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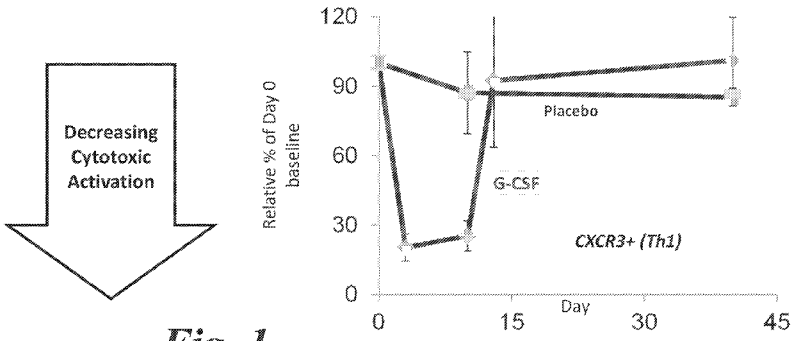


Fig. 1

(57) Abstract: Compositions and methods for preventing or reducing the likelihood of villitis of unknown etiology (VUE) in a human female are provided. The methods include administering an effective amount of granulocyte colony stimulating factor (G-CSF).

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COMPOSITIONS AND METHODS FOR TREATING OR PREVENTING VILLITIS OF UNKNOWN ETIOLOGY IN A HUMAN FEMALE

BACKGROUND

The placenta is a membranous vascular organ that develops in female mammals during pregnancy, which connects the developing fetus to the uterine wall to allow nutrient uptake, waste elimination, and gas exchange through the mother's blood supply. There are various pathologies that can affect the placenta, such as placenta accreta (abnormally deep attachment to the uterine wall muscle), placenta previa (insertion in the lower uterine segment that leads to vaginal bleeding), placental abruption (placental lining separates from the uterus). In some cases, an infectious agent is associated with or causes the pathology, such as placentitis or chorioamnionitis.

In addition, the placenta is a unique maternal-fetal interface that requires immunological tolerance to protect the fetus, which is essentially a genetically foreign semi-allogeneic graft. If there is a breakdown in the maternal-fetal tolerance, an inflammatory disease state may result. A major placental inflammatory lesion is villitis of unknown etiology (VUE), which is a relatively common lesion occurring in 2% to 33.8% term placentas and is associated with adverse pregnancy outcome (Labarrere *et al.*, *Am. J. Obstet. Gynecol. 193*:483, 2005; Redline, *Hum. Pathol. 38*:1439, 2007; Boog, *Eur. J. Obstet. Gynecol. Reprod. Biol. 136*:9, 2008).

VUE is characterized by an inflammatory cell infiltrate of placental macrophages (Höfbauer cells) and T-cells (CD4+:CD8+ median ratio of 1 to 3, as well as Tregs) within the placental villi (Kapur, *Pediatr. Dev. Pathol. 7*:453, 2004; Kim *et al.*, *Histopathology 52*:457, 2008; Katzman *et al.*, *Pediatr. Dev. Pathol. 14*:284, 2011). VUE is also associated with a systemic CXC cytokine response in both the mother and fetus (Kim *et al.*, *J. Immunol. 182*:3919, 2009). Unlike villitis caused by an infection, a major feature of VUE is the non-uniform involvement of the placental parenchyma, most commonly being localized to distal chorionic villi with areas of destructive fibrinoid necrosis, and without affecting other structures, such as the chorionic plate, proximal stem villi, and anchoring villi embedded in the basal plate. Unfortunately,

many clinicians are not aware or familiar with VUE and, therefore, its importance is significantly under-recognized.

To date, VUE requires a histopathological diagnosis, which is further classified based on distinguishable patterns of villous involvement. VUE severity is graded according to total villi involvement. For example, VUE may be classified as having low grade lesions when less than 10 villi per focus (focal or multifocal) are involved, while high grade lesions involve more than 10 villi per focus (patchy or diffuse) (*see* Redline, 2007). The severity of VUE correlates with adverse pregnancy outcomes, such as pre-term labor, intra-uterine growth restriction (IUGR), small for gestational age (SGA) infants, spontaneous miscarriage, cerebral palsy, long term neurological impairment, stillbirth, and perinatal mortality. Moreover, VUE has a tendency to recur and recur with the associated adverse pregnancy outcomes noted above (Redline, *Hum. Pathol. 16*:727, 1985). An apparent risk factor for recurrent VUE and VUE severity is having an increased body to mass index or being obese (Redline, 1985; Becroft *et al.*, *Am. J. Obstet. Gynecol. 192*:264, 2005).

There remains a need in the art for compositions and methods for treating, preventing, or reducing the risk of VUE and associated disorders. The present disclosure meets such needs, and further provides other related advantages.

SUMMARY

In one aspect, the present disclosure provides methods for preventing or reducing the likelihood of villitis of unknown etiology (VUE) in a human female subject. The method comprises administering to the subject an effective amount of granulocyte colony stimulating factor (G-CSF). For example, there are provided methods for reducing the risk of VUE by administering an effective amount of a G-CSF to a human female who previously had or is suspected of having VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day or wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg.

In another aspect, the present disclosure provides methods for preventing the recurrence of VUE in a human female subject by administering an effective amount of a G-CSF to a human female who previously had VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day or wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg.

In still another aspect, the present disclosure provides methods for reducing the progression of VUE in a human female subject by administering an effective amount of a G-CSF to a human female having VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day or wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg.

In yet another aspect, the present disclosure provides methods for treating VUE in a human female subject by administering an effective amount of a G-CSF to a human female having VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day or wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg.

In any of the aforementioned aspects, embodiments of the present disclosure include a human female subject who may have a natural pregnancy or may be pregnant due to being a recipient of an assisted reproductive procedure (such as *in vitro* fertilization, artificial insemination, frozen embryo transfer, intracytoplasmic sperm injection, zygote intrafallopian transfer, or gamete intrafallopian tube transfer, particularly when the egg used to generate the embryo is a donor egg). In further embodiments, the G-CSF is administered parenterally, enterally, subcutaneously, percutaneously, transdermally, intradermally, intravenously, topically, by inhalation, or by implantation. In certain embodiments, the G-CSF is administered at a dose ranging from about 1 μg/kg/day to about 5 μg/kg/day, or is administered at a total dose of about 500 μg to about 10 mg. The treatment may be administered to the human female when she is in the first trimester, second trimester, third trimester of pregnancy, or the first,

second, third, fourth, fifth, sixth, seventh, eighth, or ninth month of pregnancy, or during any combination of those time frames.

In further embodiments, G-CSF is administered daily or weekly, which may be daily for one to thirty-five consecutive days, daily until the end of the first trimester, daily until the end of the second trimester, daily until the end of the third trimester, daily through the 20th week of pregnancy, daily through the end of pregnancy. In certain embodiments, G-CSF is administered for one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, four weeks, five weeks, or six weeks. In certain embodiments, G-CSF is administered on consecutive days or on consecutive weeks. In certain other embodiments, G-CSF is administered daily for six weeks, five weeks, four weeks, three weeks, two weeks, or one week during the first trimester of pregnancy; daily for six weeks, five weeks, four weeks, three weeks, two weeks, or one week during the second trimester of pregnancy; or daily for six weeks, five weeks, four weeks, three weeks, two weeks, or one week during the third trimester of pregnancy.

In certain embodiments, G-CSF is formulated as a composition comprising a pharmaceutically acceptable carrier or diluent. In other embodiments, G-CSF is administered with an adjunctive agent (concurrently or sequentially), such as an immunosuppressive agent, an anti-inflammatory agent, aspirin, a corticosteroid, or progesterone, or the like.

In further embodiments, G-CSF comprises filgrastim, nartograstim, lenograstim, or a biosimilar thereof, which may optionally be formulated for extended release or modified to increase its half-life. For example, a G-CSF having an increased half-life comprises a G-CSF fusion protein (*e.g.*, fused to albumin), a G-CSF hybrid, or a G-CSF conjugated to a water soluble polymer (*e.g.*, one or more polyethylene glycol molecules, such as pegfilgrastim or a biosimilar thereof).

