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METHODS FOR PREVENTING PREMATURE FOLLICLE ACTIVATION

Inventors: Dror Meirow et al.

FIELD OF THE INVENTION

The present invention relates to compositions and methods for preventing premature follicle activation and loss induced by medical treatment or by a disease or disorder, thereby preserving fertility in a subject.

BACKGROUND OF THE INVENTION

The ovarian primordial follicle pool in human is established during embryonic development. This pool of primordial follicles constitutes the complete supply of oocytes that have the potential to ovulate through life. The population of primordial (non-growing) follicles containing diplotene oocytes is arrested in the first meiotic prophase. A ‘reserve’ of primordial follicles is the number of primordial follicles at any given age and is ultimately depleted by continuous recruitment and degeneration until exhausted. After primordial follicle development is initiated, a small number of the follicles is destined to ovulate while the rest undergo atresia. The factors that control the initiation of primordial follicle development are crucial for female fertility.

Studies of fetal, neonatal, and adult human ovaries have shown that several millions of non growing follicles (NGF) are established at around five months of gestational age. This number declines where approximately 1,000 remain at an average age of 50–51 years. It was further estimated that for 95% of women by the age of 30 years only 12% of their maximum pre-birth NGF population is present and by the age of 40 years only 3% remains. Although about one million oocytes are present at birth in the human ovary, only about 500 (about 0.05%) of these ovulate, where the rest are wasted.

Follicle reservoir destruction is a major side effect of chemotherapy treatments in young female cancer patients. The chemotherapy treatment may cause premature menopause and infertility. Alkylating agents such as cyclophosphamide (Cy) destroys the population of NGF, resulting in loss of ovarian reserve. It was shown by some of the inventors of the present invention that the loss of ovarian reserve is due to accelerated

Current options for fertility preservation in female cancer patients include oocyte/embryo cryopreservation and ovarian tissue cryopreservation. These methods are limited in their scope, as they are not available to all patients, and other factors, such, as time, cost and the invasive nature of the procedures. Ovarian cryopreservation is associated with post-treatment transplantation which risks the patient among other drawbacks. Moreover, it was recently found that ovarian tissue transplantations involve massive follicle activation and loss (Gavish et al., Hum Reprod., 29(5): 989-996, 2013).

Anti-mullerian hormone (AMH) is produced by the granulosa cells of early growing follicles. AMH serum levels are currently used as a marker of ovarian follicle reservoir. Several studies have shown that AMH participates in two critical selection points of follicle development: it inhibits the recruitment of primordial follicles into the pool of growing follicles and also decreases the responsiveness of growing follicles to FSH (Durlinger et al. Reproduction (2002) 124, 601–609).

mTOR, is a serine/threonine kinase known to regulate cellular metabolism, growth, and proliferation. Thus, mTOR inhibitors were developed through the years for anticancer therapy. PI3K pathway which mTOR is one of its components is involved in follicle activation.

There remains an unmet need for therapeutic approaches for preserving the oocyte pool and preventing undesirable and premature follicle activation and loss induced by medical treatments or by disease or disorder.

**SUMMARY OF THE INVENTION**

The present invention provides pharmaceutical compositions, kits and methods for preventing premature follicle activation and loss, through treatment with inhibitors of PI3K activity, such as, AMH, including AMH agonist or antiMIR of AMH, mTOR inhibitors, or a combination thereof. The methods of the invention are useful for inhibition of undesired or premature activation of follicles, for preserving the depot of primordial follicles, for postponing premature menopause and/or reducing the side effects associated with premature menopause, for treating diseases and disorders associated with premature
follicle activation and/or loss and for preserving follicles during treatment that induce follicle loss through activation.

The term “premature follicle activation” is interchangeable with the term “artificially induced follicle activation” and “induced follicle activation” and refers to accelerated and/or premature follicle activation and follicle loss (also termed ‘follicle burn-out’) which is induced by a medical treatment, such as, treatment with chemotherapeutic agents, radiotherapy, ovary transplantation among other medical treatment that may induce follicle burn out. These terms also encompass early, premature, follicle activation and loss includes by diseases or disorders.

Unexpectedly, AMH induced protection of primordial follicles in ovaries treated with a chemotherapeutic drug known to reduce follicle count. Moreover, ovaries exposed to AMH had an improved ratio of growing to dormant follicles. In addition, ovaries of mice treated with temsirolimus prior to their removal exhibited reduced expression of Akt and rpS6 in a dose dependent manner. Thus, the pharmaceutical compositions, kits and methods of the invention are particularly suitable for fertile women undergoing chemotherapy as it offers an advantageous platform for preserving their fertility and prolong maintenance of the ovarian function due to larger follicle stockpile which survive treatment.

Advantageously, the pharmaceutical compositions, kits and methods of the invention are further directed to prevent or attenuate premature menopause, including chemotheraphy-induced premature menopause. Accordingly, the pharmaceutical compositions, kits and methods of the invention provide a therapeutic platform for reducing complications and relate to medical treatments and disease which cause premature menopause. In addition, the pharmaceutical compositions, kits and methods of the invention are effective in providing therapy of preserving the fertility of women suffering from a disease or disorder that accelerates follicle activation.

