MELAFERT: Impact of adjuvant therapy on fertility in patients with resected melanoma at high risk of relapse. A Prospective multicenter observational study

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Protocol Code: MELAFERT
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Study Promoter: Italian Melanoma Intergroup

Dr. Francesco Spagnolo
Principal Investigator

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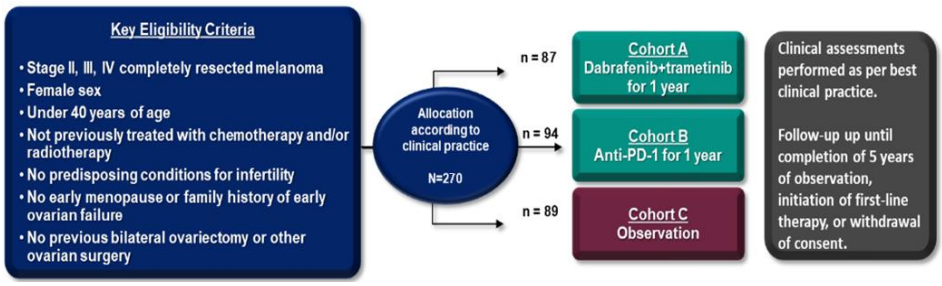
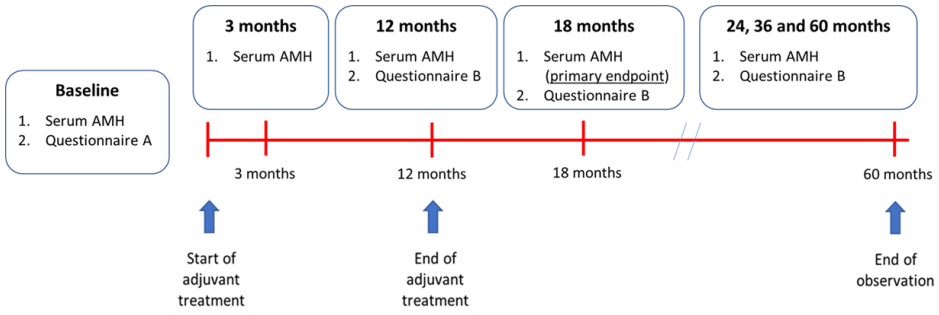
Signature **Date**

Synopsis

<table>
<tr><td>Title of Trial</td><td colspan="3">MELAFERT: Impact of adjuvant therapy on fertility in patients with resected melanoma at high risk of relapse. A Prospective multicenter observational study</td></tr>
<tr><td>Rationale for Trial</td><td colspan="3">Melanoma survivorship in reproductive-age women is increasing due to the advent of effective therapies in the curative setting. However, while the impact on fertility and ovarian function of chemotherapy agents is well known, there is still a lack of consistent data regarding novel the MAP kinase pathway inhibitors and immune-checkpoint inhibitors (ICIs) used in melanoma [1-5].
A recent study showed that a single course of anti-PD-1 or anti-CTLA-4 reduced both the number and quality of oocytes in mice through an immune-mediated mechanism. In particular, primordial follicle damage cannot be restored, leading to relevant clinical implications [6].
Our study aims to help to determine the impact of MAP kinase pathway inhibitors and ICIs on reproductive outcomes, and whether clinicians should discuss (and in what terms) fertility preservation techniques in reproductive-age women receiving ICIs and MAP kinase pathway inhibitors in the adjuvant setting.</td></tr>
<tr><td rowspan="3">Objectives</td><td></td><td>Objective</td><td>Endpoint</td></tr>
<tr><td>Primary</td><td>To evaluate, in women of childbearing age, the variation in ovarian reserve after completion of adjuvant therapy with BRAF/MEK inhibitors or anti-PD-1 agents</td><td>Serum antimullerian hormone (AMH) at 18 months after the start of adjuvant therapy.</td></tr>
<tr><td>Secondary</td><td>To assess long-term fertility preservation after completion of adjuvant therapy
To assess the early impact on fertility preservation of a short course of therapy</td><td>• correlation between baseline/post-treatment serum AMH and pregnancy rate
• correlation between baseline/post-treatment serum AMH and menstrual activity
• ratio between desired (Gd)/ obtained (Go) pregnancies
• other reproductive outcomes
• AMH at 3 months after the start of adjuvant therapy
• AMH at 12 months after the start of adjuvant therapy</td></tr>
<tr><td>Trial Design</td><td colspan="3">This is a multicenter, observational, prospective study. A total of 270 female patients will be enrolled in the study according to inclusion and exclusion criteria. BRAF mutational assessment will be performed as per clinical practice and patients with resected stage II, III and IV melanoma will then be treated with BRAF/MEK inhibitors or anti-PD-1 or observation as per clinical practice (Figure 1).</td></tr>
</table>

	All patients who meet the eligibility requirements will be assessed at baseline for fertility outcomes (AMH, FSH, Beta estradiol) and will be given a questionnaire (questionnaire A) to collect data on menstrual cycles, pregnancies, terminations of pregnancy. Data on patient characteristics, tumor characteristics, types of anticancer treatments and reproductive outcomes will be collected through eCRF. Patients will then be re-evaluated by laboratory tests (AMH, FSH, beta-estradiol) and questionnaires (questionnaire B) related to their desire to become pregnant, the course of the menstrual cycle, pregnancies and its outcomes at 12, 18, 24, 36 and 60 months from the start of therapy (Figure 2). AMH will also be re-evaluated at 3 months after the start of adjuvant therapy to assess the early impact on fertility preservation of a short course of therapy. AMH assessments will be performed centrally at the San Martino Polyclinic, Genoa, Italy. The Centers will be responsible for collecting the blood samples, processing them and sending by courier the frozen serum samples stored at -20 °C for centralized analysis. Clinical assessments will be performed as per best clinical practice. Patients will be followed-up until completion of 5 years of observation, initiation of first-line therapy or withdrawal of consent.  **Figure 1**. Study design  **Figure 2**. Key study assessments at different timepoints. AMH: antimullerian hormone
Population	Reproductive-age female patients with completely resected stage II, III, IV melanoma, irrespective of BRAF mutational status, with no previous history of chemo, radiation therapy and/or ovarian surgery.
Key Inclusion Criteria	a) Stage II, III, IV completely resected melanoma b) Female sex c) Under 40 years of age d) Not previously treated with chemotherapy and/or radiotherapy e) Being able to give written informed consent.