Another aspect of the present invention relates to a kit for treating, preventing, or reducing the likelihood of VUE or an associated disorder in a human female, wherein the kit includes an effective amount of G-CSF; and a label with instructions for using the G-CSF to prevent or reduce the likelihood of VUE or an

associated disorder, such as spontaneous miscarriage, pre-term labor, pre-term delivery, intra-uterine growth restriction (IUGR), small for gestational age (SGA) infants, long term neurological impairment, cerebral palsy, or fetal death (stillbirth).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows that the level of CXCR3 on Th1 cells are decreased in normal, healthy, non-pregnant women treated with G-CSF, indicating that such patients would have reduced response to CXCL9, CXCL10 and CXCL11 cytokines (*i.e.*, leading to a reduction in cytotoxic activation).

Figure 2 shows the level of CXCR3 on Th1 cells are decreased in normal, healthy, non-pregnant subjects treated with multiple doses of G-CSF at various dose levels.

DETAILED DESCRIPTION

In one aspect, the present disclosure provides compositions and methods for treating, preventing, or reducing the likelihood of villitis of unknown etiology (VUE) in a human female subject by administering an effective amount of granulocyte colony stimulating factor (G-CSF). A unique feature of VUE is the interaction of leukocytes from two distinct hosts (mother and fetus), which indicates that both hosts are engaged in the inflammatory response. While the nature of local and systemic inflammatory responses of acute chorioamnionitis are well documented (Murtha *et al.*, *Am. J. Obstet. Gynecol. 175*:966, 1996; Shimoya *et al.*, *Int. J. Gynecol. Obstet. 57*:153, 1997; Gomez *et al.*, *Am. J. Obstet. Gynecol. 179*:194, 1998), those relating to VUE are just beginning to be addressed (Kim *et al.*, 2009). It is shown in this description that a significant percentage of CXCR3-expressing T-cells in blood are reduced in human females treated with G-CSF, which indicated that such a treatment is useful in reducing the likelihood of VUE, particularly recurrent VUE, in a human female in need thereof.

Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein. Additional definitions are set forth throughout this disclosure.

In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. As used herein, the term "about" means ± 20% of the indicated range, value, or structure, unless otherwise indicated. The term "consisting essentially of" limits the scope of a claim to the specified materials or steps, or to those that do not materially affect the basic and novel characteristics of the claimed invention. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components. The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include," "have" and "comprise" are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

As used herein, the phrase "villitis of unknown etiology" or "VUE" refers to inflammatory placental lesions defined histologically as an inflammatory cell infiltrate predominately characterized by mononuclear cells in the absence of an infective etiology, which may include areas of destructive fibrinoid necrosis. Disorders associated with VUE include spontaneous miscarriage, pre-term labor, pre-term delivery, intra-uterine growth restriction (IUGR), small for gestational age (SGA) infants, long term neurological impairment, neonatal encephalopathy, cerebral palsy, fetal death (stillbirth), or any combination thereof. The grading and severity of VUE is determined using the method of Kraus *et al.* (Inflammation and Infection, In *Placental Pathology Atlas of Non Tumor Pathology* 3. F. T. Kraus, R. W. Redline, D. J. Gersell, D. M. Nelson, and J. M. Diche, eds. Armed Forces Institute of Pathology, Washington, DC, pp. 75–116, 2004), which method is incorporated herein by reference.

"Treatment," "treating" or "ameliorating" refers to either a therapeutic treatment or prophylactic/preventative treatment. A treatment is therapeutic if at least one symptom of disease (*e.g.*, VUE) in an individual receiving treatment improves or a

treatment may delay worsening of a progressive disease in an individual, or prevent onset of additional associated diseases (*e.g.*, spontaneous miscarriage, IUGR, SGA). The prophylactic or preventative treatment blocks or inhibits a subject from acquiring a disorder, its attendant symptoms, or associated disorders. In certain embodiments, preventative treatment refers to a method of reducing the risk of acquiring a disorder, its attendant symptoms, or associated disorders.

The term "therapeutically effective amount" or "effective amount" refers to that amount of an active agent being administered sufficient to prevent development of or alleviate to some extent one or more of the symptoms of the condition or disorder being treated, or prevent the disorder or prevent one or more symptoms of the disorder being treated in a statistically significant manner. In certain embodiments, the term "effective amount" refers to that amount of an active agent being administered sufficient to reduce the risk of the disorder or one or more symptoms of the disorder or associated disorders. When referring to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When referring to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered serially or simultaneously or whether in the same formulation or in separate formulations.

The term "subject" refers to animals such as mammals, including, but not limited to, primates (such as humans), cows, sheep, goats, horses; dogs, cats, rabbits, rats, mice and the like. In preferred embodiments, the subject is a human female.

The term "colony stimulating factor" or "CSF" relates to a growth factor that promotes and contributes to the maturity of cells, such as, hematopoietic and blood cells. Examples of CSF molecules include, but are not limited to, erythropoietin, G-CSF, GM-CSF, macrophage CSF, interleukin (IL)-3, IL-6 and stem cell factor.

The term "granulocyte-colony stimulating factor" or "G-CSF" refers to compounds or factors that stimulate proliferation, differentiation, commitment and end cell functional activation of granulocytes in an animal, including a human subject. The term G-CSF or G-CSF variant according to the present invention includes all naturally occurring variants of G-CSF (with or without a leader sequence), G-CSF biosimilars, as

well as G-CSF proteins derived therefrom which are modified by recombinant DNA technology, in particular fusion proteins which contain further polypeptide sequences apart from the G-CSF moiety. For example, one may: (1) increase half-life (or prepare an oral dosage form, for example) of the G-CSF molecule by, for example, decreasing the ability of proteases to act on the G-CSF molecule or adding chemical modifications to the G-CSF molecule, such as one or more polyethylene glycol molecules or enteric coatings for oral formulation which would act to change some characteristic of the G-CSF molecule as described above, such as increasing serum or other half-life or decreasing antigenicity; (2) prepare a hybrid molecule, such as combining G-CSF with part or all of another protein such as another cytokine or another protein which effects signal transduction via entry through the cell through a G-CSF – G-CSF receptor transport mechanism; or (3) increase the biological activity as in, for example, the ability to selectively stimulate neutrophils (as compared to a non-modified G-CSF molecule). G-CSF includes derivatives, mimetics, variants and chemically modified compounds or hybrids thereof as described in U.S. Patent Nos. 5,399,345; 5,416,195; 5,981,551; 6,166,183 and 6,261,550, the contents of which are incorporated by reference in entireties. G-CSF is commercially available under the names Neupogen® (Amgen), Tevagrastim® (Teva), Biograstim® (CT Arzneimittel), Ratiograstim® (Ratiopharm GmbH)), Zarzio® (Sandoz GmbH), Filgrastim Hexal® (Hexal AG), Neulasta® (Amgen), Granocyte® and Neutrogin® (Chugai),and Neu-up® (Kyowa Hakko).

The term "granulocyte/macrophage colony stimulating factor" or "GM-CSF" refers to compounds or factors that stimulate proliferation, differentiation, commitment and end cell functional activation of monocytes and granulocytes in an animal, including a human subject. GM-CSF includes derivatives, mimetics, variants and chemically modified compounds or hybrids thereof as described in, for example, U.S. Patent Nos. 5,895,646; 5,891,429 and 5,908,763; the contents of which are incorporated by reference in entireties. GM-CSF is commercially available under the trade names Leukine®, Berlex® and Leucomax® (Wyeth).

The term "macrophage colony stimulating factor" or "M-CSF" (also known as "CSF-1 ") refers to· compounds or factors that stimulate proliferation or promote survival of monocytes and macrophages in an animal, including a human subject. M-CSF includes derivatives, mimetics, variants and chemically modified compounds or hybrids thereof as described in, for example, U.S. Patent Nos. 5,837,230 and 5,888,495; the contents of which are incorporated by reference in entireties. M-CSF is commercially available under the trade name Leukoprol® (Kyowa).

The term "granulocyte" refers to a white blood cell containing granules, especially a lexukocyte (white blood cell or corpuscle) containing neutrophil, basophil or eosinophil granules in its cytoplasm. The term "macrophage" relates to a mononuclear, phagocytic white blood cell that can exit the circulation and enter tissue spaces.

