2 –10/01/2024)

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1. SYNOPSIS

Title	**A 3D bioprinted hormone-producing model for BRCA mutated patients after Risk Reducing Surgery: the DISC-OVARY trial**
Version	2.0 (10.01.2024)
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Promoter, coordinator center	Division of Gynecologic Oncology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; Department Woman and Child Health Sciences, Universita' Cattolica del Sacro Cuore, Rome, Italy
Collaborator centers	none
Background	Women carrying germline BReast CAncer gene (BRCA) 1/2 mutations have an increased lifetime risk of breast and ovarian cancers [1,2]. Risk-reducing salpingo-oophorectomy (RRSO) by the age of 40 (postponable to age of 45 for BRCA2mut)[3] is the standard of care in ovarian cancer risk reduction (about 80%)[4]. Although potentially lifesaving, RRSO may negatively affect quality of life and impair long-term health. To overcome these side effects, hormone replacement therapy (HRT) is crucial but remains a major concern, especially due to its negative effects (potential breast cancer risk[9], thromboembolic events[10]) and, overall, due to the long-term safety lack of data. Preliminary data from the literature revealed that only 70% of gynecologists recommend HRT after RRSO due to oncological safety concerns and low women's requests (more than 70% of cases were oral prescriptions, while only 24% preferred local administration) [5]. In conclusion, for some women, the concern of menopause risks act as a deterrent for a lifesaving procedure (RRSO). In this scenario, it is fundamental to provide a new strategy for *BRCA* mutated patients, in order to reduce menopausal drawbacks without exposing them to a higher breast cancer risk, reproducing the physiological hormonal rhythm without compliance issue.

Rationale	Selecting theca and granulosa cells from removed ovaries of *BRCA*1/2mut patients undergoing RRSO and developing a 3D bioprinted hormone-producing bioprosthetic model. If efficacy and tolerability are confirmed in vivo, this bioprosthetic model might be used to replace hormones' production in BRCA mutated patients undergoing prophylactic surgery.
Type of the study	Feasibility single-institution study
Primary endpoint	Develop a 3D bioprinted model able to produce estrogens and progesterone and confirm in vitro functionality.
Sample size	No study has previously investigated the feasibility of 3D bioprinted hormone-producing model, hence this configures as a pilot study which, as such, does not need any formal sample size calculation.
Inclusion criteria	- Women between 18-40 y.o; - BRCA1/2 germline mutations; - Completed childbearing; - Willing to undergo RRSO; - Negative final histological examination; - No previous breast cancer.
Exclusion criteria	- Other malignancies; - final histological examination reporting malignant disease (any); - desire of fertility sparing.
Bibliography	1. Antoniou et al., *Am J Hum Genet* 2003 2. Gabaldó Barrios et al., *Fam Cancer* 2017 3. Dwyer et al., NCCN Guidelines ® 2023 4. Marchetti et al., *BMC Womens Health* 2014 5. Palaia et al., *Int J Gynecol Can* 2022 6. Taneja et al., Front Immunol 2018

Background and Rationale

Women carrying germline BReast CAncer gene (BRCA) 1/2 mutations have an increased lifetime risk of breast and ovarian cancers (72% and 44% for BRCA1 and 69% and 17% for BRCA2, respectively) [1,2]. Risk-reducing salpingo-oophorectomy (RRSO) by the age of 40 (postponable to age of 45 for BRCA2mut)[3] is the standard of care in ovarian cancer risk reduction (about 80%)[4]. Although potentially lifesaving, RRSO may negatively affect quality of life and impair long-term health[5,6] (cardiovascular disease, osteoporosis[7] and impairment of immune response[8]).