There is provided, according to some embodiments, a method of inhibiting premature follicle activation comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a compound selected from the group consisting of anti-mullerian hormone, anti-mullerian hormone agonist, and antiMIR of anti-mullerian hormone, wherein the follicle activation is induced by a medical treatment or a disease.
According to some embodiments, the compound is anti-mullerian hormone. According to some embodiments, the treatment is transplantation of ovarian tissue or whole ovary. According to some embodiments, said treatment is treatment with an agent that induces follicle loss. According to some embodiments, said agent is an anti-cancer agent. According to some embodiments, said agent is chemotherapy. According to some embodiments, said medical treatment is radiotherapy. According to some embodiments, said treatment comprises chemotherapy, radiotherapy and a combination thereof. Each possibility is a separate embodiment of the invention.

According to some embodiments, said administering said pharmaceutical composition is carried out in combination with said treatment. According to some embodiments, said administering said pharmaceutical composition is carried out prior to said treatment.

According to some embodiments, the disease is an accelerated follicle activation disorder. According to some embodiments, the accelerated follicle activation disorder is endometriosis.

According to some embodiments, the subject in need thereof is a subject at perimenopause.

According to some embodiments, the method further comprises transplanting ovarian tissue or whole ovary. According to some embodiments, the graft (ovarian tissue or whole ovary) is coated prior to transplantation.

According to some embodiments, said pharmaceutical composition is administered prior to said transplanting. According to some embodiments, said pharmaceutical composition is administered during the transplantation. According to some embodiments, said pharmaceutical composition is administered during the transplantation, wherein said graft is covered with alginate.

According to some embodiments, the method further comprises administering to said subject at least one inhibitor of PI3K pathway activation. According to some embodiments, the method further comprises administering to said subject at least one mTOR inhibitor.

According to some embodiments, the method further comprises administering to said subject at least one follicle reserve protective compound.
There is provided, according to some embodiments, a pharmaceutical composition comprising anti-mullerian hormone, anti-mullerian hormone agonist or antiMIR of anti-mullerian hormone for inhibiting artificially induced follicle activation.

According to some embodiments, the pharmaceutical composition further comprises at least one inhibitor of PI3K pathway activation. According to some embodiments, the at least one inhibitor of PI3K pathway activation is an mTOR inhibitor. According to some embodiments, the pharmaceutical composition further comprises at least one mTOR inhibitor. According to some embodiments, the mTOR inhibitor is a first generation mTOR inhibitor or a second generation mTOR inhibitor. According to some embodiments, the inhibitor is a rafalog. According to some embodiments, the inhibitor is Temsirolimus.

According to some embodiments, the pharmaceutical composition is for use in combination with at least one mTOR inhibitor.

There is provided, according to some embodiments, use of anti-mullerian hormone, anti-mullerian hormone agonist, antiMIR of anti-mullerian hormone, or pharmaceutical composition comprising same, for the manufacture of a medicament for inhibiting artificially induced follicle activation.

According to some embodiments, the use further comprises use of at least one mTOR inhibitor.

According to some embodiments, the use further comprises use of an inhibitor of PI3K pathway activation (not only mTOR inhibitor) to prevent follicle activation and loss ("Burn-Out").

There is provided, according to some embodiments, a kit for inhibiting induced follicle activation in a subject in need thereof comprising:

(i) a first packaging containing a pharmaceutical composition comprising a compound selected from the group consisting of anti-mullerian hormone, anti-mullerian hormone agonist, and antiMIR of anti-mullerian hormone; and

(ii) written instructions of use of said pharmaceutical composition for inhibiting follicle activation induced in said subject.

According to some embodiments, the kit further comprises a second packaging containing a pharmaceutical composition comprising at least agent that induces follicle loss.
According to some embodiments, the kit further comprises a third packaging containing at least one follicle reserve protective compound.

According to some embodiments, the kit further comprises a forth packaging containing at least one mTOR inhibitor.

There is provided, according to some embodiments, a method of inhibiting induced follicle activation comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising an inhibitor of PI3K pathway activation, such as, an mTOR inhibitor.

According to some embodiments, the premature follicle activation is induced by a medical treatment. According to some embodiments, the medical treatment is treatment with an agent that induces follicle loss. According to some embodiments, said agent is an anti-cancer agent. According to some embodiments, said agent is chemotherapy. According to some embodiments, said administering said pharmaceutical composition is carried out in combination with said medical treatment. According to some embodiments, said administering said pharmaceutical composition is carried out prior to said medical treatment. According to some embodiments, said anti-cancer agent is other than said at least one mTOR inhibitor.

According to some embodiments, the subject in need thereof is having an accelerated follicle activation disorder.

According to some embodiments, the subject in need thereof is a subject at perimenopause.

According to some embodiments, the subject in need thereof is having ovarian disorder.

According to some embodiments, the method further comprises transplanting ovarian tissue or whole ovary in said subject. According to some embodiments, said pharmaceutical composition is administered prior to said transplanting. According to some embodiments, said pharmaceutical composition is administered during said transplanting.

According to some embodiments, the method further comprises administering to said subject at least one at least one follicle reserve protective compound.

There is provided, according to some embodiments, a pharmaceutical composition comprising at least one mTOR inhibitor for inhibiting follicle activation.

There is provided, according to some embodiments, a use of at least one mTOR inhibitor, or a pharmaceutical composition comprising same, for the manufacture of a
medicament for inhibiting premature follicle activation induced by a medical treatment or by a disease.