The term "spontaneous abortion" refers to delivery or loss of the product of conception before the 20th week of pregnancy. The term spontaneous abortion includes but is not limited to miscarriage, threatened abortion, inevitable spontaneous abortion, incomplete spontaneous abortion, habitual or recurrent spontaneous abortion or missed abortion. The term "miscarriage" is synonymous with spontaneous abortion. The term "threatened spontaneous abortion" refers to any bleeding or cramping of the uterus in the first 20 weeks of pregnancy. The term "inevitable spontaneous abortion" refers to bleeding or rupture of the membranes accompanied by pain and dilation of the cervix. The term "incomplete spontaneous abortion" refers to expulsion of part of the products of conception or rupture of the membranes. The term "habitual spontaneous abortion" or "recurrent spontaneous abortion" refers to three or more consecutive spontaneous abortions.

The term "assisted reproduction" refers to clinical and laboratory techniques used to enhance fertility in humans and animals, including *in vitro* fertilization (IVF), FET, ICSI, GIFT, ZIFT, artificial insemination, or the like.

The term *"in vitro* fertilization" refers to the procedure involving ovarian hyperstimulation (optionally), oocyte retrieval from the mother-to-be or a donor, fertilization outside the subject's body, embryo culture and embryo transfer. As used

herein, embryo transfer refers to the procedure involving transfer to a subject's uterus, of the developing or cleaving embryos or pre-embryos, also termed preimplantation embryos.

The term "artificial insemination" refers to an assisted reproduction procedure where a donor's sperm is deposited into the vagina (intravaginal insemination), cervical canal (intracervical insemination), uterine cavity (intrauterine insemination), or fallopian tubes (intratubal insemination) of the recipient. Intrauterine insemination (IUI) is a subset of artificial insemination in which sperm that have been washed of seminal fluid are placed directly into the uterus to bypass the vagina and cervix. Artificial insemination can be performed without any fertility drugs on the day of ovulation or can be performed using fertility drugs to stimulate superovulation (*i.e.,* release of more than one oocyte per cycle).

The term "label" refers to a display of written, printed or graphic matter on the immediate container of an article, for example the written material displayed on a vial containing a pharmaceutically active agent.

The term "labeling" refers to all labels and other written, printed or graphic matter on any article or any of its containers or wrappers or accompanying such article, for example, a package insert or instructional videotapes or computer data storage devices, such as CDs and DVDs, accompanying or associated with a container of a pharmaceutically active agent.

Methods for Preventing Villitis of Unknown Etiology

In certain aspects, the present disclosure provides for the use of G-CSF in methods of treating, preventing or reducing the likelihood of VUE and associated disorders. In certain embodiments, there is provided a method for reducing the risk of villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony stimulating factor (G-CSF) to a human female who previously had VUE or is suspected of having VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day or wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg. In other embodiments,

there is provided a method for reducing the risk of VUE, comprising administering an effective amount of a G-CSF to a human female who previously had VUE or is suspected of having VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day for a plurality of days, or wherein G-CSF is administered in multiple doses and multiple days in a total amount of about 50 μg to about 20 mg.

In further embodiments, there is provided a method for preventing the recurrence of villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony stimulating factor (G-CSF) to a human female who previously had VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day, or wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg. In other embodiments, there is provided a method for preventing the recurrence of VUE, comprising administering an effective amount of a G-CSF to a human female who previously had VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day for a plurality of days, or wherein G-CSF is administered in multiple doses and multiple days in a total amount of about 50 μg to about 20 mg.

In still further embodiments, there is provided a method for reducing the progression of villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony stimulating factor (G-CSF) to a human female having VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day, or wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg. In other embodiments, there is provided a method for reducing the progression of VUE, comprising administering an effective amount of a G-CSF to a human female having VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day for a plurality of days, or wherein G-CSF is administered in multiple doses and multiple days in a total amount of about 50 μg to about 20 mg.

In yet further embodiments, there is provided a method for treating villitis of unknown etiology (VUE), comprising administering an effective amount of a

granulocyte colony stimulating factor (G-CSF) to a human female having VUE, wherein G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day, or wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg. In other embodiments, there is provided a method for treating VUE, comprising administering an effective amount of a G-CSF to a human female having VUE, wherein multiple doses of G-CSF are administered and each dose ranges from about 0.1 μg/kg/day to about 500 μg/kg/day, or wherein G-CSF is administered in multiple doses and multiple days in a total amount of about 50 μg to about 20 mg.

In certain embodiments, the method comprises treating a human female who is pregnant or will become pregnant by normal conception. In other embodiments, the method comprises treating a human female who will be or was a recipient of an assisted reproduction procedure. Exemplary assisted reproduction procedures include *in vitro* fertilization, artificial insemination, frozen embryo transfer, intracytoplasmic sperm injection, zygote intrafallopian transfer, or gamete intrafallopian tube transfer. In still other embodiments, the method comprises treating a human female who will be or was a recipient of an assisted reproduction procedure when the egg is from a donor (*i.e.*, the egg is from someone other than the female being impregnated).

In vitro fertilization is an assisted reproduction procedure to overcome fertility problems caused by, for example, tubal disease, endometriosis, oligospermia, sperm antibodies and unexplained infertility. The procedure can include controlled ovarian hyperstimulation with "fertility drugs" such as ovarian stimulants like clomiphene citrate and gonadotropin-releasing hormones. Hyperstimulation of the ovaries can induce growth of the egg (oocyte) and its encasing cells, collectively also termed the ovarian follicles. After sufficient follicular growth, final follicular maturation is induced and oocytes are retrieved or harvested. The oocytes are fertilized *in vitro* with sperm and the embryos cultured. A small number of embryos, generally 2-4, are then transferred to the uterus. Despite the transfer of multiple embryos, the term pregnancy rate is only about 25%.

Frozen embryo transfer (FET) is a procedure that utilizes cryopreserved embryos from a previous cycle of in vitro fertilization or ICSI. The cryopreserved

embryos are thawed and transferred into the uterine cavity through a catheter. FET can be done with no medications or with the use of various medications including estrogen and progesterone.

Intracytoplasmic sperm injection (ICSI) involves placing a sperm inside an egg with a microscopic needle, rather than placing many sperm close to the outside of the egg, as in IVF, in a dish in a lab. Once fertilization occurs, the resulting embryo is placed in the recipient's uterus.

Gamete intrafallopian transfer (GIFT) is a procedure that combines eggs and sperm in a dish in a lab. The egg/sperm mixture is then surgically injected into the recipient's fallopian tubes using a laparoscope or fiber-thin tube. Fertilization happens inside the recipient's body, and the embryo implants naturally. Although this procedure was once commonly practiced, it's rarely used today because the success with IVF is far greater on average.

In further embodiments, a human subject treated in the methods of this disclosure includes any other person or population at risk for VUE as determined by a practitioner of skill in the art. In certain embodiments, a subject is at risk for developing VUE when she had VUE in a previous pregnancy or the subject has a high body to weight index or is obese. In other embodiments, an at risk subject is obese, morbidly obese, has overall poor health or comorbid conditions that indicate a risk of VUE to a skilled practitioner. In certain embodiments, these conditions can be inflammatory conditions, diabetes mellitus, autoimmune disease, immunologic problems, or the like.

In any of the aforementioned embodiments, a G-CSF may be administered parenterally, enterally, subcutaneously, percutaneously, transdermally, intradermally, intravenously, topically, by inhalation, or by implantation. In particular embodiments, a G-CSF is administered subcutaneously.

In any of the aforementioned embodiments, a G-CSF treatment further reduces the likelihood of disorders associated with VUE, such as spontaneous miscarriage, pre-term labor, pre-term delivery, intra-uterine growth restriction (IUGR),

small for gestational age (SGA) infants, long term neurological impairment, neonatal encephalopathy, cerebral palsy, or fetal death (stillbirth).

