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(54) Titre : FORMULATIONS PHARMACEUTIQUES ET SYSTEMES POUR L'ADMINISTRATION D'UN AGENT ANDROGENE ET D'UN INHIBITEUR DE L'AROMATASE, ET PROCEDES D'UTILISATION

(54) Title: PHARMACEUTICAL FORMULATIONS AND SYSTEMS FOR DELIVERY OF AN ANDROGENIC AGENT AND AN AROMATASE INHIBITOR AND METHODS FOR USE

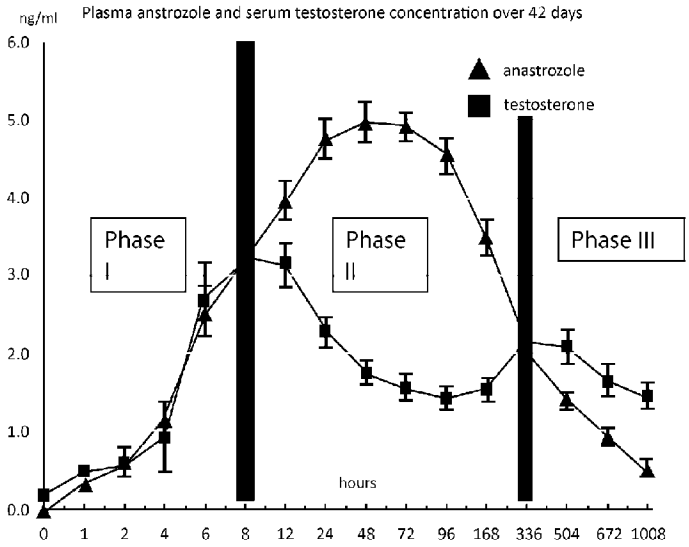


Figure 2

(57) Abrégé/Abstract:
The present disclosure is directed formulations, delivery systems and/or methods of use that have a novel a sustained release multi-phasic concentration pattern that may be used, among other things, for reducing mammographic breast density and/or breast stiffness in warm-blooded animals. For example, the formulation may comprise: the administration of an effective amount of androgenic agent and an effective amount of an aromatase inhibitor to a subject that provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgen and plasma concentration for the aromatase inhibitor and improves, amoung other things, breast tissue stabilization and/or increases of the levels of androgen receptor expression. The present disclosure is also directed to the use of an effective amount of an androgenic agent in combination with an effective amount of an aromatase inhibitor for the prophylaxis or treatment of autoimmune inflammatory mastitis (AIM) in a patient in 15 need thereof. Autoimmune inflammatory mastitis includes the conditions of idiopathic inflammatory macromastia, plasma cell mastitis, granulomatous mastitis.

Abstract

The present disclosure is directed formulations, delivery systems and/or methods of use that have a novel a sustained release multi-phasic concentration pattern that may be used, among other things, for reducing mammographic breast density and/or breast stiffness in warm-blooded animals. For example, the formulation may comprise: the administration of an effective amount of androgenic agent and an effective amount of an aromatase inhibitor to a subject that provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgen and plasma concentration for the aromatase inhibitor and improves, amoung other things, breast tissue stabilization and/or increases of the levels of androgen receptor expression. The present disclosure is also directed to the use of an effective amount of an androgenic agent in combination with an effective amount of an aromatase inhibitor for the prophylaxis or treatment of autoimmune inflammatory mastitis (AIM) in a patient in 15 need thereof. Autoimmune inflammatory mastitis includes the conditions of idiopathic inflammatory macromastia, plasma cell mastitis, granulomatous mastitis.

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Pharmaceutical Formulations and Systems for Delivery of an Androgenic Agent and an Aromatase Inhibitor and Methods of Use

FIELD

The present disclosure is directed generally to pharmaceutical formulations and/or drug delivery systems that have a novel multi-phasic release pattern when delivered to a warm-blooded animal. The compositions disclosed are useful in warm-blooded animals where it is desirable to elevate organ specific or serum androgen level without elevating, or substantially elevating, organ specific and/or serum estradiol levels. The present disclosure is also directed to providing an implant or transdermal patch comprising an effective amount of an androgenic agent and an aromatase inhibitor that delivers a multi-phasic release pattern or pharmacokentics that may be used in warm blood animals where it is desirable to elevate organ specific or serum androgen level without elevating, or substantially elevating, organ specific and/or serum estradiol levels. Methods of use are also disclosed, including methods relating to tissue specific autoimmune inflammartory conditions.

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US patent application No. 14/920,192 entitled "Methods of Reducing Mammographic Breast Density and/or Breast Cancer Risk," filed October 22, 2015.

US patent application No. 15/490,309 entitled "Methods of Reducing Mammographic Breast Density and/or Breast Cancer Risk," filed April 18, 2017.

PCT/AU2015/000633 entitled "Methods of Reducing Mammographic Breast Density and/or Breast Cancer Risk," filed October 22, 2015.

Each of these references and PCT/AU2015/000633, US patent application Nos. 62/067,297, 62/324,525, 15/490,309 and 14/920,192 are incorporated herein by reference in their entirety.

BACKGROUND

Testosterone is critical in the regulation of immune function in both men and women (Gubbels Bupp, 2018). There are several physiological and disease states where it is desirable to increase the tissue level of 5 alpha dihydrotestosterone (DHT) while at the same time reducing oestradiol level within the same tissue without causing significant perturbations in serum oestradiol levels. DHT is predominantly an intra-tissue hormone derived from testosterone which is delivered to that tissue via the circulation. Delivery of testosterone to the tissue which requires treatment in women is problematic due to the relatively small therapeutic window available to dose with testosterone without causing androgenic side-effects.

Within most tissues, there is also five alpha reductase enzyme which converts testosterone to DHT. Therefore, in a therapeutic situation where it is desirable to increase DHT without increasing oestradiol there is an unmet need which the present disclosure is directed to provide pharmaceutical formulations that work towards inhibition of aromatase enzyme and induction of 5 alpha reductase enzyme to effect an alteration in the DHT to oestradiol ratio within that tissue.

Both in men and women subcutaneous testosterone has been used to deliver zero-order kinetic dosing of testosterone. However, the use of testosterone alone in many physiological and disease states is not desirable due to the high level of aromatase enzyme within the tissue, which is undergoing therapeutic intervention. An example of this is women with high mammographic breast density where there is a very high level of aromatase enzyme within the tissue, which converts testosterone preferentially to oestradiol. It has been estimated that 43% of women in the United States of America

from 40 and 75 years of age have mammographic breast density (MBD), which is categorized as high, i.e., having a Breast Imaging-Reporting and Data System (BI-RADS®) score of 3 and 4 (or c and d). The American Cancer Foundation has suggested that this high breast density is a significant risk factor for the development of breast cancer. Traditionally, therapeutic intervention for the peri-menopausal transition is either a low dose combination oral contraceptive or continuous estradiol and a synthetic progestin delivery system to protect the uterus from both increased endometrial cancer risk and unwanted uterine bleeding. This an inappropriate treatment for women with high breast density and/or breast stiffness as they reduce an already precarious testosterone level and increase breast density and/or breast stiffness. However, these are the current recommendations of the Menopause Society of North America and the Menopause Society of Australia.

Another problem in the prior art is breast pain and its treatment. Breast pain is a significant problem in female health. It has been estimated that 45% of woman in their thirties indicated that breast pain impinges on their quality of life, and 10% indicated that they had experienced breast pain for at least half their life. There is little in the way of treatment; tamoxifen and aromatase inhibitors have been used as an off-label medication for this condition. However, tamoxifen is associated with significant side effects that impact on its compliance in patients, and aromatase inhibitors have been contraindicated in pre-menopausal women as a single agent due to resultant purterbations in the hypothalamic-pituitary-oavarian axis. There is a need in the art for better treatments to reduce breast pain in women.