<table>
<tr><td>Key Exclusion Criteria</td><td colspan="2">a) Unresectable melanoma
b) Predisposing conditions for infertility
c) Early menopause or family history of early ovarian failure (idiopathic, < 45 years)
d) Previous bilateral ovariectomy or other ovarian surgery
e) Personal history of autoimmune diseases, endocrine disorders (except for hypothyroidism)
f) Personal history of severe mental disorders associated with infertility (e.g., nervous anorexia) and/or requiring treatments that could impair fertility
g) Inability to give written informed consent.</td></tr>
<tr><td rowspan="5">Planned Trial Milestone Dates</td><td>Trial Start
(First Patient First Visit)</td><td>01/02/2025</td></tr>
<tr><td>Recruitment End
(Last Patient First Visit)</td><td>01/02/2027</td></tr>
<tr><td>Trial End
(Last Patient Last Visit)</td><td>01/08/2028 (for primary endpoint)</td></tr>
<tr><td>Completion of Trial Report</td><td>01/11/2028</td></tr>
<tr><td>Primary Publication Date</td><td>01/02/2029</td></tr>
<tr><td rowspan="3">Number of Patients and Centers</td><td>Total Planned Number of Patients</td><td>270</td></tr>
<tr><td>Planned Number of Centers</td><td>20</td></tr>
<tr><td>Planned Number of Countries
(List the Countries)</td><td>Italy, France, Poland, Spain, The Netherlands, Sweden, Denmark, UK, Belgium, Australia</td></tr>
<tr><td>Sample Size Justification and Statistical Analysis</td><td colspan="2">Primary endpoint is the serum AMH level after 18 months from the start of adjuvant therapy (or of recruitment for patients in cohort C). We estimated that the two treatment arms compared to the observation arm will have a 20% drop in AMH values compared to the observation arm.
We expected an 18-months relapse rate of:
- Cohort A: patients receiving dabrafenib+trametinib: 23%;
- Cohort B: patients receiving anti-PD-1: 29%;
- Cohort C: control arm: 25%.

The sample size has been estimated with the ANOVA method (3 groups) assuming a 5% type 1 error (statistical significance), an 80% of study power, and a mean AMH reduction of 20% in the two treatment arms compared to the untreated patients, setting the within-group variance at 18%, a total of 67 patients per arm are needed to obtain a statistically significant difference between the mean AMH. Considering different recurrence rates in the 3 groups:

- Cohort A: dabrafenib+trametinib: (x-0.23x) =67 → x=67/0.77 → 87 patients
- Cohort B: anti-PD-1: (x-0.29x) =67 → x=67/0.71 → 94 patients
- Cohort C: control: (x-0.25x) =67 → x=67/0.75 → 89 patients.</td></tr>
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References	1. Lambertini, M. et al. Cancer and fertility preservation: international recommendations from an expert meeting. BMC Med. 14, 1 (2016) 2. Lambertini, M. et. al. Fertility preservation and post-treatment pregnancies in post-pubertal cancer patients: ESMO Clinical Practice Guidelines. Ann. Oncol. 31, 1664-1678 (2020). 3. Garutti, M. et al. Checkpoint inhibitors, fertility, pregnancy, and sexual life: a systematic review. ESMO Open, Volume 6, Issue 5, 100276 4. G.V. Long et al. Adjuvant Dabrafenib plus Trametinib in Stage III BRAF-Mutated Melanoma. NEJM, Sep. 2017 5. Eggermont, A. M. M. et al. Adjuvant Pembrolizumab Versus Placebo in Resected Stage III Melanoma. N Engl J Med. 378:1789-1801 (2018). 6. Winship, A.L., Alesi, L.R., Sant, S. et al. Checkpoint inhibitor immunotherapy diminishes oocyte number and quality in mice. Nat Cancer 3, 1–13 (2022).

1. Introduction and rationale

Although the occurrence of cutaneous melanoma tends to rise as individuals age, it can also affect younger populations with notable frequency. Specifically, the incidence rate is significantly higher among females than males in the younger age groups, indicating a greater susceptibility among young women to develop this type of cancer [1].
According to the World Health Organization (WHO) GLOBOCAN, there are 30,000 new estimated cases of melanoma in the age range 0-39 years in 2020 [1]. Most of these cases are detected in Europe, Northern America, Australia and New Zealand (Figure 1) [2].