In any of the aforementioned embodiments, a G-CSF dose is administered at doses of about 0.1 μg/kg/day to to 25 μg/kg/day, about 1 μg/kg/day to 10 μg/kg/day, about 1 μg/kg/day to 5 μg/kg/day; or about 1 μg/kg/day to 2.5 μg/kg/day. In other embodiments, a total dose of at least 0.01 mg, at least 0.02 mg, at least 0.05 mg at least 0.1 mg, at least 0.2 mg, at least 0.5 mg, at least 1 mg, at least 2 mg, at least 5 mg, at least 10 mg, at least 15 mg, or at least 20 mg of G-CSF is administered (as a single dose or in multiple doses) to a human female in methods of this disclosure.

In certain embodiments, the GCSF is administered to the subject prior to pregnancy. For instance, the GCSF is administered to a subject that is planning or attempting to become pregnant. In other embodiments, the GCSF is administered to a pregnant subject. The GCSF can be administered at any time during the first, second, or third trimester of pregnancy.

A G-CSF dose in any of the aforementioned embodiments can be administered to a subject daily or weekly until the risk of VUE is reduced or eliminated and as long as no symptoms of toxicity are presented. In certain embodiments, a G-CSF dose is administered to a human female who is in the first trimester, second trimester, or third trimester of pregnancy. In further embodiments, a G-CSF dose is administered to a human female who is in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth month of pregnancy. In other embodiments, a G-CSF dose is administered daily through the first, second, or third trimester of pregnancy, or any combination thereof. In further embodiments, a G-CSF dose is administered daily through the 20^{th} week of pregnancy or through the end of pregnancy. In particular embodiments, a G-CSF dose can be administered daily for one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, four weeks, five weeks, or six weeks. In certain embodiments, a G-CSF dose is administered daily for six weeks, five weeks, four weeks, three weeks, two weeks, or one week during the first trimester of pregnancy. In certain other embodiments, a G-CSF dose is administered daily for six weeks, five weeks, four weeks, three weeks, two weeks, or one week

during the second trimester of pregnancy. In still certain other embodiments, a G-CSF dose is administered daily for six weeks, five weeks, four weeks, three weeks, two weeks, or one week during the third trimester of pregnancy.

In further embodiments, a G-CSF dose is administered for five to 42, five to 35, five to 28, five to 21, five to 14, or five to seven, eight, nine or ten consecutive days during the first trimester of pregnancy, second trimester of pregnancy, third trimester of pregnancy, or any combination thereof. In still further embodiments, a G-CSF dose is administered for at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least 14, at least 14, at least 14, at least 14, or at least 42 consecutive days during at least one to six weeks of the pregnancy. In yet further embodiments, a G-CSF dose is administered through the first trimester of pregnancy, second trimester of pregnancy, third trimester of pregnancy, or any combination thereof. In any of the aforementioned embodiments, G-CSF is administered on consecutive days or on consecutive weeks. In any of the aforementioned embodiments, G-CSF is administered in a plurality of doses, on multiple days, or a combination thereof.

G-CSF and Administration

As described in detail herein, the present disclosure provides methods of administering an effective amount of granulocyte colony stimulating factor (G-CSF) to prevent, or reduce the likelihood of, VUE and associated disorders.

The G-CSF administered in the methods of this disclosure can be any G-CSF known to one of skill in the art without limitation. Thus, a range of modifications can be made to a wild-type G-CSF molecule so long as the known immune system modulating activity of the G-CSF is maintained. There are a number of assays that can be used to ensure that anyone modified G-CSF retains the desired immune system modulating activity. Plural types of G-CSF molecules can be administered in the practice of the instant invention. The plural G-CSF molecules can be administered concurrently, consecutively, or sequentially. The term G-CSF or G-CSF variant according to the present invention includes all naturally occurring variants

of G-CSF with or without a leader sequence as well as G-CSF proteins derived therefrom which are modified by recombinant DNA technology, in particular fusion proteins which contain further polypeptide sequences apart from the G-CSF moiety. For example, one may: (1) increase half-life (or prepare an oral dosage form, for example) of the G-CSF molecule by, for example, decreasing the ability of proteases to act on the G-CSF molecule or adding chemical modifications to the G-CSF molecule, such as one or more polyethylene glycol molecules or enteric coatings for oral formulation which would act to change some characteristic of the G-CSF molecule as described above, such as increasing serum or other half-life or decreasing antigenicity; (2) prepare a hybrid molecule, such as combining G-CSF with part or all of another protein such as another cytokine or another protein which effects signal transduction via entry through the cell through a G-CSF – G-CSF receptor transport mechanism; or (3) increase the biological activity as in, for example, the ability to selectively stimulate neutrophils (as compared to a non-modified G-CSF molecule). In certain embodiments, the G-CSF can be any G-CSF or any derivative, variant, mimetic, chemically modified version or hybrid thereof, as described in U.S. Patent Nos. 5,399,345; 5,416,195; 5,981,551; 6,166,183 and 6,261,550. In further embodiments, the G-CSF can be administered in the form of a nucleotide sequence encoding G-CSF or expression vectors encoding G-CSF described in U.S. Patent No. 5,422,248. The G-CSF can be formulated according to any formulation for administration known to those of skill in the art.

In certain embodiments, G-CSF is a commercially available G-CSF available as a pharmaceutical composition or a biosimilar thereof, suitable for administration to an animal, including a human. Such commercially available pharmaceutical compositions can be, for example, filgrastim (Neupogen® (Amgen), Tevagrastim® (Teva), Biograstim® (CT Arzneimittel), Ratiograstim® (Ratiopharm GmbH), Zarzio® (Sandoz GmbH), Filgrastim Hexal® (Bexal AG), pegfilgrastim (Neulasta®, Amgen), nartograstim (Neu-Up®, Kyowwa) or lenograstim (Neutrogin®, Granocyte®, Chugai).

Filgrastim, nartograstim, and lenograstim are useful for promoting neutrophil proliferation and are generally administered to individuals in need to increased neutrophils, for example, patients undergoing chemotherapy. Filgrastim, nartograstim, and lenograstim are indicated for myelosuppressive chemotherapy, bone marrow transplant, peripheral blood progenitor cell collection and severe chronic neutropenia. Off label uses include treatment of neutropenia in AIDS patients, aplastic anemia, hairy cell leukemia, myelodysplasia, drug-induced and congenital agranulocytosis and alloimmune neonataineutropenia.

The usual treatment of neutropenia associated with myelosuppression is 5 μg/kg/day, once daily either by bolus subcutaneously or short (15-30 minute) intravenous infusion or by continuous subcutaneous or intravenous infusion. Administration is once daily starting no earlier than 24 hours after chemotherapy and continues for 14 days or until the individual's absolute neutrophil count is 10,000/mm^3. For patients undergoing bone marrow transplant, the usual dose is 10 μg/kg/day administered as an intravenous infusion over 4-24 hours or as a continuous 24 hour subcutaneous infusion. The first dose is generally administered at least 24 hours after chemotherapy and at least 24 hours after bone marrow infusion. During recovery, the dose is adjusted according to the patient's absolute neutrophil count. Filgrastim dosing for peripheral blood progenitor cells generally begins at 10 μg/kg/day subcutaneously either as a bolus or continuous infusion. It is recommended that filgrastim be given for at least four days before leukapheresis and continued until the last leukapheresis procedure. Doses of filgrastim for congenital neutropenia are 5 μg/kg subcutaneously twice daily while idiopathic or cyclic neutropenia is generally treated with a dose of 5 μg/kg subcutaneously once daily.

Pegfilgrastim is a monomethoxypolyethylene glycol conjugate of filgrastim. The pharmaceutical composition is commercially available as preservative free solutions of 10 mg/ml pegfilgratim in prefilled single-dose syringes. Pegfilgrastim is indicated to decrease infections in patients with febrile neutropenia undergoing myelosuppressive chemotherapy. Recommended dosing is a single 6 mg subcutaneous injection administered once per chemotherapy cycle.