The elasticity of breast tissue is recognized as a factor in the formation of breast cancer. It has been demonstrated that increased elasticity in breast cells results in an increased mechano-transduction across the genome of a cell, which may result in greater malignant transformation. There is a need in the art for compositions that provide pharmaceutical formulations that decreases mechano-transduction on the genome of a cell to reduce the risk of malignant transformation.

A further problem in the art is that of tissue specific autoimmune inflammatory conditions, particularly those presenting in the breast. These conditions may be termed Autoimmune Inflammatory Mastitis (AIM). The anatomical, histological, and physiological roles of salivary glands and mammary glands are similar as they both belong to the effector sites of the associated mucosal immune system leaving them

vulnerable to autoimmunity. Autoimmune breast tissue, compared to normal breast tissue, has been demonstrated to have elevated aromatase (that converts androgens to estradiol) and other factors associated with inflammation , such as IGF2, EGFR, TGF-β, PDGFR-α and β, which have been found to be upregulated. AIM is usually treated with heavy immunosuppressive therapy and/or disfiguring surgery and there is therefore a need for an improved therapy.

How a drug composition is delivered and it impacts on a body may be related to the pharmacokinetics and/or pharmacodynamics profile of the drug composition, the drug delivery system and/or where the drug is delivered to the subject's body. In broad terms, pharmacokinetics is a description of the rate and extent of uptake, distribution and/or elimination of drugs in the body. In other words, how a body, or a portion of the body, affects a drug composition. In broad terms, pharmacodynamics is a description of how drugs affect the body or a portion of the body. Drug compositions may have different pharmacokinetics and/or pharmacodynamics profiles. For example, some drug compositions may have zero order, first order, or second order kinetics. The kinetics and/or dynamics may have an impact on the efficacy of a drug composition and/or the efficacy of the on treatment. Thus, the role that pharmacokinetics and/or pharmacodynamics plays in certain drug compositions, or certain drug delivery systems may be relevant to a particular drug, drug composition, and/or outcome for a subject.

Previously implants have been produced that combine testosterone with an aromatase inhibitor so that there may be an inhibition of the conversion of testosterone to oestradiol. However, if the manufacture and concentration of the compounds result in the active ingredients being delivered via zero-order kinetics, the end result is a lowering of serum oestradiol level which is not desirable in many situations as it results in oestrogen deprivation symptoms and the potential for side effects from higher sustained levels of the aromatase inhibitor. There is an ongoing need for a pharmaceutical formulation and/or delivery system for providing a high intra-tissue DHT to oestradiol ratio. There is also an ongoing need for alternatives to currently available treatments for diseases and conditions for which an increase in the tissue level of 5 alpha-dihydrotestosterone (DHT) whilst at the same time reducing oestradiol level within the same tissue without causing significant perturbations in serum oestradiol levels is beneficial.

The present disclosure in one or more embodiments is directed to addressing and/or ameliorating one or more of the disadvantages of the prior art, or to at least provide a useful pharmaceutical formulation or therapeutic delivery system, as will become apparent from the disclosure herein. The present disclosure in one or more embodiments may also provide other advantages and/or improvements as discussed herein.

SUMMARY

To obtain high intra-tissue DHT to oestradiol ratio, the present inventor has recognised there is an urgent need for a delivery system which delivers testosterone and aromatase inhibitor in a multi-phasic release pattern. In one or more embodiments of a pharmaceutical formulation described herein there may be provided an early peak of serum testosterone, which is rapidly blocked by a high level of aromatase inhibitor allowing the induction of 5 alpha reductase conversion of testosterone to DHT. The formulation may then provide for a rapid reduction of the aromatase inhibitor to ensure that there is not a blockade globally of oestradiol production and symptomatic lowering of oestradiol level in the serum. The alteration of the axis towards an elevated DHT to oestradiol level in tissues where there is overexpression of aromatase may be achieved with the utilisation of this multi-phasic release pattern. If, for example, zero-order kinetics for both components is utilised there will not be a release of the genesis of oestradiol which will result in detrimental effects to the woman and the unwanted long-term exposure of the woman to higher levels of an aromatase inhibitor than is required for a therapeutic response.

In an aspect of the invention there is provided a pharmaceutical formulation comprising: an effective amount of an androgenic agent, an effective amount of an aromatase inhibitor and a binding agent; the formulation upon administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgenic agent and plasma concentration for the aromatase inhibitor; and the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising:

a first time period in which the androgenic agent, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma; and

a second time period in which the androgenic agent, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma.

In another aspect of the invention there is provided a pharmaceutical formulation comprising: an effective amount of an androgenic agent, an effective amount of an aromatase inhibitor and a binding agent; the formulation upon administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgenic agent and plasma concentration for the aromatase inhibitor; and the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising:

a first time period in which the androgenic agent, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma;

a second time period in which the androgenic agent, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma;

a third time period in which the androgenic agent, has a second peak concentration in the serum that is less than the Tmax and the aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the androgenic agent; and

a fourth time period in which the androgenic agenthas a gradually decreasing serum level concentration and the aromatase inhibitor has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.

In another aspect of the invention there is provided a pharmaceutical formulation comprising: an effective amount of an androgenic agent, an effective amount of an aromatase inhibitor and a binding agent; the pharmaceutical formulation is compressed into a pellet; the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the

subject over time as measured by serum concentration for the androgenic agent and plasma concentration for the aromatase inhibitor; and the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising:

a first time period in which the androgen, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma; and

a second time period in which the androgenic agent, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma.

In another aspect of the invention there is provided a pharmaceutical formulation comprising: an effective amount of an androgeic agent, an effective amount of an aromatase inhibitor and a binding agent; the pharmaceutical formulation is compressed into a pellet; the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgenic agent and plasma concentration for the aromatase inhibitor; and the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising:

a first time period in which the androgenic agent, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma;

a second time period in which the androgenic agent, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma;

a third time period in which the androgenic agent, has a second peak concentration in the serum that is less than the Tmax and the aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the androgenic agent; and

a fourth time period in which the androgenic agent has a gradually decreasing serum level concentration and the aromatase inhibitor has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.

In at least some embodiments the pharmaceutical formulation comprises: 60 mg to 120 mg of a testosterone, or an ester thereof, 2 mg to 6 mg of an aromatase inhibitor, more preferably 4 mg to 6 mg of an aromatase inhibitor, and stearic acid; the pharmaceutical formulation is compressed into a pellet that has a diameter of between 4.25 mm to 4.75 mm and a length of between 4 mm to 7 mm; the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the testosterone or an ester thereof, and plasma concentration for the aromatase inhibitor; and the sustained release multi-phasic concentration pattern comprises:

a first time period in which the testosterone or an ester thereof, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma; and

a second time period in which the testosterone or an ester thereof, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma.

In at least some embodiments the pharmaceutical formulation comprises: 60 mg to 120 mg of a testosterone, or an ester thereof, 2 mg to 6 mg of an aromatase inhibitor, more preferably 4 mg to 6 mg of an aromatase inhibitor, and stearic acid; the pharmaceutical formulation is compressed into a pellet that has a diameter of between 4.25 mm to 4.75 mm and a length of between 4 mm to 7 mm; the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the testosterone or an ester thereof, and plasma concentration for the aromatase inhibitor; and the sustained release multi-phasic concentration pattern comprises:

a first time period in which the testosterone or an ester thereof, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma;

a second time period in which the testosterone or an ester thereof, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma;

a third time period in which the testosterone or an ester thereof, has a second peak concentration in the serum that is less than the Tmax and the aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the testosterone or an ester thereof; and

a fourth time period in which the testosterone or an ester thereof, has a gradually decreasing serum level concentration and the aromatase inhibitor has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.