To overcome these side effects, hormone replacement therapy (HRT) is crucial but remains a major concern, especially due to its negative effects (potential breast cancer risk[9], thromboembolic events[10]) and, overall, due to the long-term safety lack of data. Synthetic and animal-derived hormones seem to be associated with breast cancer risk[11]. Although breast cancer risk is lower for bioidentical hormones (i.e. estriol)[12], data continue to be discordant. Several evidence still sustain estriol's impact on breast cancer (especially for lobular histotype; OR 2.0, 95% CI 1.3–3.2)[13] and endometrial disease (endometrial cancer: OR 3.0, 95% CI 2.0-4.4 and endometrial atypical hyperplasia: OR 8.3, 95% CI 4.0-17.4, respectively)[14]. Furthermore, estriol's efficacy on cardiovascular disease and osteoporosis is still unclear when compared to other estrogen compounds[15]. Side effects of estriol therapy may include breast tenderness, nausea, bloating, mood changes, headache, and vaginal bleeding or spotting[16]. Moreover, all the HRT's administration routes (oral, transdermal, and vaginal) need daily/frequent assumption, thus compliance of the patients is essential and adherence to long-term therapy in developed countries is reported around 50% with a high risk of forgetfulness or discontinuation[17–19]. Each route also has specific disadvantages (i.e. the risk of thromboembolism in the oral one[20]). Finally, HRT may influence other hormones' production: it increases T_4 dosage requirements of women being treated for primary hypothyroidism as well as alter the pituitary-thyroid axis in euthyroid women[21]. While for cortisol level findings are still inconsistent[22], hormone exogenous intake does not permit to follow the daily hormonal fluctuation. Aging and hypoestrogenism, in postmenopausal women, determine immune system changes which may play a crucial role in the development of postmenopausal diseases (diabetes or atherosclerosis) and may be negatively influenced by HRT [23]. Preliminary data of a recent MITO group survey revealed that only 70% of gynecologists recommend HRT after RRSO due to oncological safety concerns and low women's requests (more than 70% of cases were oral prescriptions, while only 24% preferred local administration) [24].

In conclusion, for some women, the concern of menopause risks act as a deterrent for a lifesaving procedure (RRSO). In this scenario, it is fundamental to provide a new strategy for *BRCA* mutated patients, in order to reduce menopausal drawbacks without exposing them to a higher breast cancer risk, reproducing the physiological hormonal rhythm without compliance issue. Our 3D bioprinted hormone-producing model from patients' own autologous cells could meet this need.

HYPOTHESIS

We hypothesize to select theca and granulosa cells from removed ovaries of *BRCA*1/2mut patients undergoing RRSO, avoiding the epithelial ones at risk of developing cancer, and use those cells to

develop a 3D bioprinted hormone-producing bioprosthetic model in order to replace patients' own production.

AIMS

Primary endpoint: Develop a 3D bioprinted model able to produce estrogens and progesterone and confirm in vitro functionality.

EXPERIMENTAL DESIGN

No study has previously investigated the feasibility of 3D bioprinted hormone-producing model, hence this configures as a pilot study which, as such, does not need any formal sample size calculation.
The proposed research project aims to select theca and granulosa cells from patients' removed ovaries, combine them with different ratios of extracted mesenchymal cells; print them onto the prosthetic model scaffolds and assess its hormone production.

The Project Team will include young, dedicated and selected members from:

- Department of Woman, Child and Public Health, Fondazione Policlinico Universitario A. Gemelli IRCCS: the "Genetic/Familial high-risk patients' clinic" (where Dr. Marchetti is already currently involved) and Operating Rooms (for blood and tissue samples' collection)
- 3D Bioprinting Facility, to enable the fabrication of three-dimensional structures using living cells and biomaterials.
- Operational Unit of Gynecologic Pathology and Breast Pathology

IMPACT ON CANCER

The results of this pilot project will provide a 3D bioprinted model able to produce estrogens and progesterone in vitro. If functionality is confirmed in vivo, our model might be used in BRCA mutated patients allowing them to avoid surgery induced menopause's side effects. Specifically, a 3D bioprinted hormone-producing bioprosthetic model would provide patients with a viable alternative to surgery-induced menopause and traditional HRT.