In any of the aforementioned methods, G-CSF is administered in an effective amount, *i.e.*, an amount effective to treat, prevent, or reduce or eliminate the risk of, VUE as well as associated disorders as described herein. The amount can be determined by the skilled practitioner guided by the description herein and knowledge in the art. In particular embodiments, G-CSF is administered at doses of about 0.1 μg/kg/day to 1,000 μg/kg/day, about 0.5 μg/kg/day to 500 μg/kg/day, about 1 μg/kg/day to 250 μg/kg/day, about 1 μg/kg/day to 100 μg/kg/day, about 1 μg/kg/day to 50 μg/kg/day, about 1 μg/kg/day to 25 μg/kg/day, about 1 μg/kg/day to 10 μg/kg/day, about 1 μg/kg/day to 5 μg/kg/day; about 1 μg/kg/day to 2.5 μg/kg/day; or about 1.67 μg/kg/day. In other embodiments, a total dose of at least 0.01 mg, at least 0.02 mg, at least 0.05 mg at least 0.1 mg, at least 0.2 mg, at least 0.5 mg, at least 1 mg, at least 2 mg, at least 3 mg, at least 4 mg, at least 5 mg, at least 6 mg, at least 7 mg, at least 8 mg, at least 9 mg, at least 10 mg, at least 15 mg, at least 20 mg at least 25 mg, at least 50 mg, at least 75 mg, at least 100 mg, at least 125 mg, at least 150 mg, at least 175 mg, at least 200 mg, at least 300 mg, at least 400 mg, at least 500 mg, or at least 600 mg of G-CSF is administered (as a single dose or in multiple doses) to a human female.

In certain embodiments described above, the present invention provides methods of administering to a subject in need thereof an effective amount of G-CSF as a monotherapy. In other embodiments, the present invention provides methods of administering to a subject an effective amount of G-CSF in combination with at least one adjunctive agent or therapy. Adjunctive agents include immunosuppressive agents, such as cytokines, that suppress Th1 immune response, cytokines that enhance Th2 immune response, and non-myeloablative immunosuppressive agents. Adjunctive agents may also be anti-inflammatory agents or inhibitors of pro-inflammatory cytokines. Anti-inflammatory agents include those that reduce leukocyte populations or inhibit leukocyte function. In further examples, corticosteroids, such as prednisone or methylprednisolone, or aspirin or a combination thereof can be used as adjunctive therapies. An adjunctive therapy can be another CSF, erythropoietin or stem cell factor. Exemplary CSFs include G-CSF, GM-CSF or M-CSF.

The G-CSF can be administered according to any method of administration known to those of skill in the art. Methods of administration include subcutaneous administration, parenteral administration, enteral administration, topical administration. G-CSF may also be administered by inhalation. In the compositions administered, the G-CSF can be formulated in any manner known to those of skill in the art for formulating and administering effective amounts of G-CSF.

Filgrastim or non-glycosylated G-CSF is available as a preservative-free pharmaceutical composition comprising 300 μg/ml and 480 μg/ml vials or 300 μg/0.5ml and 480 μg/0.5ml self-injectors. The composition can be administered subcutaneously without further admixture. Intravenous preparations require dilution with proper diluent, such as 5% dextrose, diluted to a final concentration of filgrastim of 5 to 15 μg/ml. Saline is not recommended as a diluent due to product precipitation. Mixture with albumin is recommended to prevent adsorption to plastic or glass materials during preparation and infusion. The final concentration of human albumin should be 2 mg/ml. It is highly recommended that filgrastim be refrigerated at 2°C to 8°C.

The presently available pharmaceutical composition contains a small amount of acetate, TWEEN® 80, sodium, arid sorbitol. These excipients are used to achieve and maintain characteristics that are physiologically acceptable to the body and pharmaceutically practical. Such characteristics include tonicity, osmoticity, osmolality, osmolarity, viscosity and shelf life. Aqueous pharmaceutical compositions of G-CSF with increased serum half-life have been described, for example, in U.S. Patent No. 5,919,757.

In certain embodiments, pharmaceutical compositions can comprise G-CSF in a salt form. For example, because proteins can comprise acidic and/or basic termini side chains, proteins can be included in the pharmaceutical compositions in either the form of free acids or bases, or in the form of pharmaceutically acceptable salts. Pharmaceutically acceptable salts can include, suitable acids which are capable of forming salts with the proteins of the present invention including, for example, inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid and the like; and organic acids such

as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, cinnamic acid, anthranilic acid, citric acid, naphthalene sulfonic acid, sulfanilic acid and the like. Suitable bases capable of forming salts with the subject proteins can include, for example, inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide or the like; and organic bases such as mono-, di-and tri-alkyl amines (*e.g.*, triethyl amine, diisopropyl amine, methyl amine, dimethyl amine or the like) or optionally substituted ethanolamines (*e.g.*, ethanolamine, diethanolamine or the like).

Administration of G-CSF compounds of this disclosure, or their pharmaceutically acceptable salts, in pure form or in an appropriate pharmaceutical composition, can be carried out using any mode of administration for agents serving similar utilities. The pharmaceutical compositions of this disclosure can be prepared by combining a compound of this disclosure with an appropriate pharmaceutically acceptable carrier, diluent or excipient, and may be formulated into preparations in solid, semi-solid, or liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. Exemplary routes of administering such pharmaceutical compositions include oral, topical, subcutaneous transdermal, percutaneous, inhalation, parenteral, enteral, sublingual, buccal, rectal, vaginal, intranasal and implantation.

Parenteral administration refers to any route of administration that is not through the alimentary canal, including, but not limited to, injectable administration, i.e., intravenous, intramuscular and the like as described below. Enteral administration refers to any route of administration which is oral, including, but not limited to, tablets, capsules, oral solutions, suspensions, sprays and the like, as described below. For purposes of this invention, enteral administration also refers to rectal and vaginal routes of administration. Topical administration refers to any route of administration through the skin, including, but not limited to, creams, ointments, gels and transdermal patches, as described below (see also, Pharmaceutical Sciences, 18th Edition (Gennaro et al., eds., Mack Printing Company, Easton, Pennsylvania, 1990).

Parenteral pharmaceutical compositions of the present invention can be administered by injection, for example, into a vein (intravenously), an artery (intraarterially), a muscle (intramuscularly) or under the skin (intradermally or subcutaneously) or in a depot composition.

Injectable pharmaceutical compositions can be sterile suspensions, solutions or emulsions of the G-CSF in aqueous or oily vehicles. The compositions can also comprise formulating agents or excipients, such as suspending, stabilizing and/or dispersing agents. The formulations for injection can be presented in unit dosage form, in ampules or in multidose containers, and can comprise added preservatives. In certain embodiments, the pharmaceutical compositions contain buffers such as citrate, acetate, phosphate, tris (hydroxymethyl) amino methane or THAM (tromethamine).

Depot or sustained release pharmaceutical compositions can be used in the methods of the invention. For example, continuous release of G-CSF can be achieved by the conjugation of the G-CSF with a water soluble polymer as described in U.S. Patent No. 5,320,840. G-CSF may be contained in an inert matrix or device for slow release after implantation of the matrix or device.

Injectable compositions can be pharmaceutically appropriate compositions for any route of injectable administration, including, but not limited to, intravenous, intraarterial, intracoronary, pericardial, perivascular, intramuscular, subdermal, subcutaneous and intraarticular.

Alternatively, the injectable pharmaceutical composition can be provided in powder form for reconstitution with a suitable vehicle, including but not limited to sterile pyrogen free water, buffer, dextrose solution, etc., before use. To this end, the GCSF can be lyophilized as appropriate. The pharmaceutical compositions can be supplied in unit dosage forms and reconstituted prior to use *in vivo*.

For prolonged delivery, the pharmaceutical composition can be provided as a depot preparation, for administration by implantation; e.g., subcutaneous, intradermal, or intramuscular injection. Thus, for example, the pharmaceutical composition can be formulated with suitable polymeric or hydrophobic materials as an emulsion in an acceptable oil or ion exchange resins, or as sparingly soluble derivatives;

as a sparingly soluble salt form of the G-CSF, or derivative, mimetic or variant thereof. The G-CSF can be present in an inert matrix or device for implantation to achieve prolonged release.

Alternatively, transdermal delivery systems manufactured as an adhesive disc or patch that slowly releases the active ingredient for percutaneous absorption can be used. To this end, permeation enhancers can be used to facilitate penetration of the GCSF. A particular benefit may be achieved by incorporating the G-CSF into a transdermal patch.