In certain exemplary embodiments, in the pharmaceutical formulation during the first time period the aromatase inhibitor does not exhibit zero-order release.

In certain exemplary embodiments, in the pharmaceutical formulation during the first time period the aromatase inhibitor exhibits first order release.

In certain exemplary embodiments, in the pharmaceutical formulation during the second time period the aromatase inhibitor does not exhibit zero order release.

In certain exemplary embodiments, in the pharmaceutical formulation during the second time period the androgenic agent (e.g., testosterone or an ester thereof), does not exhibit zero order release.

In certain exemplary embodiments, in the pharmaceutical during the third time period the aromatase inhibitor does not exhibit zero order release.

In certain exemplary embodiments, in the pharmaceutical formulation during the third time period the androgenic agent, does not exhibit zero order release.

In certain exemplary embodiments, in the pharmaceutical formulation during the third time period the aromatase inhibitor exhibits first order release.

In certain exemplary embodiments, in the pharmaceutical formulation during the third time period the androgenic agent, exhibits first order release.

In certain exemplary embodiments, in the pharmaceutical formulation during the fourth time period the aromatase inhibitor does not exhibit zero order release.

In certain exemplary embodiments, in the pharmaceutical formulation during the fourth time period the androgenic agent, does not exhibit zero order release.

In certain exemplary embodiments, in the pharmaceutical formulation during the fourth time period the aromatase inhibitor exhibits first order release.

In certain exemplary embodiments, in the pharmaceutical formulation during the fourth time period the androgenic agent, exhibits first order release.

In certain exemplary embodiments of the pharmaceutical formulation the first time period ends right after the androgen has a first peak in concentration (Tmax) in the serum. In certain exemplary embodiments of the pharmaceutical formulation the first time period ends between 5 hours to 14 hours. In certain exemplary embodiments of the pharmaceutical formulation the first time period ends between 5.5 hours to 13 hours.

In certain exemplary embodiments of the pharmaceutical formulation the second time period ends right after the aromatase inhibitor has its Tmax. In certain exemplary embodiments of the pharmaceutical formulation the second time period ends between 23 hours to 80 hours.

Moreover, certain embodiments are directed to a pharmaceutical formulation comprising: approximately 80 mg of a testosterone or an ester thereof, approximately 4 mg anastrozole and approximately 2 mg of a stearic acid; the pharmaceutical formulation is compressed into a pellet that has a diameter of between 4.4 mm to 4.6 mm and a length of between 4 mm to 7 mm; the pellet upon subcutaneously administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the testosterone or an ester thereof, and plasma concentration for the anastrozole; the sustained release multi-phasic concentration pattern comprising: a first time period of in which the testosterone or an ester thereof, has a first peak in concentration (Tmax) in the serum and the anastrozole is increasing in concentration in the plasma but is below its Tmax concentration in the plasma; a second time period in which the testosterone or an ester thereof, has initially a decreasing serum level concentration and then an increasing serum level concentration and the anastrozole has its Tmax concentration in the plasma; a third time period in which the testosterone or an ester thereof, has a second peak concentration in the serum that is less than the Tmax and the anastrozole is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the testosterone or an ester thereof; and a fourth time period in which the testosterone or an ester thereof, has a gradually decreasing serum level concentration and the anastrozole has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other. Methods of using the pharmaceutical formulation are also disclosed.

Further embodiments are directed to a pharmaceutical formulation comprising: an effective amount of an androgenic agent an effective amount of an aromatase inhibitor and a binding agent; the pharmaceutical formulation is compressed into a pellet; the pellet upon subcutaneously administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgenic agent and plasma concentration for the aromatase inhibitor; and the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising: a first time period in which the androgenic agent has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor does not have its Tmax concentration in the plasma; a second time period in which the androgenic agent has a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma; a third time period in which the androgenic agent has a second peak concentration in the serum that is less than its Tmax and the aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the androgenic agent and a fourth time period in which the androgenic agent has a decreasing serum level concentration and the aromatase inhibitor has a decreasing concentration in the plasma.

Further embodiments are directed to methods of reducing mammographic breast density, as measured by AVBD and/or VBD%, in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to a use of a pharmaceutical formulation in methods of reducing VBD% in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing AVBD in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing AVBD and VBD% in a patient in need thereof, comprising

administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of treating mammographic breast density, as measured by AABD and/or ABD%, in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing ABD% in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing AABD in a patient in need thereof, comprising administering to the patient subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing AABD and ABD% in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing mammographic breast density in a patient having a breast with a mammographic breast density of 7.5% or greater, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing VBD% in a patient having a breast with a VBD% of 7.5% or greater, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing ABD% in a patient having a breast with an ABD% of 7.5% or greater, comprising administering to the patient a subcutaneous pellet comprising: i) an

effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of treating mammographic breast density in a patient having a breast with a BI-RADS® score of 3 or 4 (or c or d), comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing mammographic breast density in a patient having a breast with a BI-RADS® score of 3 or 4 (or c or d), comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of inducing breast involution in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of inducing net cell death over proliferation in a breast of a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of inducing net extracellular matrix degradation over development of extracellular matrix in a breast of a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reversing cell number and mammographic breast density in a breast of a peri-menopausal patient, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing mammographic breast density and peri-menopausal symptoms in a

patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods that may be used in pre-menopausal and/or peri-menopausal women for reducing the risk of breast cancer and at substantially the same time not causing perturbations in the hypothalamic-pituitary axis and/or other endocrine axis, for example, adrenal gland and/or ovary gland.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing breast stiffness in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing breast pain in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of decreasing breast elasticity in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of decreasing mechano-transduction on the genome of a cell in order to reduce the risk of malignant transformation in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of increasing the ratio of fibro-glandular and adipose tissue in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of increasing CD36 in a patient in need thereof, comprising administering to

the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of stabilizing and/or an increase in levels of androgen receptor expression in breast tissue of a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing and/or treating macromastia in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of increasing GCDFP15 in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing breast pain associated with having a mammography image taken in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of increasing mammographic sensitivity in a patient, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing ABD% and/or AABD in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing BPE in an MRI image of a patient, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments are directed to use of a pharmaceutical formulation in methods of reducing the size and/or quantity of cysts in a patient in need thereof, comprising administering to the patient a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Additionally, further embodiments are directed to the prophylaxis or treatment of autoimmune inflammatory mastitis in a patient in need thereof.

In particular, in another aspect of the invention there is provided a method for the prophylaxis or treatment of autoimmune inflammatory mastitis in a patient in need thereof, comprising administering to the patient 1) an effective amount of an androgenic agent and 2) an effective amount of an aromatase inhibitor.

In this embodiment, the androgenic agent and the aromatase inhibitor may be administered to the patient in the same pharmaceutical formulation or in separate formulations to one another. Accordingly, the present dislosure extends to all combination treatments of the patient for autoimmune inflammatory mastitis. By combination treatment is meant the androgenic agent and the aromatase inhibitor may be administered together at the same time or sequentially one after the other by the same or different routes whereby the androgenic agent and the aromatase inhibitor exert their respective therapeutic effect in overlapping therapeutic windows, as may be determined by a medical physician or attendant.

Typically, in at least some embodiments as described herein for the treatment of autoimmune inflammatory mastitis, the androgenic agent and the aromatase inhibitor are administered in the same or in different sustained-release pharmaceutical formulations.