There is provided, according to some embodiments, a kit for inhibiting induced follicle activation in a subject in need thereof comprising:

(i) a first packaging containing a pharmaceutical composition comprising at least one mTOR inhibitor; and

(ii) written instructions of use of said pharmaceutical composition for inhibiting in said subject follicle activation induced by a medical treatment or a disease.

Further embodiments, features, advantages and the full scope of applicability of the present invention will become apparent from the detailed description and drawings given hereinafter. However, it should be understood that the detailed description, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

**BRIEF DESCRIPTION OF THE FIGURES**

Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive. The figures are listed below.

**Fig. 1** shows count of primordial, primary and secondary+ follicles in whole ovaries cultured with medium alone (control; No Rx), medium and 100ng/ml AMH (AMH), phosphoramid mustard (PM) and initially cultured with PM following culture with 100ng/ml AMH (PM+AMH). *p<0.05 compared with PM, **p<0.01 compared with No Rx.

**Fig. 2** shows the ratio of growing to dormant (primordial stage) follicles in each of the treatment groups presented in Fig. 1. *p<0.05 compared with PM, **p<0.001 compared with No Rx.

**Fig. 3** shows Western blot analysis of Akt and rpS6 expression in ovaries extracted from Balb/C mice treated with 0.1, 1 or 10 mg/kg Temsirolimus, compared to a control protein (β-actin).
DETAILED DESCRIPTION OF THE INVENTION

The present invention provides pharmaceutical compositions, kits and use thereof for inhibiting or preventing follicle activation induced by medical treatment(s) or disease(s) and disorder(s). The compositions and kits of the invention comprise at least one inhibitor of PI3K pathway activation. In some embodiments, the at least one inhibitor of PI3K pathway activation comprise (1) AMH, including AMH agonist or antiMIR of AMH; (2) an mTOR inhibitor; and (3) a combination of (1) and (2). The compositions and kits of the invention may be used prior to or in combination with anti-cancer therapy. The compositions of the invention may be used in any disorder or therapeutically procedure that involves premature or undesired follicle activation and protect the PI3K pathway by inhibiting, attenuating or preventing its activation.

The terms “follicle activation”, “initiation of follicle growth” and “initial recruitment of follicle” as used herein are interchangeable. Premature or early follicle activation and eventually loss of fertility are also termed herein follicle burn out.

Anti-mullerian hormone, also termed hereinafter “AMH”, is a protein hormone typically refers to a protein designated by NCBI Accession No.: P03971. It has been also termed Müllerian inhibiting factor (MIF), Müllerian-inhibiting hormone (MIH), and Müllerian-inhibiting substance. The present invention encompasses the full AMH sequence, homologs, analogs, variants and derivative of the AMH protein or a fragment thereof, with the stipulation that the AMH activity is preserved. A mathematical model simulating the female reproductive cycle, predicted that AMH could be used to delay menopasuse (Margolskee et al., J. Theor. Biol., 326:21-35, Feb. 2013).

Without being bound by any theory or mechanism, AMH inhibits or prevents follicle activation by inhibiting or preventing recruitment of primordial follicles into the pool of growing follicles, thereby preventing undesired acceleration effect on growing follicle resulting in follicle exhaustion, as for example induced by a disease, a syndrome, invasive procedures and/or medicaments, such as, chemotherapy.

Mammalian target of rapamycin, also known as mTOR, is a serine/threonine kinase known to regulate cellular metabolism, growth, and proliferation. mTOR effects downstream pathway, forming two complexes - mTORC1 and mTORC2.
mTOR inhibitors are molecules and agents that disrupt the activity of mTOR. The first generation of mTOR inhibitors consists of rapamycin, a natural antibiotic (also termed “sirolimus”), and its analogs (collectively termed rapalogs), which includes temsirolimus (CCI-779), everolimus (RAD001), and ridaforolimus (AP-23573). The rapalogs have been shown to have a modest anticancer effect. The second generation of mTOR inhibitors, known as ATP-competitive mTOR kinase inhibitors, compete with ATP in the catalytic site of mTOR. This group of agents inhibits all of the kinase-dependent functions of mTORC1 and mTORC2 and therefore, block the feedback activation of PI3K/AKT signaling.

In some embodiments, the present invention provides a method of inhibiting induced follicle activation comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a compound selected from the group consisting of anti-mullerian hormone, anti-mullerian hormone agonist, and antiMIR of anti-mullerian hormone.

The term "agonist" as used herein refers to any chemical substance, a fragment of AMH protein, a derivative of AMH or a modified AMH protein, which capable of activating the AMH receptor, resulting with the inhibition of follicle activation. As used herein and further detailed below, the term "inhibiting follicle activation" or "preventing follicle activation" refers to a transient or permanent condition wherein some or all follicles are maintained in their primordial stage.

The term "inhibiting" as used herein includes, but is not limited to, preventing, attenuating, impeding, reducing to a certain extent, complete inhibition and/or partial inhibition.

The present invention further provides a method for inhibiting or preventing the induced follicle activation in a subject in need thereof by increasing the activity of the AMH receptor. Increasing the activity of an AMH receptor may be obtained, for example, by elevating the AMH or AMH agonist amounts. Administering AMH per se is one approach for elevating AMH amount. Another approach is by overexpressing a gene encoding for AMH. Overexpression of AMH could be achieved by gene therapy mediated by adenovirus and lentivirus vectors, however significant hurdles still exist with the implementation of gene therapy as a practical and safe therapeutic strategy.
The term "antiMIR" refers to contiguous nucleic acids, DNA or RNA, which are complementary to micro-RNA or miRNA. The antiMIR binds to the miRNA and inhibits the silencing/degrading activity it has upon the mRNA of a target gene. This results in elevation of the target gene expression. The antiMIR of the invention is targeted for miRNA that silence the AMH gene. In some embodiments, the present invention provides a method of preventing artificially induced follicle activation, comprising the step of administering antiMIR of AMH.