For oral administration, the pharmaceutical formulations can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulfate). The tablets may be coated by methods well known in the art (see, Remington's Pharmaceutical Sciences, 18th edition (Gennaro *et al.*, eds.) Mack Printing Company, Pennsylvania, 1990).

Liquid pharmaceutical compositions for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be a dry product for constitution with water or other suitable vehicle before use. Such liquid pharmaceutical compositions can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid).

The pharmaceutical compositions can also comprise buffer salts, flavoring, coloring and sweetening agents as appropriate. Pharmaceutical compositions for oral administration can be suitably prepared to provide controlled release of the G-CSF.

Enteral pharmaceutical compositions can be suitable for buccal administration, for example, in the form of tablets, troches or lozenges. For rectal and vaginal routes of administration, the G-CSF can be prepared as solutions (*e.g.*, for retention enemas), suppositories or ointments. Enteral pharmaceutical compositions can be suitable. for admixture in feeding mixtures, such as, for mixture with total parenteral nutrition (TPN) mixtures or for delivery by a feeding tube (*see* Dudrick *et al.*, 1998, Surg. Technol. Int. VII:174-l84; Mohandas et al., 2003, Nat'l. Med. J. India 16(1):29-33; Bueno et al., 2003, Gastrointest. Endosc. 57(4):536-40; Shike et al., 1996, Gastrointest. Endosc. 44(5):536-40).

For administration by inhalation, the G-CSF can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator can be formulated comprising a powder mix of the compound and a suitable powder base such as lactose or starch. Inhaled pharmaceutical compositions can be those, for example, described in U.S. Patent Nos. 5,284,656 and 6,565,841.

The compositions can, if desired, be presented in a pack or dispenser device that can comprise one or more unit dosage forms comprising the G-CSF. The pack can, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration.

The pharmaceutical compositions can be for a single, one time use or can contain antimicrobial excipients, rendering the composition suitable for multiple, extended use with greater shelf stability; for example, a multi-use bottle. In another embodiment, the pharmaceutical composition of interest can be in unit dose or unit-of use packages. As known in the art, a unit dose is targeted for a single use. The unit dose form can be in a vial, which can contain a solution or a desiccated form for reconstitution, a pre-filled syringe, a transdermal patch and the like.

As is known to those of skill in the art, a unit-of-use package is a convenient prescription size, patient ready unit labeled for distribution by health care providers. The package contains as much active ingredient as necessary for a typical treatment regimen.

The pharmaceutical composition can be labeled and have accompanying labeling to identify the composition contained therein and other information useful to health care providers and end users. The information can include instructions for use, dose, dosing interval, duration, indication, side effects and other contraindications, warnings, precautions, storage recommendations and the like.

Various embodiments of the pharmaceutical compositions have been described. The descriptions and examples are intended to be illustrative of the invention and not limiting. Indeed, it will be apparent to those of skill in the art that modifications to the pharmaceutical compositions can be made to the various embodiments of the invention described without departing from the spirit of the invention.

The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification of listed in the Application Data Sheet, including but not limited to U.S. Patent Application No. 61/890,867, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

In one aspect the G-CSF compositions can be administered parenterally, for example, subcutaneously or intravenously. The parenteral administration can be in a single bolus or as a continuous infusion. In one aspect, the parenteral administration can be a single intravenous infusion given over 15-30 minutes. In another aspect the parenteral administration can be a continuous infusion of G-CSF diluted in 5% dextrose.

The methods provide for administration of G-CSF for a therapeutically or prophylactically effective time. In certain embodiments, the G-CSF is administered prior to the onset or observation of the disorder or symptoms accompanying the disorder. In further embodiments, the G-CSF is administered during the disorder or during the time period that symptoms accompanying the disorder are observed. In other embodiments, the G-CSF is administered for a time after the disorder had cleared. For example, the GCSF can be administered about one day, about two days, about three days, about four days, about one week, about two weeks and up to about eight weeks, following resolution of threatened abortion or after confirmation of pregnancy during assisted reproduction.

Kits

In another aspect, the present invention provides kits for carrying out the methods of this disclosure. In certain embodiments, the present disclosure provides kits for treating, preventing, or reducing the likelihood of, VUE as well as associated disorders. The kits comprise one or more effective doses of G-CSF along with a label or labeling with instructions on using the G-CSF to prevent or reduce the likelihood of VUE according to the methods of this disclosure. In certain embodiments, kits can comprise components useful for carrying out the methods, such as devices for delivering the G-CSF. In certain embodiments, a kit can comprise components useful for the safe disposal of devices for delivering the G-CSF, *e.g.*, a sharps container for used syringes.

In other embodiments, the present disclosure provides kits for treating, preventing or reducing the likelihood of VUE in females following normal conception or in females who were recipients of IVF, FET, ICSI, GIFT or ZIFT, particularly when the egg is from a donor. Exemplary kits may comprise one or more effective doses of G-CSF along with a label or labeling with instructions on using the G-CSF to prevent or reduce the likelihood of VUE during or following IVF, FET, ICSI, GIFT and ZIFT. In certain embodiments, kits may comprise components useful for carrying out the methods, such as devices for delivering the G-CSF (*e.g.*, needle). In certain

embodiments, a kit may comprise components useful for the safe disposal of devices after delivering G-CSF to a human female, *e.g.*, a sharps container for used needles or syringes.

In one embodiment, G-CSF in a kit is formulated for subcutaneous administration. In another embodiment, G-CSF in a kit is formulated for intramuscular administration. In another embodiment, G-CSF in a kit is formulated for intravascular administration.

The kit may further contain other active compounds, such as CSFs (*e.g.*, G-CSF, GM-CSF, and macrophage CSF), erythropoietin, stem cell factors, anti-inflammatory agents, immunosuppressive agents, aspirin, progesterone, or the like.

In certain embodiments, G-CSF in a kit is contained in an implantable device for slow release after implantation of the device. In other embodiments, G-CSF in a kit is contained in a transdermal patch for slow release after application of the transdermal device. In further embodiments, G-CSF in a kit is a G-CSF conjugated to a water soluble polymer (*e.g.*, polyethylene glycol), such as pegfilgrastim.

In certain embodiments, the present disclosure provides a transdermal patch comprising G-CSF as an active ingredient. In other embodiments, the present disclosure provides an implantable device comprising G-CSF.as an active ingredient. In yet other embodiments, the present disclosure provides an implantable device G-CSF embedded in an inert matrix. In certain embodiments, the present disclosure provides a vaginal ring comprising G-CSF as an active ingredient and in some cases with another complimentary compound as an adjunctive agent.

The present disclosure is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and Tables are incorporated herein by reference.

EXAMPLES

EXAMPLE 1

G-CSF REDUCES PERCENTAGE OF CXCR3 EXPRESSING T-CELLS *IN VIVO*

A Phase 1, randomized, double-blind, placebo controlled dose escalation study was conducted to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of subcutaneous doses of G-CSF in healthy female volunteers (pre-menopausal females ages 18-40). The study included 6 dose cohorts with 8 subjects in each dose cohort. Subjects were randomized in a 3:1 ratio to receive either G-CSF or placebo. Cohorts 1, 3, and 5 received a single subcutaneous injection of study drug (G-CSF or placebo) at doses of 65 μg, 130 μg, and 260 μg, respectively. Cohorts 2, 4, and 6 received daily subcutaneous injections of study drug (G-CSF or placebo) for 10 days at doses of 65 μg, 130 μg, and 260 μg, respectively. The G-CSF used was an *E. coli*-derived human granulocyte colony stimulating factor (rhG-CSF) manufactured using recombinant DNA technology (rDNA). The G-CSF comprises a single peptide chain of 175 amino acids, with a molecular mass of 18,799 g/mol in the native oxidized form and lacks glycosylation.