In particularly preferred embodiments, the sustained release pharmaceutical formulation(s) are for subcutaneous delivery of the androgenic agent and the aromatase inhibitor to the patient, e.g., in solid-dosage form(s) such as in the form of pellet(s).

Most typically, the androgenic agent and the aromatase inhibitor are provided in the same sustained-release pharmaceutical formulation. The sustained-release formulation can, for example, be a pharmaceutical formulation as described herein for providing a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgen and plasma concentration for the aromatase inhibitor.

Hence, further embodiments are directed the use of a pharmaceutical formulation in methods of treating autoimmune inflammatory mastitis to a patient in need thereof, comprising administering the pharmaceutical formulation to the patient in the form of a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

The autoimmune inflammatory mastitis for which the patient is treated in accordance with the present disclosure may, for example, be selected from the group consisting of idiopathic inflammatory macromastia, plasma cell mastitis, granulomatous mastitis, and combinations of the foregoing.

Further embodiments of the present disclosure are therefore directed to the use of a pharmaceutical formulation in methods of treating idiopathic inflammatory macromastia associated with autoimmune disease in a patient in need thereof, comprising administering the pharmaceutical formulation to the patient in the form of a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments of the present disclosure are directed to the use of a pharmaceutical formulation in methods of treating plasma cell mastitis in a patient in need thereof, comprising administering the pharmaceutical formulation to the patient in the form of a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

Further embodiments of the present disclosure are directed to the use of a pharmaceutical formulation in methods of treating granulomatous mastitis in a patient in need thereof, comprising administering the pharmaceutical pellet to the patient in a the form of a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

The present invention further extends to the use of an androgenic agent in the manufacture of a medicament for the prophylaxis or treatment of autoimmune inflammatory mastitis in a patient in need thereof in combination with an effective amount of an aromatase inhibitor.

In yet another emobodiment, there is provided the use of an aromatase inhibitor in the manufacture of a medicament for the prophylaxis or treatment of autoimmune inflammatory mastitis in a patient in need thereof in combination with an effective amount of an androgenic agent.

Typically, the androgenic agent administered or included in a pharmaceutical formulation for use in the treatment of autoimmune inflammatory mastitis (AIM) or an AIM condition as described herein is a testosterone, or a pharmaceutically acceptable salt or an ester thereof. The aromatase inhibitor may also be utilised in the form of a pharmaceutically acceptable salt or an ester thereof.

From the above, it will be apparent the instant disclosure expressly extends to methods for the administration of an effective amount of a pharmaceutical formulation as described herein to a subject for purposes and/or treatments as described. Further, in certain embodiments there is provided the use of an androgen and an aromatase inhibitor in the manufacture of a medicament as described herein. The summary provided above is not intended to be limiting as to the embodiments disclosed herein and other embodiments are disclosed in this specification. In addition, limitations of one embodiment may be combined with limitations of other embodiments to form additional embodiments.

DEFINITIONS

Terms are used herein are as generally used in the art unless otherwise defined in the following:

The term "absolute area of breast density" (AABD) means the measurement of the surface area of fibro-glandular tissue in a subject's mammogram in square centimeters. For example, this may be measured using CUMULUS software algorithms or visual inspection of a mammogram. Several other tests may be used to measure AABD, including but not limited to, VOLPARA, QUANTRA, and methods taking into account the surface area of fibro-glandular tissue in a mammogram.

The term "androgenic agent" means an agent that increases androgenic activity and/or synthesis. For example, an androgenic agent may be a steroid hormone that binds with high affinity (in the pM or nM range) and specificity to its intracellular mediator, the androgen receptor resulting in receptor conformational change allowing cofactor inclusion, nuclear transportation and/or stimulation of transactivation activity. Thus, regulating the expression of target genes. The androgenic agent may, for example, be an androgen such as an androgen selected from the group consisting of: testosterone, methyltestosterone, and dehydroepiandrosterone. Combinations of these

androgenic agents may also be contemplated. In addition to the pharmaceutically acceptable esters of testosterone, esters may include but are not limited to, the enanthate, propionate, cypionate, phenylacetate, acetate, isobutyrate, buciclate, heptanoate, decanoate, undecanoate, caprate and/or isocaprate esters.

The term "area breast density percentage" (ABD%) means the proportion or percentage of fibro-glandular (dense) tissue relative to the total surface area of the breast on a mammogram. For example, this may be measured using CUMULUS software algorithms or visual inspection of a mammogram. Several other tests that may be used to measure ABD%, including but not limited to, VOLPARA, QUANTRA, and methods taking into account the surface area of fibro-glandular tissue in a mammogram.

The term "aromatase inhibitor" means a chemical compound, hormone or polypeptide that blocks and/or inhibits the activity of aromatase, which is an enzyme that converts androgens to estrogens. The aromatase inhibitor may, for example, be selected from the group consisiting of: anastrozole, exemestane, and letrozole.

The term "autoimmune inflammatory mastitis (AIM)" means a condition in which non-infective, non-lactational inflammation of breast tissue occurs as the result of autoimmune reaction to elements within the breast tissue.

The term "breast cancer" means a malignant proliferation of epithelial cells lining the ducts or lobules of the breast.

The term "breast elasticity" means a measurement of the pressure required to achieve a given fractional deformation of the breast or a part of the breast. For example, Elasticity = Pressure/Fractional change in radius of the breast, where pressure is measured in, for example, kilo-pascals and fractional change in radius of the breast = (R1-R2)/R1, where R1 is the un-compressed radius and R2 is the compressed radius. Another example may be to directly measure the elasticity of breast tissue by applying shear waves through the breast tissue, for example, using the SuperSonic Imagine Aixplorer™ that uses SuperSonic Imagine's ShearWave™ Elastography.

The term "breast stiffness" means, in its broadest sense, as the measurement of the resistance of a breast to deformation. Factors that may influence the degree of breast stiffness include but is not limited to, physical forces generated by interactions between cells and between cells and the extracellular matrix, the number of cells and the extent of collagen present in the breast, the degree of fluid retention within the breast, the degree of proteoglycan expression and/or combinations thereof. One

example of measuring breast stiffness includes the use of the formula force/deformation (dN/cm), where dN denotes deca-Newtons and cm centimetres, wherein the deformation may be determined as the difference between the radius of the mammographic area semicircle and the radius of the volumetric hemisphere, and the compression force is recorded from the mammogram, such as a digital mammogram. For example, under Boyd, et al., the deformation may be determined as the difference R1-R2, where R1 is the un-compressed radius, and R2 is the compressed radius. (Boyd, et al., "Evidence that Breast Tissue Stiffness is Associated with Risk of Breast Cancer" PloS One 2014 Jul10;9(7):e100937)

The term "breast tissue" means the collection of epithelial cells, stromal cells, extracellular matrix, and/or migratory cells, located within and/or in the vicinity of the breast.

The term "effective amount" or "pharmaceutically effective amount" of an agent or compound means a sufficient amount of the agent or compound to provide the desired therapeutic effect and is nontoxic, has an acceptable nontoxic profile and/or an acceptable side effects profile. The amount required may vary from patient to patient, depending, for example, on age, the general condition of the patient, the severity of the condition being treated, the particular agent or compound administered, and/or one or more combinations of these factors. An appropriate "effective amount" typically in an individual case may be determined by one of ordinary skill in the art by reference to the pertinent texts and literature and/or using routine experimentation.