The term "antiMIR of AMH" refers to a molecule that inhibits AMH silencing by miRNA.

The term "complementary" in the context of the present invention refers to antiMIR sequence that has at least 90%, 95%, or 100% identity to a complementary sequence of miRNA of AMH.

The AMH protein hormone may be isolated and purified by methods selected on the basis of properties revealed by its sequence. Purification can be achieved by protein purification procedures such as chromatography methods (gel-filtration, ion-exchange and immunoaffinity), by high-performance liquid chromatography (HPLC, RP-HPLC, ion-exchange HPLC, size-exclusion HPLC, high-performance chromatofocusing and hydrophobic interaction chromatography) or by precipitation (immunoprecipitation). Polyacrylamide gel electrophoresis can also be used to isolate the AMH protein based on the molecular weight of the protein, charge properties and hydrophobicity. For example, Picard et al. (1984) describes an improved method for the purification of anti-Müllerian hormone from incubation medium of bovine fetal testes (Mol Cell Endocrinol. 1984 Jan;34(1):23-9).

According to alternative embodiments, AMH or its equivalents may be produced by the use of recombinant DNA techniques as are well known to one skilled in the art. Nucleic acid sequences which encode for the proteins of the invention may be incorporated in a known manner into appropriate expression vectors (i.e. recombinant expression vectors). Possible expression vectors include (but are not limited to) cosmids, plasmids, or modified viruses (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses, lentiviruses, herpes viruses, poxviruses), so long as the vector is compatible with the host cell used. The expression "vector...compatible with the host cell" is defined as contemplating that the expression vector(s) contain a nucleic acid molecule
of the invention and attendant regulatory sequence(s) selected on the basis of the host
cell(s) to be used for expression, said regulatory sequence(s) being operatively linked to
the nucleic acid molecule. "Operatively linked" is intended to mean that the nucleic acid is
linked to regulatory sequence(s) in a manner which allows expression of the nucleic acid.
Suitable regulatory sequences may be derived from a variety of sources, including
bacteria, fungal, or viral genes (for example, see the regulatory sequences described in
Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press,
San Diego, Calif. (1990)). Selection of appropriate regulatory sequence(s) is dependent on
the host cell(s) chosen, and may be readily accomplished by one of ordinary skill in the
art. Examples of such regulatory sequences include the following: a transcriptional
promoter and enhancer, RNA polymerase binding sequence, or a ribosomal binding
sequence (including a translation initiation signal). Depending on the host cell chosen and
the expression vector employed, other additional sequences (such as an origin of
replication, additional DNA restriction sites, enhancers, and sequences conferring
inducibility of transcription) may be incorporated into the expression vector.

In some embodiments, the present invention provides a method of inhibiting
follicle activation induced by one or more medical treatments comprising administering to
a subject a therapeutically effective amount of a pharmaceutical composition comprising a
compound selected from the group consisting of anti-mullerian hormone, anti-mullerian
hormone agonist, antiMIR of anti-mullerian hormone, at least one mTOR inhibitor and a
combination thereof.

In some embodiments, the present invention provides a method of inhibiting
follicle activation induced by a disease or disorder comprising administering to a subject a
therapeutically effective amount of a pharmaceutical composition comprising a compound
selected from the group consisting of anti-mullerian hormone, anti-mullerian hormone
agonist, antiMIR of anti-mullerian hormone, at least one mTOR inhibitor and a
combination thereof.

It is to be understood that the pharmaceutical compositions, kits and methods of
the invention are directed for treating women. Thus, the terms ‘subject’ and ‘subject in
need thereof’ refer to a female patient, including, a woman and an adolescent children.

In some embodiment, the subject is a subject having a disease or disorder
associated with loss of follicle activation and/or loss or reduced fertility.
In some embodiments, the subject is a subject having a disease or disorder associated with loss of follicle activation and/or loss or reduced fertility which are induced by a medical treatment.

In some embodiments the subject is undergoing treatment with an agent that induces follicle loss. In some embodiments, said agent is a chemotherapeutic agent.

In some embodiments, the subject is a subject having cancer. In some embodiments, the subject having cancer is being treated with anti-cancer therapy prior to and/or in parallel to treatment for inhibiting follicle activation. In some embodiments, the anti-cancer therapy is chemotherapy.

In other embodiments, the anti-cancer therapy is radiotherapy. In some embodiments, the anti-cancer treatment results in accelerated or premature follicle activation.