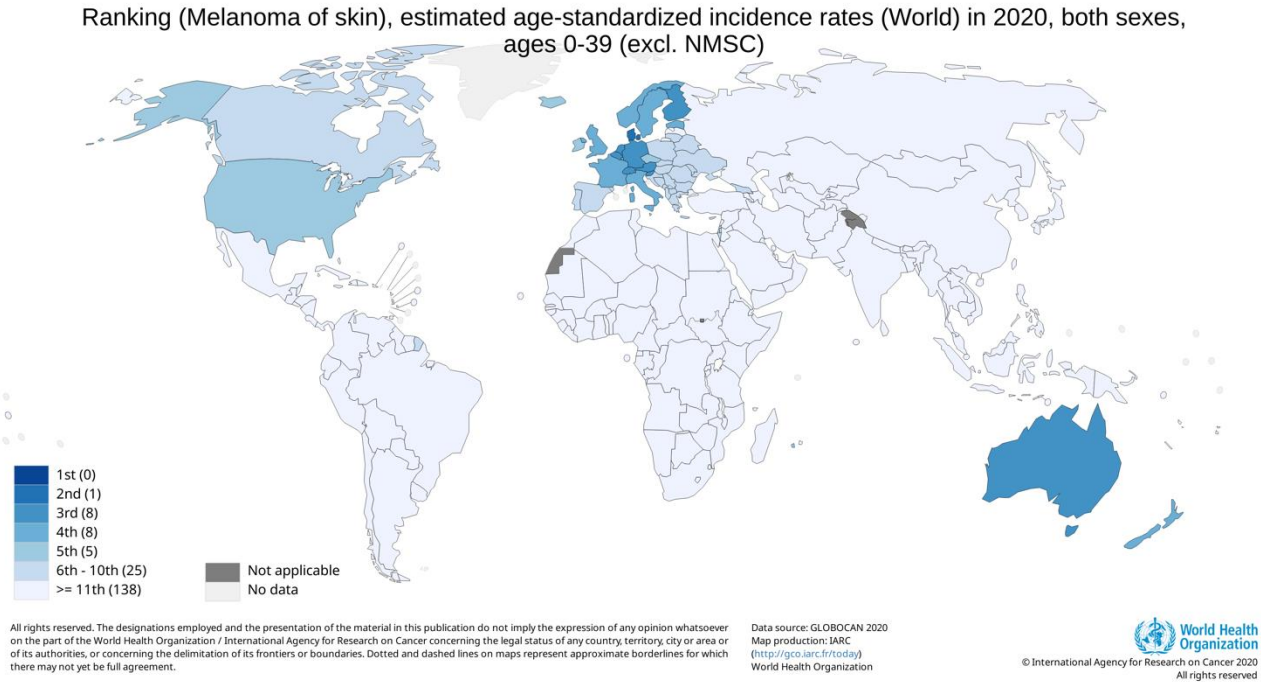

Figure 1. Ranking of the estimated incidence rates of cutaneous melanoma worldwide in 2020 [2].

In Europe, melanoma ranks as the third most common cancer type among individuals aged 0-39, following breast and thyroid cancer, with an estimated 16,481 new cases of melanoma in 2020 in this age group [1]. The majority occur in females, with around 10,000 new cases annually [1].
In Australia and New Zealand, within the same age range, it is estimated that there are 1,200 new cases annually, making it the second most prevalent cancer type after breast cancer [1]. Similarly, there is a higher occurrence of melanoma in females, with approximately 730 new cases reported each year [1] (Figure 2).

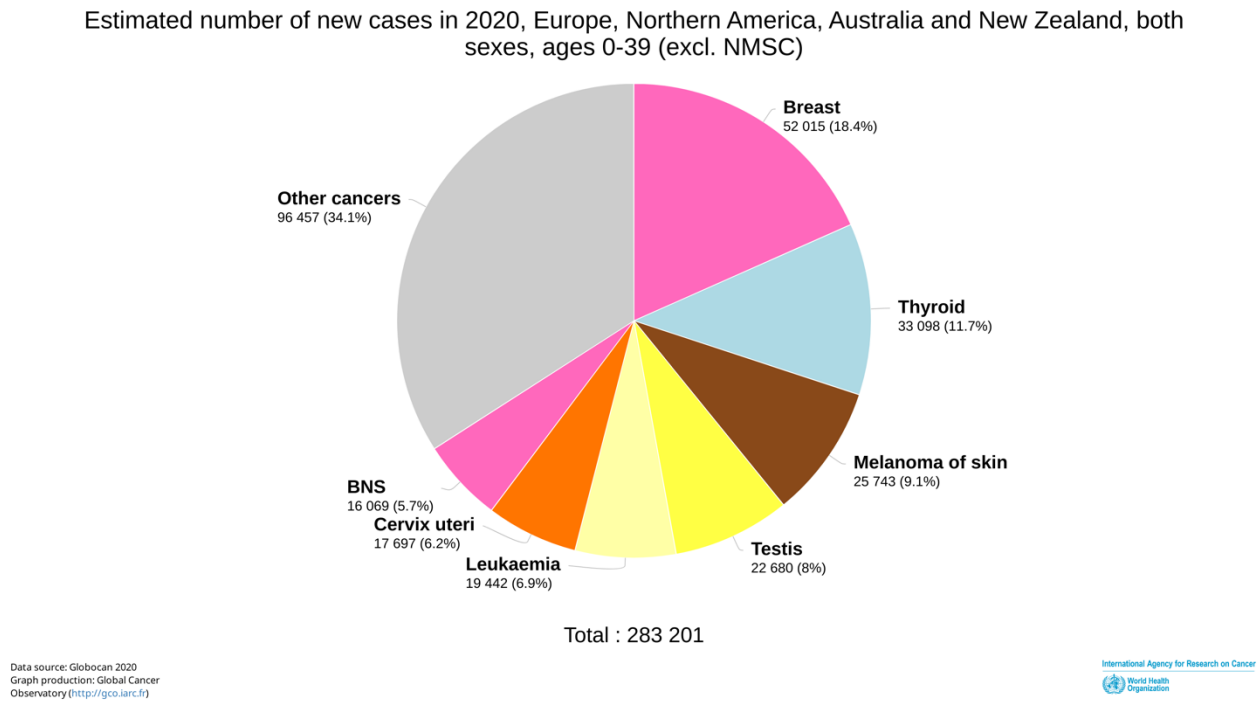

Figure 2. Estimated new cases of cancer in 2020 in Europe, Northern America, Australia and New Zealand in people aged 0-39 [2].