Blood samples (20mL) were obtained from subjects treated with G-CSF at multiple points before, during, and post treatment. The PBMC were counted and viable cells were frozen using 10% DMSO + 40% FCS + 50% RPMI containing freezing media and were stored at -140°C for long term storage. The following antibody staining panel was used on the collected PBMC and analyzed by flow cytometry (LSR-II, BD Biosciences): T cell marker CD3 and CD4, CD8 and memory marker CD45RO, Treg marker CD25 in conjunction with the following chemokine receptors: CXCR3, CCR5, CCR6, CRTH2, CCR9, and CLA. The percentage of lymphoid and myeloid subsets of cells was determined using FlowJo FACS software.

For the FACS analysis, cells were stained with relevant antibodies on ice for 30 minutes in PBS buffer containing 2% FCS and 0.1% sodium azide. Cells were washed twice before being analyzed by Becton Dickinson (BD) LSRII flow cytometer. Live cells were gated based on forward and side scatter profiles or based on exclusion

with propidium iodide (PI) staining, and analysis was performed using FlowJo software (Tree Star). To stain for cytokines intracellularly, cells were activated by PMA and ionomycin (Sigma) for 5 hours, followed by fixation and permeabilization using a commercial kit (BD Biosciences), according to the manufacturer's instructions. The following antibodies were used for staining: IFN-γ and IL-17 (eBioscience); CD45RO, CD4, CCR6, and CXCR3 (all from BD Biosciences). Briefly, cells were washed and resuspended in 1x fixation / permeabilization buffer (eBioscience) and incubated at 4°C for 30–60 minutes. Cells were then washed twice with 1x permeabilization buffer and stained with anti-CXCR3 and the other antibodies noted above for 30 minutes at 4°C. Cells were washed twice with 1x permeabilization buffer and analyzed by LSR II.

As shown in Figures 1 and 2, G-CSF treatment led to a reduction in the percentage of CXCR3 expressing T-cells in the blood; thus, showing that G-CSF treatment can be used to reduce the risk of or prevent VUE and related disorders.

CLAIMS

What is claimed is:

1. A method for reducing the risk of villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony stimulating factor (G-CSF) to a human female who previously had VUE or is suspected of having VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day.

2. A method for reducing the risk of villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony stimulating factor (G-CSF) to a human female who previously had VUE or is suspected of having VUE, wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg.

3. A method for preventing the recurrence of villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony stimulating factor (G-CSF) to a human female who previously had VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day.

4. A method for preventing the recurrence of villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony stimulating factor (G-CSF) to a human female who previously had VUE, wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg.

5. A method for reducing the progression of villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony

stimulating factor (G-CSF) to a human female having VUE, wherein the G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day.

6. A method for reducing the progression of villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony stimulating factor (G-CSF) to a human female having VUE, wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg.

7. A method for treating villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony stimulating factor (G-CSF) to a human female having VUE, wherein G-CSF is administered at a dose ranging from about 0.1 μg/kg/day to about 500 μg/kg/day.

8. A method for treating villitis of unknown etiology (VUE), comprising administering an effective amount of a granulocyte colony stimulating factor (G-CSF) to a human female having VUE, wherein a total amount of G-CSF administered ranges from about 50 μg to about 20 mg.

9. The method according to any one of claims 1-8, wherein the G-CSF is administered parenterally, enterally, subcutaneously, percutaneously, transdermally, intradermally, intravenously, topically, by inhalation, or by implantation.

10. The method according to any one of the preceding claims, wherein the G-CSF is administered subcutaneously.

11. The method according to any one of the preceding claims, wherein the human female was a recipient of an assisted reproduction procedure.

12. The method according to claim 11, wherein the assisted reproduction procedure is *in vitro* fertilization, artificial insemination, frozen embryo

transfer, intracytoplasmic sperm injection, zygote intrafallopian transfer, or gamete intrafallopian tube transfer, optionally when the egg is from a donor.

13. The method according to any one of the preceding claims, wherein the G-CSF is administered at a dose ranging from about 1 μg/kg/day to about 5 μg/kg/day.

14. The method according to any one of the preceding claims, wherein the G-CSF is administered at a total dose of about 500 μg to about 10 mg.

15. The method according to any one of the preceding claims, wherein the human female is in the first trimester, second trimester, or third trimester of pregnancy.

16. The method according to any one of the preceding claims, wherein the human female is in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth month of pregnancy.

17. The method according to any one of the preceding claims, wherein the dose is administered daily or weekly.

18. The method according to any one of the preceding claims, wherein the human female is administered G-CSF in a plurality of doses.

19. The method according to any one of the preceding claims, wherein the human female is administered G-CSF in a plurality of doses on multiple days.

20. The method according to any one of the preceding claims, wherein the dose is administered daily through the end of pregnancy.

21. The method according to any one of the preceding claims, wherein the dose is administered daily through the 20th week of pregnancy.

22. The method according to any one of the preceding claims, wherein the dose is administered for one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, four weeks, five weeks, or six weeks.

23. The method according to claims 18-22, wherein the dose is administered on consecutive days or on consecutive weeks.

24. The method according to any one of the preceding claims, wherein the dose is administered daily for six weeks, five weeks, four weeks, three weeks, two weeks, or one week during the first trimester of pregnancy.

25. The method according to any one of the preceding claims, wherein the dose is administered daily for six weeks, five weeks, four weeks, three weeks, two weeks, or one week during the second trimester of pregnancy.

26. The method according to any one of the preceding claims, wherein the dose is administered daily for six weeks, five weeks, four weeks, three weeks, two weeks, or one week during the third trimester of pregnancy.

27. The method according to any one of the preceding claims, wherein the dose is administered for at least five to ten consecutive days during the first trimester of pregnancy, second trimester of pregnancy, third trimester of pregnancy, or any combination thereof.

28. The method according to any one of the preceding claims, wherein the dose is administered for at least five consecutive days during at least one to six weeks of the pregnancy.

29. The method according to any one of the preceding claims, wherein the dose is administered through the first trimester of pregnancy, second trimester of pregnancy, third trimester of pregnancy, or any combination thereof.

30. The method according to any one of the preceding claims, wherein the G-CSF is administered with an adjunctive agent.

31. The method according to claim 30, wherein the adjunctive agent is an immunosuppressive agent, an anti-inflammatory agent, aspirin, corticosteroids, or progesterone.

32. The method according to any one of the preceding claims, wherein the G-CSF is formulated as a composition comprising a pharmaceutically acceptable carrier or diluent.

33. The method according to any one of the preceding claims, wherein the G-CSF comprises filgrastim, nartograstim, lenograstim, or a biosimilar thereof.

34. The method according to any one of the preceding claims, wherein the G-CSF has an increased half-life.

35. The method according to claim 34, wherein the G-CSF having an increased half-life comprises a G-CSF fusion protein, a G-CSF hybrid, or a G-CSF conjugated to a water soluble polymer.

36. The method according to claim 35, wherein the G-CSF conjugated to a water soluble polymer comprises one or more polyethylene glycol molecules.

37. The method according to claim 35, wherein the G-CSF having an increased half-life is a G-CSF conjugated to a water soluble polymer, wherein the G-CSF comprises filgrastim, nartograstim, lenograstim, or a biosimilar thereof.

38. The method according to claim 35, wherein the G-CSF conjugated to a water soluble polymer is pegfilgrastim or a biosimilar thereof.

39. The method according to any one of the preceding claims, wherein the human female previously had one or more spontaneous abortions, two or more spontaneous abortions, or recurrent spontaneous abortions.

40. The method according to any one of the preceding claims, wherein the human female previously had VUE.

41. The method according to any one of the preceding claims, wherein the G-CSF treatment further reduces the likelihood of disorders associated with VUE.

42. The method according to claim 41, wherein the disorders associated with VUE comprise spontaneous miscarriage, pre-term labor, pre-term delivery, intra-uterine growth restriction (IUGR), small for gestational age (SGA) infants, long term neurological impairment, neonatal encephalopathy, cerebral palsy, or fetal death (stillbirth).