The term "cancer" is used herein in its broadest sense and refers to a family of diseases characterized by uncontrolled cell growth. It includes, but is not limited to, adrenocortical carcinoma, anal cancer, bladder cancer, brain tumor, brain stem glioma, brain tumor, cerebellar astrocytoma, cerebral astrocytoma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal, pineal tumors, hypothalamic glioma, breast cancer, carcinoid tumor, carcinoma, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, extrahepatic bile duct cancer, ewings family of tumors (pnet), extracranial germ cell tumor, eye cancer, intraocular melanoma, gallbladder cancer, gastric cancer, germ cell tumor, extragonadal, gestational trophoblastic tumor, head and neck cancer, hypopharyngeal cancer, islet cell carcinoma, laryngeal cancer, leukemia, acute lymphoblastic, leukemia, oral cavity cancer, liver cancer, lung cancer, small cell, lymphoma, AIDS-related, lymphoma, central nervous system (primary), lymphoma, cutaneous T-cell, lymphoma, hodgkin's disease, non-hodgkin's disease, malignant mesothelioma, melanoma, merkel cell carcinoma, metastatic squamous carcinoma, multiple myeloma, plasma cell neoplasms, mycosis fungoides, myelodysplastic syndrome, myeloproliferative disorders, nasopharyngeal cancer, neuroblastoma, oropharyngeal cancer, osteosarcoma, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, exocrine, pancreatic cancer, islet cell carcinoma, paranasal sinus and nasal cavity cancer, parathyroid cancer, pheochromocytoma cancer, pituitary cancer, plasma cell neoplasm,
rhabdomyosarcoma, rectal cancer, renal cell cancer, salivary gland cancer, sezary syndrome, skin cancer, cutaneous T-cell lymphoma, skin cancer, kaposi's sarcoma, skin cancer, melanoma, small intestine cancer, soft tissue sarcoma, soft tissue sarcoma, thymoma, malignant, thyroid cancer, urethral cancer, uterine cancer, sarcoma, unusual cancer of childhood, vaginal cancer, vulvar cancer, or wilms' tumor, benign conditions associated with chemotherapy treatments, such as,lupus, rheumatoid arthritis and skin diseases.

In some embodiments, the subject in need is having an accelerated follicle activation disease or disorder. In some embodiments, the disease is a genetic disorder such as Turner syndrome. In other embodiments, the disease is Galactosemia. In other embodiments, the disease is endometriosis.

Turner syndrome refers to a chromosomal condition that affects development in females. Turner syndrome occurs when one normal X chromosome is present in a female's cells and the other sex chromosome is missing or structurally altered. Turner syndrome is characterized by an early loss of ovarian function and accelerated follicle activation may be one of the causes for this phenomenon.

Galactosemia is an inherited disorder which is characterized by inability to metabolize the sugar galactose properly. One of the symptoms of Galactosemia is accelerated follicle activation.

According to some embodiments, the pharmaceutical composition is administered by direct delivery to the ovary. In some embodiments, the pharmaceutical composition is delivered to each ovary. In some embodiments, the pharmaceutical composition is delivered to the each ovary prior to said medical treatment.

Transplantation of frozen thawed or fresh ovarian tissue or whole ovary is a delicate procedure aimed to restore fertility to patients that had lost ovarian follicle reserve or have poor quality follicles by delivering a stock of resting non growing follicles that can serve in the future to restore and maintain follicular activity and ovulations that may enable future reproduction. However, high portion of follicles delivered back to the body by transplantation disappear rapidly due to premature follicle activation. The present invention provides pharmaceutical compositions, kits and methods directed to increase graft survival, enable future pregnancy and prolong hormone secretion. Thus, in some
embodiments, the pharmaceutical compositions, kits and methods of the invention are
directed to subjects undergoing ovarian tissue transplantation or whole ovary
transplantation.

In some embodiments, the present invention provides a method of inhibiting
follicle activation in subject undergoing ovary transplantation, the method comprises
administering to a subject a therapeutically effective amount of a pharmaceutical
composition comprising a compound selected from the group consisting of anti-mullerian
hormone, anti-mullerian hormone agonist, and antiMIR of anti-mullerian hormone,
following, prior to, or in combination with ovarian tissue or whole ovary transplantation.

The term ‘ovary transplantation’ as used herein refers to transplantation of the
whole ovary or of parts of the ovary, also termed herein ‘ovarian tissue’.

In some embodiments, said pharmaceutical composition is introduced to the patient
together with the transplanted tissue/ovary. In some embodiments, the graft is protected by
to transplantation. In some embodiments, said pharmaceutical composition is delivered
topically, directly to the ovary. In some embodiments, said graft (ovarian tissue or whole
ovary) is covered with alginate, encapsulated within alginate, or otherwise coated with
alginate. In some embodiments, said pharmaceutical composition is administered by
systemic administration.

In some embodiments, the pharmaceutical composition further comprises a
pharmaceutically acceptable carrier, excipient or diluent.

As used herein, a “pharmaceutical composition” refers to a preparation of one or
more of the active ingredients described herein, for example, AMH molecule, AMH
agonist, antiMIR of AMH, at least one mTOR inhibitor and temsirolimus, with non-active
(inert) components, such as, physiologically suitable carriers and excipients. The purpose
of a pharmaceutical composition is to facilitate administration of a compound to a subject.

As used herein, the term “therapeutically effective amount” refers to an amount of
a formulation or composition which is effective to inhibit or prevent, at least partially,
follicle activation in a living organism to whom it is administered over some period of
time.

Herein, the phrases "therapeutically acceptable carrier" and "pharmaceutically
acceptable carrier", which may be used interchangeably, and refer to a carrier or a diluent
that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

Herein, the term “excipient” refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils, and polyethylene glycols.