The introduction of effective therapies in the curative setting has significantly improved the survival rates of melanoma patients, including women of reproductive age. Important advancements have been made in recent years, with phase 3 clinical trials published since 2017 leading to the incorporation of anti-PD-1 inhibitors (nivolumab and pembrolizumab) and BRAF/MEK inhibitors (dabrafenib/trametinib) into adjuvant therapy for high-risk operated melanoma [3–5].
Furthermore, ongoing studies are exploring the use of pembrolizumab [6] and encorafenib/binimetinib (NCT05270044) in even earlier stages of melanoma, with some trials still recruiting participants and others already in the follow-up phase. In parallel, investigations are underway to evaluate the combination of anti-PD-1 and anti-CTLA-4 inhibitors as well as BRAF/MEK inhibitors in the neoadjuvant setting [7].

However, while the impact on fertility and ovarian function of chemotherapy agents (used in the treatment of breast cancer or in other hematologic malignances) is well known [8], there is still a lack of consistent data regarding novel the MAP kinase pathway inhibitors and immune-checkpoint inhibitors (ICIs) used in melanoma.

These treatments may have an impact on several steps of fertility. Conception may be affected due to a decrease in libido and potential endocrinopathies involving the pituitary hormones axis or directly causing immune-related gonadotoxicity. Additionally, these therapies may also affect a potential pregnancy through dysregulation of the maternal-fetal immune tolerance, particularly with immune checkpoint inhibitors (ICIs), or through the teratogenic risk associated with BRAF/MEK inhibitors. Lastly, possible risks of pregnancy on melanoma recurrence are still unknown.

In addition, the impact of fertility preservation techniques or pregnancy on melanoma and its treatment is still not fully understood [9]. The correlation between melanoma and pregnancy or fertility treatments

remains uncertain, and further research is needed to gain a comprehensive understanding of this relationship [9,10]. For individuals diagnosed with melanoma who are considering fertility preservation through methods like oocyte or embryo cryopreservation, it is important to consider that this process may introduce a delay of 2-5 weeks before starting anticancer therapy [10]. Therefore, it may not be suitable for those who require immediate treatment. This underscores the need for conducting thorough studies to better comprehend the effects of ICI therapy on fertility potential. Such research is essential in providing informed guidance and making appropriate decisions regarding fertility preservation options for melanoma patients.

The use of ICIs in cancer treatment has the potential to affect fertility. Immunotherapy can lead to both primary hypogonadism, which refers to decreased function of the gonads (testes in males and ovaries in females), and secondary hypogonadism, which occurs due to abnormalities in the hypothalamus or pituitary gland [11]. Furthermore, immunotherapy may have an impact on sexuality and libido. The precise mechanisms by which ICIs can alter these aspects are not yet fully understood, but it is recognized that immune-related adverse events can influence sexual function and desire [11]. Given these considerations, it is important for healthcare providers to discuss and monitor fertility, as well as sexual health, when administering immunotherapy to patients.

Guidelines recommend patients to receive counseling regarding the importance of avoiding pregnancy during their treatment [12]. It is generally recommended to abstain from conceiving for a period of 4-5 months following the completion of immunotherapy or combined BRAF/MEK inhibitors.
The dysregulation of PD-1/PD-L1, a key pathway targeted in melanoma treatment, may have an impact on the pathophysiology of various pregnancy-related conditions [12]. This includes conditions like preeclampsia, peripartum cardiomyopathy, and gestational diabetes mellitus [12]. Consequently, it is crucial to be aware of these potential effects when considering pregnancy after undergoing ICI treatments.

During pregnancy, ICIs could cross the placental interface, particularly in the third trimester. However, there is currently a lack of studies evaluating the impact of ICI therapy on the developing fetus's immune system [11]. Fetomaternal tolerance is a crucial process that enables the embryo and fetus to evade maternal immune surveillance, thereby avoiding rejection during normal pregnancy. This tolerance involves both innate and adaptive immune responses, which are mediated by mechanisms such as nonclassical major histocompatibility complex (MHC) expression by trophoblast cells and regulation of the complement system [11].
When it comes to MAPK pathway inhibitors, the potential teratogenic effects cannot be completely ruled out. Germline mutations in the RAS/MAPK pathway can lead to a group of syndromes collectively known as RASopathies [12]. These syndromes are characterized by distinct congenital defects (such as facial features, cardiopathies, skeletal abnormalities), developmental delay, mental retardation, and a predisposition to tumors. RASopathies are generally attributed to the hyperactivation of the RAS/MAPK signaling pathway. While there is currently no clinical evidence suggesting that BRAF and/or MEK inhibitors specifically have teratogenic effects, the possibility cannot be completely excluded either.

Concerning the direct gonadotoxicity of ICIs, a monocenter, cross-sectional pilot study aimed to assess the impact of systemic treatments for melanoma on fertility [13]. The study involved male patients under the age of 60 who had been previously or currently treated with ICIs. The investigation included spermiogram analysis, assessment of sexual hormones, and questionnaires regarding sexual function and activity. A total of 25 patients were included in the study, with a median age of 49 years [13]. Among these patients: four individuals displayed abnormal spermiogram results [13]. However, it was determined that three of them

had significant confounding factors unrelated to ICI treatment. One case of azoospermia, a condition characterized by the absence of sperm in semen, was likely attributed to ICI therapy [13]. Another case experienced a deterioration in the parameters of oligoasthenoteratospermia, a condition characterized by low sperm count, poor motility, and abnormal morphology [13]. This deterioration was possibly associated with ICI treatment.