The term "mammographic breast density" or "MBD" means a qualitative estimate of the proportion or percentage of radiopaque, or fibro-glandular ("dense") elements and/or tissue in the breast relative to total breast area (via 2-D determination) or volume (via 3-D determination). Mammographic Breast Density (MBD) may be the qualitative or quantitative estimate of the amount of the fibro-glandular tissue (FGT) within the breast. It may be either the absolute amount of FGT or the relative amount of FGT to the amount of non-FGT (mainly adipose or fat). The absolute amount of FGT may be either estimated as a function of surface area (cm^2) (i.e., AABD) or volume (cm^3) (i.e. AVBD). The relative amount of FGT may be an estimate of the surface area of FGT relative to non-FGT (as a percentage (i.e. ABD%)) or an estimate of the volume of the FGT to the volume of the breast (i.e., VBD%). Another way may be to determine absolute volumetric breast density (i.e., AVBD) which may be the

measured volume of fibro-glandular tissue in a subject's breast in cubic centimeters. Mammographic breast density may be determined by various methods, including but not limited to, mammography, digital mammography, magnetic resonance imaging (MRI), ultrasound, digital breast tomosynthesis (DBT), virtual touch tissue imaging quantification (VTIQ), and combinations thereof. MBD may be qualitatively assessed, via 2-D determinations and/or using the BI-RADS® density categories, with 1 (or a) being least dense and 4 (or d) being the densest. MBD may also be qualitatively and/or quantitatively assessed via 3-D determinations and/or using volumetric measurements of the breast, such as determining the volumetric breast density, which is the proportion of fibro-glandular (dense) tissue relative to the total volume of tissue in the breast (e.g., fibro-glandular (dense) tissue and fat in the breast). Another way is to determine absolute volumetric breast density (AVBD), which is the measured volume of fibro-glandular tissue in a subject's breast in cubic centimeters. Assessments of MBD via 3-D determinations may also account for the heterogeneity of dense tissue within the breast. Several tests that may be used to measure MBD, including but not limited to, VOLPARA, QUANTRA, CUMULUS, and methods taking into account the volume of fibro-glandular tissue (cm^3).

The term "patient" or "subject" means an animal, including the human species that is treatable with the compositions, methods, and kits of the present disclosure. The term "patient" or "patients" is intended to refer to both the male and female gender unless one gender is specifically indicated or clear from the context. The term "patient" may also refer to a female to male transgender.

The term "peri-menopause" or "menopausal transition" means the period of time around menopause during which a woman's body makes its natural transition toward permanent infertility (menopause). Women may start peri-menopause at different ages, and may notice signs of progression toward menopause, such as menstrual irregularity, during their 40's, or even as early as their mid-30's. During peri-menopause, estrogen levels may rise and fall unevenly, menstrual cycles may lengthen or shorten, and menstrual cycles may begin in which the ovaries do not release an egg (ovulate). During peri-menopause, other menopause-like symptoms may be experienced, including, but not limited to, hot flashes, sleep problems, and/or vaginal dryness.

The term "peri-menopausal symptoms" is understood to include but is not limited to, menstrual irregularity; hot flashes and sleep problems; mood changes; mood swings; irritability; depression; vaginal dryness; urinary or vaginal infections; urinary incontinence; decreasing fertility; changes in sexual arousal or desire; bone loss; fragile bones; osteoporosis; or changing cholesterol levels, such as an increase in low-density lipoprotein (LDL) cholesterol, or a decrease in high-density lipoprotein (HDL) cholesterol and combinations thereof.

The term "pharmaceutically acceptable" means those compounds, agents, materials, compositions, excipients, and/or dosage forms that are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and/or animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit and/or risk ratio.

The term "pellet" means a solid formulation implant comprising an effective amount of an androgen and an effective amount of an aromatase inhibitor. The pellet or implant may have different suitable shapes and sizes, for example, spherical, cylindrical, rectangular, square or combintations thereof. It may have angular edges or round edges. In certain embodiments, the pellet or implant may be compressed.

The term "pharmaceutical formulation" means a formulation comprising an effective amount of an androgen and an effective amount of an aromatase inhibitor when the formulation is administered to a warm-blood subject the formulation provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgen and plasma concentration for the aromatase inhibitor. There may be at least 2, 3 or 4 different time periods. These time periods vary depending on one or more of the following: formulation, delivery system, drug concentrations, and individual phyiological variabilities during the time to complete, or substantially complete, formulation absorption. Typically, the formulation's absorption is not less than 3 months and not more than 5 months. In certain embodiments, the formulation may be delivered via a transdermal patch. In certain embodiments, the formulation may be delivered via a solid form such as a subcutaneous pellet. In certain embodiments, the formulation may be delivered via a solid form such as a compressed subcutaneous pellet.

The term "post-menopausal woman" is understood to include not only a woman of advanced age who has passed through menopause but also a woman who has had her

ovaries removed or destroyed by other means or for some other reason has suppressed estrogen production, such as one who has undergone long-term administration of corticosteroids, suffer from Cushing's syndrome or has gonadal dysgenesis.

The term "subject" is an animal including the human species that is treatable with the compositions, methods, and kits of the present disclosure. The term "subject" or "subjects" is intended to refer to both the male and female gender unless one gender is specifically indicated or clear from the context. The term "subject" may also refer to a female to male transgender.

The term "treatment" or "therapy" as used herein includes preventative (*e.g.*, prophylactic) treatment and/or palliative treatment and "treating" as used herein refers to the act of providing preventative and/or palliative treatment.

The term "volumetric breast density percentage (VBD%)" means the proportion or percentage of fibro-glandular (dense) tissue in volume relative to the total volume of tissue in the breast. For example, this may be measured using the Volpara Solution ™ software algorithms. I n the Volpara Solution ™ software algorithms, VBD% is referred to as volumetric breast density percentage. There are several other tests that may be used to measure VBD%, including but not limited to, QUANTRA, CUMULUS, and methods taking into account the volume of fibro-glandular tissue.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

For a better understanding of the disclosure, and to show more clearly how it may be carried into effect according to one or more embodiments thereof, reference will now be made, by way of example, to the accompanying figures. provides a sustained release concentration pattern in the blood of the subject over time as measured by serum concentration for the testosterone or an ester thereof, and plasma concentration for the aromatase inhibitor.

Figure 1 shows the data of the serum concentration for the androgen and plasma concentration for the aromatase inhibitor over 84 days, according to certain exemplary embodiments.

Figure 2 shows the data of Fig. 1 but expanded to further illustrate the first 42 days.

Figure 3 shows the serum concentration of testosterone in 11 subjects up to 7 days post dose, according to certain exemplary embodiments.

Figure 4 shows the serum concentration of testosterone in 11 subjects up to 85 days post dose, according to certain exemplary embodiments.

Figure 5 shows the plasma concentration of anastrozole in 11 subjects over 336 hours post dose, according to certain exemplary embodiments.

Figure 6 shows the plasma concentration of anastrozole in 11 subjects over 1680 hours post dose, according to certain exemplary embodiments.

Figures 7A-B illustrate the anastrozole absorption rates, according to certain embodiments.

Figures 8A-B illustrate the plasma anastrozole concentration post implantation, according to certain embodiments.

Figure 9 shows the frequency of observation post implantation in Example 3.

Figure 10 illustrates an example of a flow chart of models tested in Example 3.

Figure 11 shows the final structure model used in Example 3.

Figures 12A-B shows the population and individual predictions used in Example 3.

Figure 13 illustrates 11individual plots of observed and predicted plasma concentrations of anastrozole, according to certain embodiments.

Figure 14 shows a visual predictive check (VPC) of anastrozole concentration post implantation used in Example 3.

Figures 15A-B shows one versus two compartment model used in Example 3.

Figure 16 displays predictions for patient 10 from several different input models used in Example 3.