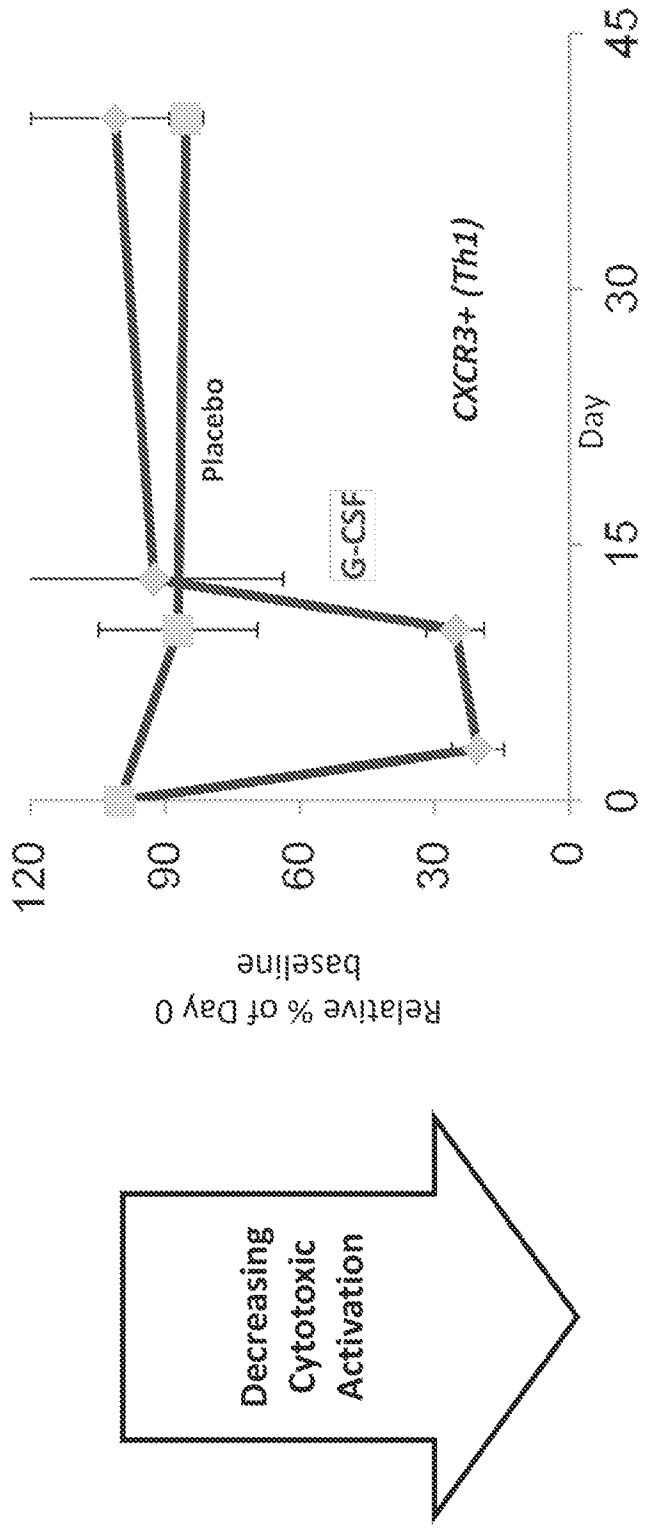

Relative % of Day 0 baseline
120
90
60
30
0
0
15
30
45
Day
CXCR3+ (Th1)
G-CSF
Placebo
Decreasing Cytotoxic Activation

Fig. 1

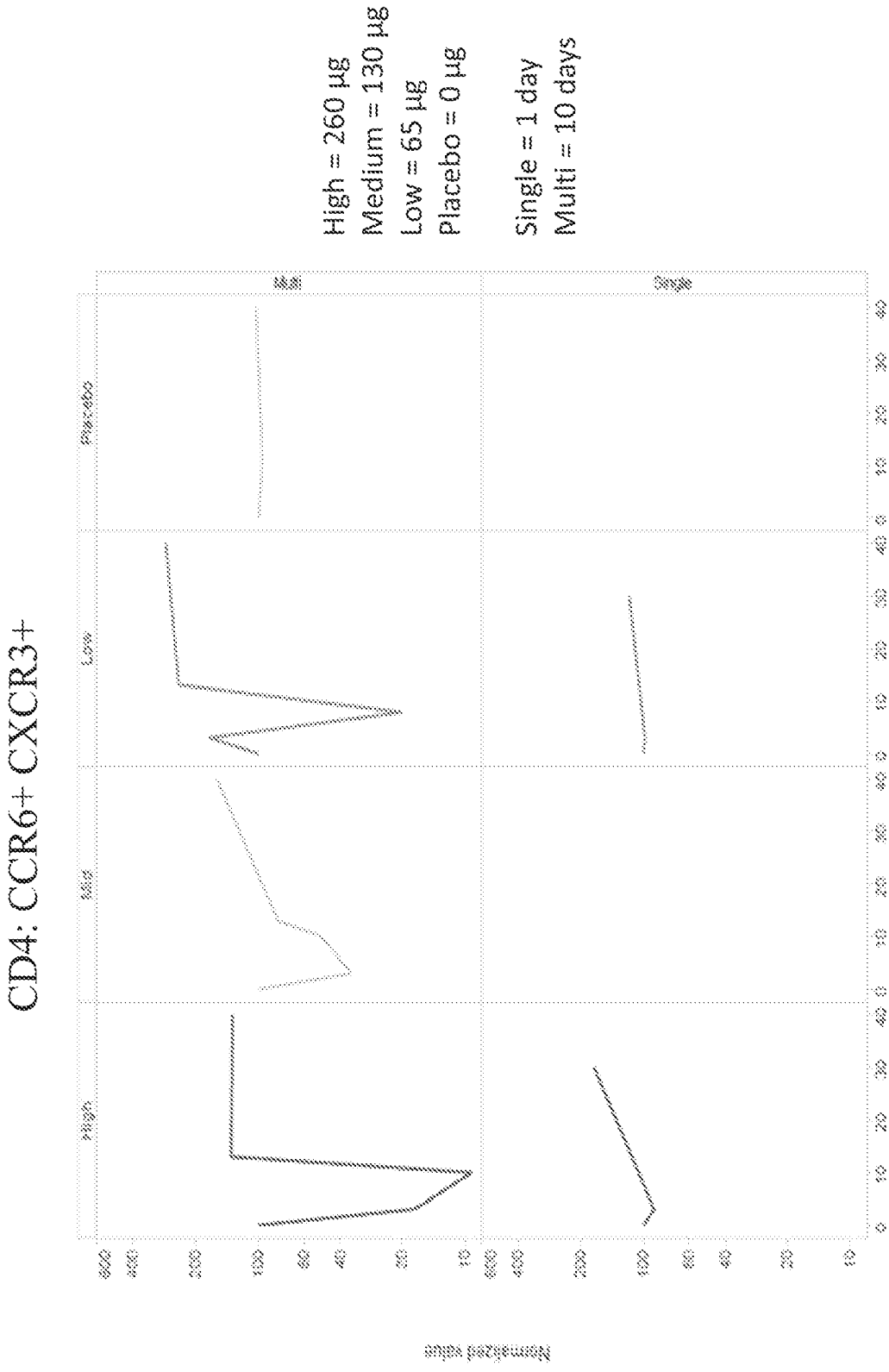

CD4: CCR6+ CXCR3+
High = 260 µg
Medium = 130 µg
Low = 65 µg
Placebo = 0 µg
Single = 1 day
Multi = 10 days
High
Med
Low
Placebo
Multi
Single
Normalized value
Day

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/060507

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/19 A61K47/48 A61P15/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/039505 A2 (NORA LLC [US]; CARTER DARRYL L [US]) 6 May 2005 (2005-05-06) see claims and examples -----	1-42
A	KIM MI JEONG ET AL: "Villitis of Unknown Etiology Is Associated with a Distinct Pattern of Chemokine Up-Regulation in the Feto-Maternal and Placental Compartments: Implications for Conjoint Maternal Allograft Rejection and Maternal Anti-Fetal Graft-versus-Host Disease", JOURNAL OF IMMUNOLOGY, vol. 182, no. 6, March 2009 (2009-03), pages 3919-3927, XP002733199, ISSN: 0022-1767 cited in the application the whole document ----- -/--	1-42

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 December 2014	22/12/2014

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/060507

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/060507

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