The impact of anti-PD-1 and anti-CTLA-4 immunotherapies on ovarian function was investigated through experiments using both tumor-bearing and tumor-free mouse models [14]. The findings revealed that immune checkpoint inhibition leads to increased infiltration of immune cells and the expression of TNF-α, a crucial cytokine involved in various ovarian processes such as follicle atresia, ovulation, and corpus luteum regression [14]. These effects of immune checkpoint inhibitors (ICIs) were observed to diminish the ovarian follicular reserve and impair the maturation and ovulation capacity of oocytes [14]. Consequently, there is a potential risk of immediate and future fertility impairment associated with ICIs. Considering the possible adverse effects on fertility, it is crucial to prioritize studies focusing on the impact of these immunotherapies in women. Fertility preservation measures should be strongly considered for women undergoing treatment with ICIs, and further research should investigate preventative strategies to mitigate potential fertility-related consequences [15].
These findings highlight the importance of understanding and addressing the impact of ICIs on ovarian function to provide appropriate counseling, fertility preservation options, and potentially develop interventions to safeguard fertility in women receiving these immunotherapies.

Measurement of anti-Mullerian hormone (AMH) allows detection and quantification of incomplete ovarian function loss (unlike amenorrhea, follicle-stimulating hormone, and oestradiol, which only reliably reflect total ovarian failure) [16,17]. This project aims to address this clinical need by assessing (through AMH [16,17]) the ovarian reserve of women of childbearing age undergoing curative treatments for operated melanoma. This project aims to address this clinical need by assessing (through anti-Müllerian hormone [16]) the ovarian reserve of women of childbearing age undergoing curative treatments for operated melanoma.

1.2 Rationale of AMH as a surrogate of ovarian reserve

Anti-Müllerian Hormone (AMH) has gained considerable attention as a reliable marker for ovarian reserve. The rationale behind its use lies in its direct correlation with the number of primordial follicles in the ovary. This hormone is secreted by the granulosa cells of small, growing follicles in the ovary. Serum levels of AMH are highly correlated with the count of growing follicles, making AMH a valuable marker for ovarian reserve and receiving increasing attention for this purpose [18]. By measuring AMH levels, clinicians can estimate the ovarian reserve, aiding in the prediction of ovarian response to stimulation during assisted reproductive technologies. The advantage of using AMH as a surrogate marker for ovarian reserve is its relatively stable expression throughout the menstrual cycle, making it a reliable predictor [19]. In fact, AMH levels are less influenced by external factors such as hormonal fluctuations, thus providing a more accurate reflection of ovarian reserve compared to other hormonal markers. Consequently, AMH has become an essential tool in reproductive medicine for counseling and individualizing treatment strategies for patients seeking fertility preservation or undergoing assisted reproductive treatments [20].

2. Objectives

This project is a non-profit, multicentre, prospective, observational study with the primary objective to evaluate, in women of childbearing age, the variation in ovarian reserve after completion of adjuvant therapy with BRAF/MEK inhibitors or anti-PD-1 agents.

Secondary objectives are an assessment long-term fertility after completion of adjuvant therapy and the early impact on fertility preservation of a short course of therapy.

2.1 Primary endpoint

Serum anti-Müllerian hormone (AMH) at 18 months after the start of adjuvant therapy.

2.2 Secondary endpoints

- correlation between baseline/post-treatment serum AMH and pregnancy rate
- correlation between baseline/post-treatment serum AMH and menstrual activity
- ratio between desired (DP)/ obtained (OP) pregnancies
- AMH at 3 months after the start of adjuvant therapy
- AMH at 12 months after the start of adjuvant therapy
- Other reproductive outcomes.

The 3-month time point was chosen as it represents the median time from the onset of immune-related endocrine adverse events [21], and to allow evaluating a potential early impact of treatments. Meanwhile, the 18-month time point was selected to ascertain whether, in the case of fertility impairment, it is reversible or not, considering that the duration of therapy is 12 months, and to allow evaluating a potential late effect of immunotherapy.

3. Methods

3.1 Study design

This is a multicentre, observational, prospective study. A total of 270 patients will be enrolled in the study according to inclusion and exclusion criteria. BRAF mutational assessment will be performed as per clinical practice and patients with resected stage II, III and IV melanoma will then be treated with BRAF/MEK inhibitors or anti-PD-1 or observation as per clinical practice (Figure 3).

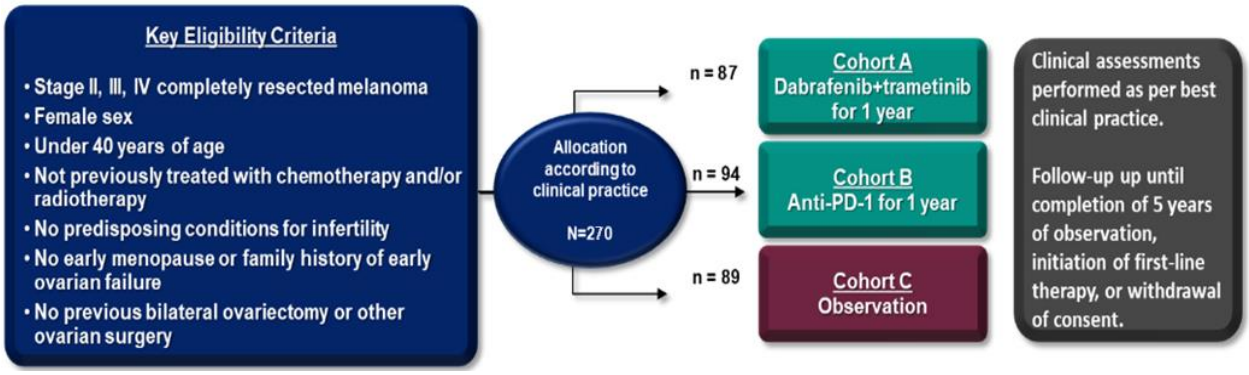

Figure 3. Study design

All patients who meet the eligibility requirements will be assessed at baseline for fertility outcomes (AMH, FSH, 5-beta oestradiol) and will be given a questionnaire (questionnaire A) to collect data on menstrual cycles, pregnancies, timing of pregnancies. Data on patient characteristics, tumor characteristics, types of anticancer treatments and reproductive outcomes will be collected through eCRF. Patients will then be re-evaluated by laboratory tests (AMH, FSH, 17beta-estradiol) and questionnaires (questionnaire B) related to their childbearing desire, menstrual cycles, pregnancies, and their outcomes at 12, 18, 24, 36 and 60 months from the start of therapy (Figure 4).