Figure 17 diagramatically illustrates the effect of the androgenic agent testosterone and the aromatase inhibitor (T+Ai) on breast tissue immunoreactivity.

Figure 18 shows the results of western blot analysis of CD36 protein in three explant samples of normal breast tissue obtained from three peri-menopausal women at baseline and after 24 hours cultivation.

Figure 19A are mammographic images before (left) and after (right) treatment in accordance with the present disclosure showing a substantial reduction in breast size and density.

Figure 19B are Volpara Solution™ breast volume and density measurements (left) and after (right) treatment for the patient of Fig. 19A showing a breast volume reduction (58%) back to pre-morbid size and fibro-glandular tissue reduction (71%).

Figure 19C are magnetic resonance images (MRI) of the breast of the patient of Fig. 19 before treatment (left) and after treatment for three subsequent years showing a reversal of the extreme background parenchymal enhancement on MRI for the subsequent 3 year period following treatment cessation.

Figure 20A are mammographic images for another patient before (left) and after (right) treatment in accordance with the present disclosure showing a substantial reduction in breast size and density.

Figure 20B are Volpara Solution™ breast volume and density measurements before (left) and after (right) treatment for the patient of Fig. 20A showing a breast volume reduction (10%) back to pre-morbid size and a reduction in fibro-glandular volume (41%).

Figure 21A shows mammographic images for another patient before (left) and after (right) treatment in accordance with the present disclosure showing a substantial reduction in breast size and density.

Figure 21B are Volpara Solution™ breast volume and density measurements before (left) and after (right) treatment for the patient of Fig. 21A showing a breast volume reduction (32%) back to pre-morbid size and a reduction in fibro-glandular volume (52%).

Figure 22A are magnetic resonance images (MRI) of the breast of further patient before treatment (left) and after treatment (right) in accordance with the present disclosure showing a complete reversal of MRI extreme background parenchymal enhancement.

Figure 22B are mammographic images before (left) and after (right) of the breast of the patient of Fig. 22B showing a reduction in breast size and density.

Figure 22C are Volpara Solution™ breast volume and density measurements before (left) and after (right) treatment for the patient of Fig. 22B showing a breast volume reduction (23%) back to pre-morbid size and a reduction in fibro-glandular volume (36%).

Figures 23A shows extreme breast swelling in another patient demonstrated by mammography before treatment treatment in accordance with the present disclosure.

Figure 23B shows a magnetic resonance image (MRI) of intense breast metabolic activity with little fat demonstrated as shown by extreme background parenchymal enhancement in the breast of the patient of Fig. 23A.

Figure 24A shows a magnetic resonance image (MRI) of another patient with granuloma formation in the retroareolar space with an enlarged reactive axillary lymphnode.

Figure 24B is an MRI image of the breast of the patient of Fig. 24A showing resolution of the granulomata and reduction in size of the axillary lymph node after treatment in accordance with the present disclosure.

It will be apparent to one skilled in the art, in view of the following detailed description and the claims appended hereto, that various substitutions and/or modifications may be made to the present disclosure without departing from the scope of the inventions as claimed.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

The following description is provided in relation to several embodiments that may share common characteristics and features. It is to be understood that one or more features of one embodiment may be combined with one or more features of other embodiments. In addition, a single feature or combination of features in certain of the embodiments may constitute additional embodiments. Specific structural and functional details disclosed herein are not to be interpreted as limiting, but merely as a representative basis for teaching one skilled in the art to variously employ the disclosed embodiments and variations of those embodiments.

The subject headings used in the detailed description are included only for the ease of reference of the reader and should not be used to limit the subject matter found throughout the disclosure or the claims. The subject headings should not be used in construing the scope of the claims or the claim limitations.

The present disclosure is directed, at least in part, to providing drug delivery systems with a multi-phasic sustained release pattern comprising an androgenic agent and an aromatase inhibitor in a compressed pellet that unexpectedly overcomes the problem of obtaining high intra-tissue DHT to oestradiol ratio when subcutaneously delivered to a subject. The desired effect is to have an early peak of serum testosterone

which is rapidly blocked by a high level of aromatase inhibitor allowing the induction of 5 alpha reductase conversion of testosterone to DHT. Then it is desirable to have a rapid reduction of the aromatase inhibitor to ensure that there is not a blockade, or substantially blockage, globally of oestradiol production and symptomatic lowering of oestradiol level in the serum. The alteration of the axis towards a high DHT to oestradiol level in tissues where there is overexpression of aromatase may be achieved with the utilisation of certain exemplary embodiments that exhibit this multi-phasic sustain release pattern.

There are several examples of overexpression of aromatase resulting in disease processes where woman and men may benefit from this testosterone and an aromatase inhibitor combination. Therefore, within these tissues, when there may be overexpression of the aromatase enzyme and the coexpression of the 5 alpha reductase enzyme this enzymatic combination may be pharmacologically manipulated to bring about an orderly transition towards alteration in DHT/oestradiol ratio. These disease conditions include but not exclusively, high mammographic breast density, breast pain, endometriosis, male gynaecomastia, perimenopausal and premenstrual tissue aberrations, autoimmune inflammatory mastitis (which includes conditions such as idiopathic inflammatory macromastia, plasma cell mastitis, and granulomatous mastitis).

Figure 2 illustrates an exemplary of a combination of testosterone and aromatase inhibitor and the impact this pattern has on several subjects (N =11) that have been administered a subcutaneous pellet regime.

In this exemplary, phase I shows a rapid increase in both serum testosterone and plasma anastrozole in the subjects. This increase results in a rapid tissue induction of five alpha reductase and the profound inhibition of aromatase. The high tissue level of testosterone is rapidly converted to DHT with conversion to oestradiol being completely blocked, or substantially blocked. In this example, this first phase lasts for approximately 8 days as illustrated by the vertical bar separating phase 1 from phase 2. The time period for phase 1 may vary depending on a number of factors, including, but not limited to, the biology of a particular subject, the formulation of the pellet and/or the manufacturing parameters used for making the pellet.

In the exemplary, the phase II time period lasts for approximately 14 days. In phase 2 it is desirable to recondition the enzymatic system to cause a sustained high

intra-tissue level of anastrozole that continues to allow the induction of 5 alpha reductase to work unopposed, or substantially unopposed, to aromatase conversion of testosterone and oestradiol. This reconditioning leads to a fall in serum testosterone avoiding excessive androgenic side-effects and persistent adduction of five alpha reductase. In this example, this second phase lasts for approximately 14 days as illustrated by the vertical bar separating phase 2 from phase 3. The time period for phase 2 may vary depending on a number of factors, including, but not limited to, the biology of a particular subject, the formulation of the pellet and/or the manufacturing parameters used for making the pellet.

In this exemplary, phase III the anastrozole level falls rapidly allowing some conversion of testosterone to oestradiol and avoiding significant usage and depletion symptoms which may result from sustained high levels of anastrozole which would be observed, for example, in a zero-order release pattern. During this time the induced 5 alpha reductase continues to convert the elevated testosterone to DHT within the target tissue until the implant runs out of the substrate and is replaced. In this example, this third phase lasts at least until the pellet is depleted. As shown in Figure 1 in this example the subjects were tracks for another 70 days. The time period for phase 3 may vary depending on a number of factors, including, but not limited to, the biology of a particular subject, the formulation of the pellet and/or the manufacturing parameters used for making the pellet.