As used herein, a “carrier” refers to any substance suitable as a vehicle for delivering an amino acid or a nucleic acid molecule of the present invention to a suitable in vivo or in vitro site. As such, carriers can act as a pharmaceutically acceptable excipient of a therapeutic composition containing a molecule of the present invention. Carriers of the present invention include: (1) excipients or formularies that transport, but do not specifically target a nucleic acid molecule to a cell (referred to herein as non-targeting carriers); and (2) excipients or formularies that deliver an amino acid or nucleic acid molecule to a specific site in a subject or a specific cell (i.e., targeting carriers). Examples of non-targeting carriers include, but are not limited to, water, phosphate buffered saline, Ringer’s solution, dextrose solution, serum-containing solutions, Hank’s solution, other aqueous physiologically balanced solutions, oils, esters and glycols. Aqueous carriers can contain suitable auxiliary substances required to approximate the physiological conditions of the recipient, for example, by enhancing chemical stability and isotonicity.

Suitable auxiliary substances include, for example, sodium acetate, sodium chloride, sodium lactate, potassium chloride, calcium chloride, and other substances used to produce phosphate buffer, Tris buffer, and bicarbonate buffer. Auxiliary substances can also include preservatives, such as thimerosal, m- and o-cresol, formalin and benzol alcohol. Therapeutic compositions of the present invention can be sterilized by conventional methods.

The pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g. by means of conventional mixing, dissolving, granulating, grinding, pulverizing, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

The compositions described herein may be prepared by known methods for the preparation of pharmaceutically acceptable compositions intended for administration to a
subject, such that an effective quantity of the active substance (e.g. AMH, mTOR inhibitor or their combination) is combined in a mixture with a suitable pharmaceutically acceptable vehicle as known in the art. On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and may be contained in buffered solutions with a suitable pH and/or be iso-osmotic with physiological fluids.

Furthermore, the pharmaceutical compositions according to the invention may comprise one or more stabilizers, such as, for example, carbohydrates including sorbitol, mannitol, starch, sucrose, dextrin and glucose, proteins such as albumin or casein, and buffers like alkaline phosphates.

In some embodiments, administering the pharmaceutical composition comprises administering via a route selected from the group consisting of: subcutaneous, topical, transdermal, oral, buccal, sublingual, sublabial, intradermal, intravaginal or combinations thereof. Each possibility is a separate embodiment of the invention. In some embodiments, administering the pharmaceutical composition comprises direct delivery to the ovary. In some embodiments, administering the pharmaceutical composition comprises direct injection to the ovary.

Administration of an “effective amount” of the pharmaceutical compositions of the present invention refers to administration of an amount effective at dosages and for periods of time, necessary to elicit a desired therapeutic response in a human. A therapeutically effective amount of a substance may vary according to the follicle activator factor or cause, age, sex, and weight of the recipient. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or on at periodic intervals, and/or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. The amount pharmaceutical composition for administration will depend on the route of administration, time of administration and varied in accordance with individual subject responses.

There is provided, in some embodiments, a kit for the inhibiting or preventing follicle activation in a subject in need thereof, the kit comprising:
(i) a first packaging containing a pharmaceutical composition comprising a compound selected from the group consisting of anti-mullerian hormone, anti-mullerian hormone agonist, and antiMIR of anti-mullerian hormone; and

(ii) written instructions for of use of said pharmaceutical composition for inhibiting follicle activation in said subject.

In some embodiments, the kit further comprises a second packaging containing a pharmaceutical composition comprising at least one anti-cancer agent.

In some embodiments, the pharmaceutical composition further comprises at least one follicle reserve protective compound and a pharmaceutically acceptable carrier, diluent or excipient.

In some embodiments, the at least one follicle reserve protective compound comprises sphingosine-1-phosphate, Tamoxifene, GnRH, trichloro(dioxoethylene-O-O') or a combination thereof. ???

In some embodiments, the pharmaceutical composition in the first packaging may further comprise at least one anticancer agent.

In some embodiments, the kit further comprises at least one PI3K inhibitor. In some embodiments, the kit further comprises at least one mTOR inhibitor. In some embodiments, the at least one mTOR inhibitor is provided in a third packaging. In some embodiment, the at least one mTOR inhibitor is provided in the first packaging. In some embodiments, the at least one mTOR inhibitor is provided in the second packaging. In some embodiment, the at least one mTOR inhibitor is different from the at least one anticancer agent. In some embodiments the at least one anticancer agent is a first mTOR inhibitor and the at least one mTOR inhibitor is a second mTOR inhibitor. In some embodiments, said first mTOR inhibitor and said second mTOR inhibitor are different. In some embodiment, said first mTOR inhibitor and said second mTOR inhibitor are the same mTOR inhibitor.

The term “kit” as used herein is interchangeable with the term package, and refers to packages of pharmaceutical formulations containing any one or more of anti-mullerian hormone, anti-mullerian hormone agonist, antiMIR of anti-mullerian hormone, at least one mTOR inhibitor and further containing, together, or in a different packaging, the anticancer agent. Accordingly, the kit may be organized to indicate a single formulation or
combination of formulations to be taken at each desired treatment regimen as specified in written instructions encompassed in the kit.