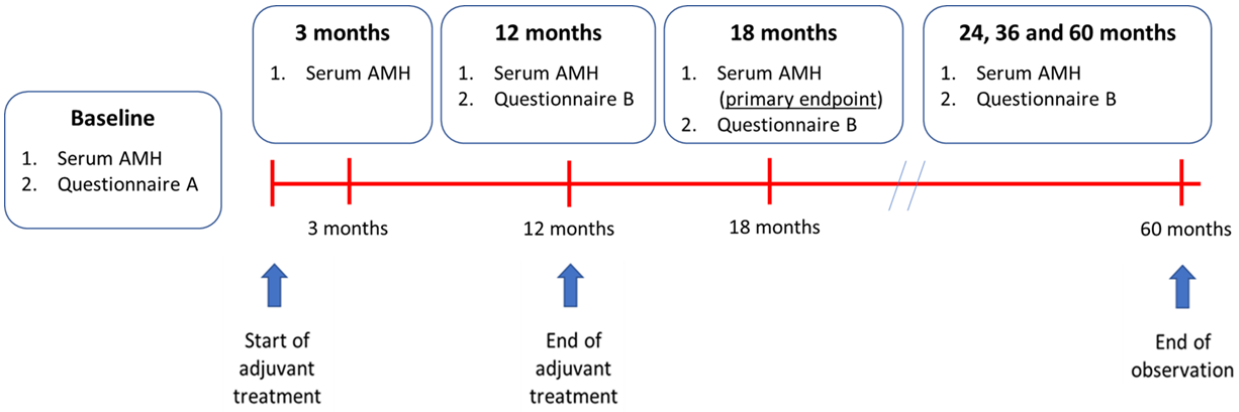

Figure 4. Key study assessments at different timepoints. AMH: antimullerian hormone

AMH will also be re-evaluated at 3 months after the start of adjuvant therapy (or observation) to assess the early impact on fertility of a short course of therapy (Table 1).

	Screening	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**
	Within 28 days to the start of adjuvant therapy or observation	3 months +/- 15 days	12 months +/- 15 days	18 months +/- 15 days	24 months +/- 15 days	36 months +/- 30 days	60 months +/- 30 days
Informed consent	x						
Eligibility criteria	x						
Age	x						
Medical history	x						
PS ECOG	x		x	x	x	x	x
Concomitant therapies	x		x	x	x	x	x
Weight	x		x	x	x	x	x
Height	x						
Questionnaire A	x						
Questionnaire B			x	x	x	x	x
Serum collecting	x	x	x	x	x	x	x
Endocrine adverse events			x	x	x	x	x

Table 1. Study procedures. ECOG PS: Eastern Cooperative Oncology Group Performance Status.

AMH assessments will be performed centrally at the IRCCS Ospedale Policlinico San Martino, Genoa, Italy. Every Institution will be responsible for collecting blood samples, processing them, and sending by courier the frozen serum samples stored at -20 °C for centralized analysis.
Clinical assessments will be performed as per best clinical practice. Patients will be followed-up until completion of 5 years of observation, initiation of first-line therapy (for advanced disease) or withdrawal of consent.

3.2 Study population

Reproductive-age female patients with completely resected stage II, III, IV melanoma, irrespective of BRAF mutational status, with no previous history of chemo, radiation therapy and/or ovarian surgery.

All patients meeting the eligibility criteria eligible belonging to the participating centers will undergo a fertility assessment (through AMH, FSH, 5-beta oestradiol testing) and will be included in one of the three cohorts in the study.

Cohort A: patients starting adjuvant therapy with dabrafenib + trametinib (BRAF/MEK inhibitors).
Cohort B: patients starting adjuvant therapy with nivolumab or pembrolizumab (anti-PD-1)
Cohort C: patients who will not initiate adjuvant therapy, but will undergo observation (due to refusal, comorbidities, other reasons).

3.3 Inclusion criteria

a) Stage II, III, IV completely resected melanoma
b) Female sex
c) Under 40 years of age
d) Not previously treated with chemotherapy and/or radiotherapy
e) Being able to give written informed consent.

3.4 Exclusion criteria

a) Unresectable melanoma
b) Predisposing conditions for infertility
c) Early menopause or family history of early ovarian failure (idiopathic, < 45 years)
d) Previous bilateral ovariectomy or other ovarian surgery
e) Personal history of autoimmune diseases, endocrine disorders (except for hypothyroidism)
f) Personal history of severe mental disorders associated with infertility (e.g., nervous anorexia) and/or requiring treatments that could impair fertility
g) Inability to give written informed consent.

4. Evaluation criteria

Patients will be recruited by investigators from participating Institutions, upon obtaining written informed consent, according to the inclusion criteria of the study. Clinical and data on histopathologic characteristics, surgery, medical therapy, follow-up, and any other proposed interventions included in the study objectives, will be recorded within a database, for which software will be selected, in compliance with current regulations regarding the processing of personal data. Each patient will be matched with an alphanumeric code. Patients will be followed-up according to clinical practice for assessment of response to treatment, disease progression and overall survival. Tumor stage will be determined according to the TNM (Tumor, Node, Metastasis) classification system of the AJCC (American Joint Committee on Cancer) [22].[22]. Adverse events will be collected according to the CTCAE (Common Terminology Criteria for Adverse Events) version 5.0.

The analysis will be conducted by comparing the following endpoints: ovarian reserve measured over time as variations in AMH values and the ratio of achieved pregnancies in relation to expected pregnancies in the different cohorts. Patients will be followed-up until completion of 5 years of observation, withdrawal of informed consent, or initiation of first-line therapy.