Certain embodiments may be used for treating extreme mammographic breast density. Serum reproductive hormone levels poorly correlate with intra-tissue levels due to significant metabolism to either inactive or active metabolites of prohormones such as testosterone. In certain disease states such as extreme mammographic breast density, there is overexpression of enzymes known to convert testosterone to either estrogen or dihydrotestosterone-10 times more potent as an androgen than testosterone. By altering the androgen to estrogen ratio in high mammographic breast density that there is the potential for reduction of that density and therefore reduce the incidence of breast cancer. The reduction in density is also applicable to situations such as gynecomastia in men where there is an excessive estrogen to androgen within the tissue. To facilitate the alteration in androgen to estrogen ratio, a subcutaneous pellet has been designed to facilitate a multi-phasic sustained release pattern. When anastrozole and testosterone are combined in a specific fashion and compression into a pellet that has

undergone specific compression the resultant pharmacokinetics of facilitates this multi-phasic sustained release of both active ingredients. Again, as discussed elsewhere, it is desirable that there is an early increased of anastrozole to obtain the minimal concentration required to facilitate the blockade, or substantial blockage, of aromatase within the tissue expressing high levels of aromatase at approximately the time as peak testosterone levels are delivered to that tissue. The early increased of anastrozole facilitates the conversion of testosterone through five alpha reduction to dihydrotestosterone and facilitates an androgenic excess over estrogenic environment. Once this initial high levels of anastrozole are obtained a significantly low level is maintained so as to not cause alterations in either hypothalamic-pituitary function or induction of total body aromatase inhibition as is seen in higher concentrations of aromatase inhibitors such as when they are taken orally. This then allows slow of testosterone into the tissues in which there has been an induction of the five-alpha reductase in the somatic system to continue the perturbation of the antigenic environment. This unique multi-phasic sustained release pattern is useful to establish alteration in tissue hormone levels without causing significant physiological alterations in other serum reproductive hormone levels and the resultant potential for adverse side-effects.

Certain embodiments may be used for treating one or more of the following: high breast density and breast stiffness. High breast density and/or breast stiffness is not normal, rather it is pathological; and it is something which may be addressed by effective amounts of an androgenic agent and/or an effective amount of an aromatase inhibitor rather than being treated as a lifestyle modification (e.g., diet and exercise) which has not proven to be successful in pre-menopausal, peri-menopausal and/or post-menopausal women.

Certain embodiments are directed to pharmaceutical formulations that may be used in methods for providing an individualized reduction of one or more of the following: mammographic breast density and breast stiffness in a patient in need thereof, comprising (i) determining the patient's MBD and/or breast stiffness; (ii) optionally, measuring the patient's free androgenic index and/or alterations in the patient's free androgenic index over a period of time of at least one month; (iii) determining adjusted doses of an androgenic agent and aromatase inhibitor taking into account the patient's body weight, total body fat, MBD, age, and free androgenic index;

and (iv) administering the adjusted dose to said patient. In certain embodiments, the measuring the patient's free androgenic index and/or alterations in the patient's free androgenic index over a period of time of at least one month may include taking a blood sample and measuring the amount of free androgenic agent (or testosterone) in the patient's serum.

Certain embodiments are directed to pharmaceutical formulations may be used in methods for providing an individualized reduction of one or more of the following: VBD%, AVBD and breast stiffness in a patient in need thereof, comprising (i) determining the patient's VBD% and/or AVBD and/or breast stiffness; (ii) optionally, measuring the patient's free androgenic index and/or alterations in the patient's free androgenic index over a period of time of at least one month; (iii) determining adjusted doses of an androgenic agent and/or aromatase inhibitor taking into account the patient's body weight, total body fat, VBD% and/or AVBD, age, and free androgenic index; and (iv) administering the adjusted dose to said patient. In certain embodiments, the measuring the patient's free androgenic index and/or alterations in the patient's free androgenic index over a period of time of at least one month may include taking a blood sample and measuring the amount of free androgenic agent (or testosterone) in the patient's serum.

Certain embodiments are directed to pharmaceutical formulations may be used in methods for providing an individualized reduction of one or more of the following: ABD%, AABD, and breast stiffness in a patient in need thereof, comprising (i) determining the patient's VBD% and/or AVBD and/or breast stiffness; (ii) optionally, measuring the patient's free androgenic index and/or alterations in the patient's free androgenic index over a period of time of at least one month; (iii) determining adjusted doses of an androgenic agent and/or aromatase inhibitor taking into account the patient's body weight, total body fat, ABD% and/or AABD, age, and free androgenic index; and (iv) administering the adjusted dose to said patient. In certain embodiments, the measuring the patient's free androgenic index and/or alterations in the patient's free androgenic index over a period of time of at least one month may include taking a blood sample and measuring the amount of free androgenic agent (or testosterone) in the patient's serum.

Certain embodiments are directed to pharmaceutical formulations may be used for reducing one or more of the following: mammographic breast density and breast

stiffness in a patient in need thereof, wherein the pharmaceutical formulation increases sensitivity of breast imaging detections by mammography, digital mammography, magnetic resonance imaging (MRI), ultrasound, digital breast tomosynthesis (DBT), virtual touch tissue imaging quantification (VTIQ), or combinations thereof.

Certain embodiments are directed to pharmaceutical formulations may be used for reducing one or more of the following: mammographic breast density and breast stiffness in a patient in need thereof, wherein the pharmaceutical formulation increases detection of breast cancer developing in the patient.

Certain embodiments are directed to pharmaceutical formulations may be used for reducing one or more of the following: VBD%, AVBD, and breast stiffness in a patient in need thereof, wherein the pharmaceutical formulation increases detection of breast cancer developing in the patient.

Certain embodiments are directed to pharmaceutical formulations may be used for reducing one or more of the following: ABD%, AABD, and breast stiffness in a patient in need thereof, wherein the pharmaceutical formulation increases detection of breast cancer developing in the patient.

Pharmaceutical formulations as disclosed herein may be used to affect one or more of the following in a patient: reducing mammographic breast density; treating mammographic breast density; reducing breast stiffness; treating breast stiffness; reducing mammographic breast density in a patient having a breast with a mammographic breast density of 7.5% or greater; reducing mammographic breast density in a patient having a breast with a BI-RADS® score of 3 or 4 (or c or d); inducing breast involution in a patient; inducing net cell death over proliferation in a breast of a patient; inducing net extracellular matrix degradation over development of extracellular matrix in a breast of a patient; methods of reversing cell number and mammographic breast density in a breast of a peri-menopausal patient; reducing mammographic breast density and peri-menopausal symptoms in a patient, and prophylaxis or treatment of autoimmune inflammatory mastitis (e.g., idiopathic inflammatory macromastia, plasma cell mastitis, granulomatous mastitis, and combinations of the foregoing).

These pharmaceutical formulations and combination treatments of an androgenic agent and an aromatase inhibitor (Ai) as described hereinmay be useful in pre-menopausal, peri-menopausal woman, and/or post-menopausal woman.

For example, high breast density in peri-menopause woman is known as a risk for developing breast cancer. The dense tissue in peri-menopausal women is not considered normal and has pathological implications. This increase in breast density may be due to lifelong exposure to high levels of estrogen and progesterone in the presence of a low testosterone environment. The present inventors have discovered, among other things, that pre-menopausal, peri-menopausal and/or post-menopausal woman who receive via a multi-phasic pattern formulation an effective amount of an androgenic agent such as testosterone and an effective amount of an aromatase inhibitor (such as anastrozole) may show a reduction in breast density and/or breast stiffness. The present inventors have also discovered that pre-menopausal, peri-menopausal and/or post-menopausal woman who receive via a multi-phasic pattern formulation an effective amount of testosterone and an effective amount of an aromatase inhibitor (such as anastrozole) may show induction of breast involution and/or net cell death over proliferation. The present inventors have also discovered that an effective amount of an aromatase inhibitor delivered to a subject via a multi-phasic pattern formulation to the subject's breast tissue may be used to stop the conversion of testosterone to estrogen and thus allow testosterone to invoke an involution of the breast cells.