In some embodiments, the kit contains packaging or a container with each of said first and second and third pharmaceutical compositions, formulated for the desired delivery route. Suitably, the kit contains instructions on dosing and an insert regarding the active agent. Optionally, the kit may further contain instructions for monitoring circulating levels of product(s) and material(s) that may be used for evaluating treatment efficacy. For performing such evaluation assays that kit may further include reagents, well plates, containers, markers or labels, and the like. Such kits are readily packaged in a manner suitable for treatment of a desired indication. The kit may also contain instructions for use of a delivery device. Other suitable components to include in such kits will be readily apparent to one of skill in the art, taking into consideration the desired indication and the delivery route.

The compositions described herein can be a single dose or for continuous or periodic discontinuous administration. For continuous administration, the package or kit may include each of the pharmaceutical compositions in their dosage unit (e.g., solution, lotion, tablet, pill, or other unit described above or utilized in drug delivery), and optionally instructions for administering the doses daily, weekly, or monthly, for a predetermined length of time or as prescribed. When the pharmaceutical compositions are to be delivered periodically in a discontinuous fashion, the package or kit can include placebos during periods when the pharmaceutical compositions are not delivered. When varying concentrations of a composition, of the components of the composition, or the relative ratios of the components of the pharmaceutical composition or the ratio of the first pharmaceutical composition to the second pharmaceutical composition over time is desired, the package or kit may contain a sequence of dosage units which provide the desired variability.

A number of packages or kits are known in the art for dispensing pharmaceutical agents for periodic oral use. In some embodiments, the package has indicators for each period. In other embodiments, the package is a labeled blister package, dial dispenser package, or bottle.

The packaging means of a kit may itself be geared for administration, such as an inhaler, syringe, pipette, eye dropper, or other such apparatus, from which the
pharmaceutical composition(s) may be applied to an affected area of the body, such as the arms, injected into a subject, or even applied to and mixed with the other components of the kit.

The compositions of the kit of the invention also may be provided in dried or lyophilized forms. When reagents or components are provided as a dried form, reconstitution generally is by the addition of a suitable solvent. It is envisioned that the solvent also may be provided in another packaging of the kit.

The kit of the present invention also will typically include a means for containing the vials in close confinement for commercial sale such as, e.g., injection or blow-molded plastic containers into which the desired vials are retained. Irrespective of the number or type of packages and as discussed above, the kit also may include, or be packaged with a separate instrument for assisting with the injection/administration or placement of the composition within the body. Such an instrument may be an inhaler, syringe, pipette, forceps, measuring spoon, eye dropper or any such medically approved delivery means.

In some embodiments, the pharmaceutical compositions of the kit are provided in the presence or absence of one or more of the carriers or excipients described above. The kit may optionally contain instructions for administering the pharmaceutical composition to a subject having a disease associated with premature follicle activation or having a condition requiring to inhibit or attenuate follicle activation in order to protect fertility.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.
EXAMPLES

Example 1: AMH prevents follicle loss in ovaries treated with a chemotherapy drug.

To examine the effect of AMH on ovaries, ovaries of neonatal mice were cultured in-vitro in the presence of the cyclophosphamid metabolite, phosphoramid mustard (PM) for 2 hours. The ovaries then cultured with or without 100ng/ml AMH (PM+AMH or PM, respectively). Control ovaries were cultured with medium alone (No Rx) or with only AMH (AMH). Ovaries were removed after day 4 in culture and processed for histological analysis. The number of primordial, primary, and secondary+ stage follicles was counted. Ovaries exposed to PM alone had significantly reduced numbers of primordial follicles on day 4 culture, compared with untreated ovaries (Figure 1). Ovaries exposed to PM and AMH had significantly greater numbers of primordial follicles than with the PM alone.

Example 2: AMH improves the ratio of growing to dormant follicles following a treatment with a chemotherapy agent.

The ratio of growing/dormant follicles was examined in ovaries treated with the chemotherapy drug and/or AMH. Significant differences between the treatments were observed on day 4. The ratio of growing to dormant follicles was greatest in the ovaries exposed to PM alone. The ratio was significantly improved in ovaries exposed to PM + AMH.

Overall, the results indicate that AMH reduces chemo-induced follicle activation, suggesting its potential in protecting follicle reserve in young female cancer patients.

Example 3: mTOR inhibitors protect fertility

Post-natal female Balb/C mice (12 days) were injected IP with 0.1,1 or 10 mg/kg Temsirolimus or with an equivalent volume of vehicle control every other day beginning on day 1 through day 11, and their ovaries were removed on day 12. Ovaries were snap-frozen in liquid Nitrogen and stored at -80°C for Western blotting. Protein analysis showed decreasing expression of Akt and pS6 with increasing doses of Temsirolimus, indicating that IP treatment with Temsirolimus effectively inhibits the PI3K/PTEN/Akt pathway in vivo (Fig. 3).
Weights of mice in all groups were not significantly different and no adverse health effects were observed in Temsirolimus treated mice.

The results suggest use of Temsirolimus, as well as other mTOR inhibitors and agents capable of inhibiting PI3K/PTEN/Akt pathway, for preserving fertility.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.
FIGURE 1
FIGURE 2
FIGURE 3
**INVENTOR(S)**

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Additional inventors are being named on the separately numbered sheets attached hereeto.