The data analysis will involve describing the variability of clinical parameters in the entire observed sample and producing descriptive analyses of individual clinical parameters on the entire sample and subgroups of patients identified based on demographic variables (e.g., age).

5. Sample Size and Statistical Analysis

Primary endpoint is the serum AMH level after 18 months from the start of adjuvant therapy (or of enrolment for patients in cohort C). We estimated that the two treatment arms compared to the observation arm will have a 20% drop in AMH values compared to the observation arm.

We expected an 18-months relapse rate of:

- Cohort A: patients receiving dabrafenib+trametinib: 23% ([4]);- Cohort A: patients receiving dabrafenib+trametinib: 23% ([4]);
- Cohort B: patients receiving anti-PD-1: 29% ([3,5]);- Cohort B: patients receiving anti-PD-1: 29% ([3,5]);
- Cohort C: control arm: 25% ([3–6,22]). The control group will mainly consist of stage II and stage IIIA patients with favourable prognostic features (in whom adjuvant therapy may not be administered).- Cohort C: control arm: 25% ([3–6,22]). The control group will mainly consist of stage II and stage IIIA patients with favourable prognostic features (in whom adjuvant therapy may not be administered).

The sample size has been estimated with the ANOVA method (3 groups) assuming a 5% type-1 error (statistical significance), an 80% of study power, and a mean AMH reduction of 20% in the two treatment arms compared to the untreated patients, setting the within-group variance at 18%, a total of 67 patients per arm are needed to obtain a statistically significant difference between the mean AMH. Considering different recurrence rates in the 3 groups:

- Cohort A: dabrafenib+trametinib: (x-0.23x) =67 → x=67/0.77 resulting in 87 patients
- Cohort B: anti-PD-1: (x-0.29x) =67 → x=67/0.71 resulting in 94 patients
- Cohort C: control: (x-0.25x) =67 → x=67/0.75 resulting in 89 patients.

6. Data management and quality control

The CRF data will be managed by a CRA. The data will be cleaned, edited, and queries will be issued queries to the investigators. The CRF will be designed to uniquely collect only the data needed for the study and to allow a simple process in searching for data of interest. It will be used an electronic data capture system rather than a paper CRF so that the majority of the checks can be performed at the time of entry, reducing the need for follow-up and correction of data through a subsequent manual query process. It will be developed a data validation plan to document the steps taken to ensure the completeness and accuracy of the data collected.

Information will be collected on: demographic characteristics, including age at diagnosis of stage III melanoma and primary melanoma characteristics, such as tumor Breslow's thickness, primary site, presence or absence of ulceration, mitotic rate, BRAF mutational status; staging according to AJCC 8th edition classification; surgical treatment modality; adjuvant treatment modality, including type used, duration, and reason for discontinuation of therapy; and therapy; presence and characteristics of melanoma recurrence, including stage of recurrence and their treatment; survival status of the patient. Moreover, information will be collected through questionnaires regarding concomitant therapies, biometric data, tobacco smoking and alcohol drinking habits; menstrual status, childbearing intention, the use of contraceptive methods (and types), history of pregnancies, miscarriages, voluntary terminations of pregnancy, deliveries (and delivery characteristics with associated complications).

The study will be conducted according to applicable ICH and GCP guidelines. Follow-up will be conducted to ensure protocol adherence, data quality and regulatory compliance.

7. Protection of human subjects

The investigator must ensure that the necessary ethics committee approvals are obtained before starting the study. The investigator must ensure that the consent form is obtained from each patient enrolled. The investigator, or the person designated by the investigator, must inform the Subject regarding all relevant aspects. Each patient must be informed about the voluntary nature of participation in the study and the possibility of withdrawing consent at any time. The language used in oral and written information should be simple, non-technical, and understandable to each subject enrolled. Before informed consent can be obtained, the investigator or the person designated by the investigator must provide the patient with ample time and give the opportunity to reflect and discuss the details of the study with her family and her general practitioner before deciding whether or not to participate. Prior to the Subject's participation in the study, the written and personally dated informed consent form must be signed by the Subject and the person who conducted the informed consent discussion. The patient will be given copies of all the informed consent forms with a letter to the general practitioner.

8. Confidentiality and access to data

The confidentiality of information that could identify patients within the database must be protected, in compliance with privacy and confidentiality regulations in accordance with regulatory requirements. For the purpose of protecting patient identity, each patient will be assigned a univocal code, such as a series of numbers and/or letters. The data recorded with the code assigned to the patient are called "key-coded data". Key-coded study data will be managed by the sponsor and/or its delegates in a study-specific electronic

database (the “study database”). Only the investigator and the site staff have access to the link between the code assigned to the patient and the identity of the patient. However, in the event of an audit or inspection, in compliance with local laws and regulations, official’s government, internal review board, ethical committee representatives, and promoter representatives will be able to access this information at the study site.
All parties involved in the development of the study will maintain strict confidentiality to ensure that neither the person nor the privacy of the patient family participating in the registries are violated. The data will be processed only by authorized staff in a manner that complies with local law on privacy and the General Data Protection Regulation 2016/679 (GDPR) of the European Union. In order to protect the identity of persons involved in the study, in accordance with privacy regulations (GDPR 2016/679; DL 30/06/2003 N.196; DL101 of 10/08/2018), the patient must have consented in writing to the processing of personal data and direct access to their related medical files by personnel authorized by the sponsor and regulatory authorities. This consent must be provided with the written informed consent.
Patients identity will be kept confidential even if the study is published or in case of presentation of the study at scientific congresses. All patient information will be covered by strict confidentiality in accordance with the EU General Data Protection Regulation (GDPR) and local law (GDPR 2016/679; DL 30/06/2003 No.196; DL101 of 10/08/2018) on the protection of privacy.
Signing this protocol implies that the investigator agrees to conduct the study according to the standards of good clinical practice (GCP, as defined in CPMP/ICH/135/95). The investigator agrees to retain all essential documents required by GCP, all source documents, the subject identification list, all informed consent forms signed by the parties for the period of time established by the applicable laws.

9. Pharmacovigilance

The sponsor is responsible for collecting all Serious Adverse Events (SAEs), reports of drug exposure during pregnancy, and reports of drug abuse and misuse (irrespective of whether a clinical event has occurred). The collection of individual non-serious AEs and reports describing a special scenario other than drug abuse/misuse should follow local regulations. In addition, a summary should be included in the interim and final study reports to Novartis.
In accordance with local regulations, all collected SAEs in subjects exposed to a drug, reports of drug exposure during pregnancy, and reports of drug abuse and misuse must be transferred to the respective pharmaceutical company. This transfer should occur within 15 days of awareness of these events. Additionally, the sponsor may choose to periodically transfer non-serious AEs and non-serious Adverse Drug Reactions (ADRs) to the pharmaceutical company, provided that local regulations are met.
Individual transfer of reports describing a "special scenario" other than drug abuse/misuse is not required unless these are associated with an SAE or a non-serious ADR.

10. Insurance

Given the observational nature of the study and in accordance with Italian guidelines for observational studies (03/31/2008 in G.U. No. 76), no additional insurance policies will be required in addition to those already required by routine clinical practice.

11. Study duration

The planned trial milestone dates are:

Trial Start (First Patient First Visit): 01/02/2025

Recruitment End (Last Patient First Visit): 01/02/2027

Trial End (Last Patient Last Visit) for primary endpoint: 01/08/2028

Completion of Trial Report: 01/11/2028

Primary Publication Date: 01/02/2029.

12. Potential risks and benefits

No specific risks are expected from participation in this study, nor any benefits. Through the collected data, however, it will be possible to determine new diagnostic and/or therapeutic strategies that may benefit future patients.

13. Publication and data communication plan

Data from this study will be disseminated at international conferences and in international peer reviewed scientific journals in adherence to the ICMJE Authorship Guidelines (www.icmje.org). The Principal Investigator and Steering Committee members will be included in publications, and all other study participants will be included as co-authors based on the number of patients enrolled up to the maximum number of authors granted by the journal and/or scientific society.

14. Drugs supply

Drugs will not be supplied by the Sponsor, but will be delivered according to clinical practice and local regulations.

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Questionnaire A

Dear Madam,

Below are some questions of fundamental importance for the study you are participating in. Please answer them accurately, knowing that your responses will only be viewed by the attending physicians and will be recorded anonymously (as stated in the personal data processing information). Thank you for your participation.

1. Age __
2. Weight __ Height __
3. Do you smoke? □ YES □ NO □ Former smoker
4. For how many years did you smoke in total? __
5. How many cigarettes per day (20 cigarettes = 1 pack)? __
6. At what age did you have your first period (age of menarche)? __
7. Maternal menopause age: __
8. Date of last period: **__/__/__**
9. Do you use hormonal contraceptives? (e.g., birth control pill, ring, IUD, etc.)

   □ YES (which one? __)

   □ NO
10. If YES, for how long? □ stopped more than 6 months ago □ stopped less than 6 months ago □ still taking them
11. Menstrual cycle duration:

    □ no menstrual cycle for at least 3 months

    □ < 25 days

    □ 25 - 35 days

    □ >35 - 40 days
12. Have you tried (2-3 unprotected intercourse per week) to conceive in the past without success?

    □ YES (if yes, specify for how long__)

    □ NO
13. Previous pregnancies? □ YES □ NO
14. If YES:

    □ total number of pregnancies__

□ number of miscarriages__

□ number of ectopic pregnancies__

□ number of voluntary terminations__

□ number of full-term pregnancies__

15. If YES, were there any complications during pregnancy or childbirth?

    □ YES (specify__)

    □ NO

16. Were there any health problems with the newborn? □ YES □ NO

17. Have you recently experienced signs of menopause onset (hot flashes, decreased libido, vaginal dryness, mood swings)? □ YES □ NO

Questionnaire B

Dear Madam,

Below are some questions of fundamental importance for the study you are participating in. Please answer them accurately, knowing that your responses will only be viewed by the attending physicians and will be recorded anonymously (as stated in the personal data processing information). Thank you for your participation.

1. Age__
2. Weight__
3. Have you noticed any changes in weight?

   □ Increase (by how much?) __

   □ Decrease (by how much?) __
4. Do you smoke? □ YES □ NO □ Former smoker
5. If YES: □ Started after the last evaluation □ Already a smoker
6. If YES, how many cigarettes per day (20 cigarettes = 1 pack)? __
7. Date of last period: **__/__/__**
8. Do you use hormonal contraceptives? (e.g., birth control pill, ring, IUD, etc.)

   □ YES (which one? __)

   □ NO
9. If YES, for how long? □ discontinued more than 6 months ago □ discontinued less than 6 months ago □ still taking them
10. Menstrual cycle duration:

    □ no menstrual cycle for at least 3 months

    □ < 25 days

    □ 25 - 35 days

    □ >35 - 40 days
11. Have you had regular unprotected intercourse in the past months?

    □ YES (for how long?) __

    □ NO
12. Have you tried to conceive (2-3 unprotected intercourse per week) since the last visit until now?

□ YES □ NO

13. If NO:

□ Advised against by the oncologist

□ No desire for motherhood (or new pregnancies)

□ Other__

14. If YES:
□ number of pregnancies__
□ number of miscarriages__
□ number of ectopic pregnancies__
□ number of voluntary terminations__
□ number of full-term pregnancies__

15. If YES, were there any complications during pregnancy or childbirth?

□ YES (specify__)

□ NO

16. Were there any health problems with the newborn? □ YES □ NO

17. Have you recently experienced signs of menopause onset (hot flashes, decreased libido, vaginal dryness, mood swings)? □ YES □ NO