**TITLE OF THE INVENTION (500 characters max):**

METHODS FOR PREVENTING PREMATURE FOLLICLE ACTIVATION

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

- The address corresponding to Customer Number: 125762

**ENCLOSED APPLICATION PARTS (check all that apply)**

- Application Data Sheet. See 37 CFR 1.76
- Drawing(s) \ Number of Sheets 3
- Specification (e.g. description of the invention) \ Number of Pages 26

**Fees Due:** Filing Fee of $250 ($125 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(a).

**METHOD OF PAYMENT OF THE FILING FEE AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT**

- Applicant claims small entity status. See 37 CFR 1.27.
- A check or money order made payable to the Director of the United States Patent and Trademark Office is enclosed to cover the filing fee and application size fee (if applicable).
- Payment by credit card. Form PTO-2038 is attached.
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This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 10 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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SIGNATURE /Cynthia Webb/ Date August 31, 2014

TYPED or PRINTED NAME Cynthia A. Webb

TELEPHONE +972 (8) 948-4666 Docket Number: SBA/013 USP

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Drawings-only black and white line drawings

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Total Files Size (in bytes): 360305
CLAIMS

1. A method of inhibiting premature follicle activation comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a compound selected from the group consisting of anti-mullerian hormone, anti-mullerian hormone agonist, and antiMIR of anti-mullerian hormone, wherein the premature follicle activation is induced by a medical treatment or by a disease.

2. The method of claim 1, wherein the compound is anti-mullerian hormone.

3. The method of claim 1, wherein said treatment is transplantation of ovarian tissue or whole ovary.

4. The method of claim 1, wherein said treatment is treatment with a follicle loss inducing agent.

5. The method of claim 1, wherein said treatment comprises chemotherapy, radiotherapy and a combination thereof.

6. The method of claim 1, wherein said administering said pharmaceutical composition is carried out in combination with said medical treatment.

7. The method of claim 1, wherein said administering said pharmaceutical composition is carried out prior to said medical treatment.

8. The method of claim 1, wherein the disease is an accelerated follicle activation disorder.

9. The method of claim 1, wherein the subject in need thereof is a subject at perimenopause.

10. The method of claim 2, further comprising transplanting ovarian tissue or whole ovary.

11. The method of claim 10, wherein said pharmaceutical composition is administered prior to said transplanting.

12. The method of claim 10, wherein said pharmaceutical composition is administered during the transplantation.

13. The method of claim 1, further comprising administering to said subject at
least one mTOR inhibitor.

14. The method of claim 1, further comprising administering to said subject at least one follicle reserve protective compound.

15. A pharmaceutical composition comprising anti-mullerian hormone, anti-mullerian hormone agonist or antiMIR of anti-mullerian hormone for inhibiting premature follicle activation.

16. The pharmaceutical composition of claim 15, further comprising at least one mTOR inhibitor.

17. The pharmaceutical composition of claim 15, for use in combination with at least one mTOR inhibitor.

18. A kit for inhibiting premature follicle activation in a subject in need thereof comprising:

(i) a first packaging containing a pharmaceutical composition comprising a compound selected from the group consisting of anti-mullerian hormone, anti-mullerian hormone agonist, and antiMIR of anti-mullerian hormone; and

(ii) written instructions of use of said pharmaceutical composition for inhibiting in said subject premature follicle activation induced by a medical treatment or a disease.

19. The kit of claim 18, said treatment is treatment with follicle loss inducing agent and the kit further comprises a second packaging containing a pharmaceutical composition comprising said follicle loss inducing agent.

20. The kit of claim 18, further comprising a third packaging containing at least one follicle reserve protective compound.

21. The kit of claim 18, further comprising a forth packaging containing at least one mTOR inhibitor.

22. A method of inhibiting premature follicle activation comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising an mTOR inhibitor, wherein said follicle loss is induced by medical treatment or a disease.
23. The method of claim 22, wherein said treatment is transplantation of ovarian tissue or whole ovary.

24. The method of claim 22, wherein said treatment is treatment with a follicle loss inducing agent.

25. The method of claim 22, wherein said treatment comprises chemotherapy, radiotherapy and a combination thereof.

26. The method of claim 24, wherein said administering said pharmaceutical composition is carried out in combination with said medical treatment.

27. The method of claim 24, wherein said administering said pharmaceutical composition is carried out prior to said medical treatment.

28. The method of claim 24, wherein said anti-cancer agent is other than said at least one mTOR inhibitor.

29. The method of claim 22, wherein the disease is an accelerated follicle activation disorder.

30. The method of claim 22, wherein the subject in need thereof is a subject at perimenopause.

31. The method of claim 29, wherein the subject in need thereof is having an ovarian disorder.

32. The method of claim 23, further comprising transplanting ovarian tissue or whole ovary.

33. The method of claim 32, wherein said pharmaceutical composition is administered prior to said transplanting.

34. The method of claim 32, wherein said pharmaceutical composition is administered during said transplanting.

35. The method of claim 22, further comprising administering to said subject at least one follicle reserve protective compound.

36. A pharmaceutical composition comprising at least one mTOR inhibitor for inhibiting premature follicle activation.

37. Use of at least one mTOR inhibitor, or a pharmaceutical composition
comprising same, for the manufacture of a medicament for inhibiting premature follicle activation.

38. A kit for inhibiting premature follicle activation in a subject in need thereof comprising:

(i) a first packaging containing a pharmaceutical composition comprising at least one mTOR inhibitor; and

(ii) written instructions of use of said pharmaceutical composition for inhibiting premature follicle activation in said subject, as induced by medical treatment or a disease.
ABSTRACT

The present invention provides composition and methods for inhibiting or preventing premature follicle activation in a subject in need thereof.