Certain further embodiments are directed to the prophylaxis or treatment of autoimmune inflammatory mastitis (AIM), which includes the conditions of idiopathic inflammatory macromastia, plasma cell mastitis and granulomatous mastitis as described above.

Breast tissue may be a target tissue in autoimmune diseases, this process being favoured by the hormonal milieu (Touraine, 2005). Autoimmune breast tissue, compared to normal breast tissue, has been demonstrated to have elevated aromatase (that converts androgens to estradiol) and other factors associated with inflammation, such as IGF2, EGFR, TGF-β, PDGFR-α and β (Das, 2019).

The breast is unique in that it requires an immunopriviledged environment for lactation and a rapid remodelling of tissue following lactation (called lactational involution) that requires immunosuppression to avoid autoimmunity (Dawson, 2020). Central to this is the formation of regulatory T (Treg) cells that are able to inhibit proliferation and cytokine production in effector T cells and play a major role in immune responses and prevention of autoimmune disease. During T cell development, T cell receptor (TCR) gene segments are rearranged to generate a diverse TCR

repertoire necessary for immunity to invading pathogens. An unintended consequence of this diversity is the recognition of self-antigen, which can result in autoimmunity. For T cells, two fundamental processes promote tolerance to self in the thymus (referred to as central tolerance) prior to their release into the periphery: (1) negative selection, whereby autoreactive T cells are eliminated; and (2) generation of $CD4^+$ forkhead box P3 $(Foxp3)^+$ regulatory T (Treg) cells. Central to the process is that CD36 facilitates transfer of cell-surface antigens to promote tolerance to host antigens during homeostais. A master regulator of Treg cell development and function is transcription factor *Foxp3*. Testosterone treatment induces a strong increase in the Treg cell population both *in vivo* and *in vitro* (Walecki, 2015). High levels of Treg cells in breast tumours have reduced the capacity of immunotherapies due to Treg cell immunosuppressive capacity. Targeting Treg cells has resulted in unacceptable autoimmunity side-effects that limits the use of this pathway. The impact of testosterone and an aromatase inhibitor on breast tissue immunoreactivity is diagrammatically illustrated in Fig. 17

The manifestation of AIM is associated with dense stromal replacement of breast adipose tissue clearly demonstrated by MRI of the breasts in those affected by this disease (Touraine, 2005). One of the main factors in this inflammatory cascade is a member of the Class B scavenger receptor family of cell surface proteins, CD36. CD36 is found on platelets, erythrocytes, monocytes, differentiated adipocytes, skeletal muscle, mammary epithelial cells, spleen cells and some skin microdermal endothelial cells. It has been demonstrated that a) CD36 is downregulated by estradiol control in hormonally sensitive breast cancer cell lines (Uray, 2004); b) overexpression of CD36 in fibroblasts inhibits the formation of solid tumors in subtypes of breast cancer models (Cheng, 2020); and c) CD36 is profoundly suppressed in non-malignant tissue of women at higher risk of developing breast cancer due to high mammographic density (HMD) (i.e., low fat containing breast tissue) (DeFilippis, 2014).

Thea Tlsty's group (DeFilipis, 2014) has suggest the following cascade: i) elevated basal DNA damage in HMD epithelial cells results in increased activin A secretion; ii) activin A binds to its receptor on adjacent fibroblasts and activates the MAPK pathway; iii) MAPK pathway activation results in PPARγ phosphorylation and inhibition; iv) PPARγ inhibition leads to decreased CD36 transcription and

subsequently, the induction of the desmoplastic-like phenotypes observed in HMD tissues.

As described herein, induction of CD36 expression in breast tissue may be obtained by treating the tissue with an effective amount of an androgenic agent in combination with an effective amount of an aromatase inhibitor, and the present disclosure expressly extends to such methods and use in a patient e.g., a peri-menopausal, menopausal or post-menopausal woman.

A subcutaneous pellet is described herein for facilitating a multi-phasic sustained release pattern for alteration in androgen to estrogen ratio in the prophylaxis or treatment of autoimmune diseases or conditions. Anastrozole and testosterone can be combined and compressed into a pellet as described herein to provide pharmacokinetics facilitating this multi-phasic sustained release of both active ingredients. This release pattern ensures that early high levels of testosterone are associated with very high levels of aromatase inhibitor, so only DHT is presented to the inflamed breast tissue and that the testosterone is not converted to estradiol (which is pro-inflammatory). In the second phase, after the peak of the testosterone level is reached, the induction of 5 alpha reductase from the first phase continues to ensure maintenance of high levels of DHT to exert an anti-inflammatory effect, whilst there is a more rapid decline in anastrozole. Continued high levels of anastrozole could lead to adverse events due to an excessive drop in estradiol systemically, however this risk is reduced by the rapid reduction in anastrozole delivery. The third delivery phase then allows slow release of testosterone into the tissues in which there has been an induction of the five-alpha reductase in the somatic system to continue the perturbation of the antigenic environment. This unique multi-phasic sustained release pattern may establish alteration in tissue hormone levels substantially without causing significant physiological alterations in other serum reproductive hormone levels and the resultant potential for adverse side-effects. The impact of the androgen testosterone and the aromatase inhibitor is anastrozole as described herein on breast tissue immunoreactivity is illustrated in Figure 17.

One of the advantages found in one or more of the disclosed compositions, delivery systems and/or methods of use is the rapid early induction of conversion of testosterone to dihydrotestosterone in the tissue where an early response may reset the

homeostatic mechanism in a beneficial way and reduce the estradiol to androgen ratio in the tissue. This may result in one or more of the following advantages:

A. Enhanced mammographic detection due to reduced breast density enabling the mammogram to visualize malignancy at an earlier and/or less aggressive stage.

B. Reduced risk of interval breast cancer, such as those that may occur between mammographic screening rounds. These cancers are common in breasts with high MBD.

C. Reduction in breast stiffness.

D. Reduced pain during mammographic breast compression.

E. Ability to achieve better mammographic compression due at least in part to reduced pain.

F. Because better mammographic compression is achieved and that the breast tissue is less dense the amount of energy required to expose the image on the mammogram is therefore reduced thus reducing the radiation of the breast tissue. Reducing the risk of radiation induced breast cancer.

G. Ability to achieve better patient compliance in having regular mammographic check-ups.

H. Ability to treat patient and at the same time not causing perturbations in the hypothalamic-pituitary axis and/or other endocrine axis.

I. Reduced breast pain in a patient.

J. Reduced breast elasticity in a patient.

K. Decreased mechano-transduction on the genome of a cell in order to reduce the risk of malignant transformation in a patient.

L. Increased the ratio of fibro-glandular and adipose tissue in a patient.

M. Increased CD36 in a patient.

N. Stabilization and/or an increase in levels of androgen receptor expression in the breast tissue of a patient.

O. Treatment of macromastia in a patient.

P. Increased GCDFP15 in a patient.

Q. Reduced BPE in an MRI image of a patient.

R. Reduction in the size and/or quantity of cysts in a patient.

S. Reduce the risk of breast cancer.

T. Treatment of autoimmune inflammatory mastitis (AIM) (which includes e.g., the conditions of idiopathic inflammatory macromastia, plasma cell mastitis and granulomatous mastitis).

There are a number of categories used by diagnosticians and physicians to characterize the type and/or degree of mammographic breast density of a breast of a patient.

A diagnosing or treating physician may use one or more exams/tests to evaluate, characterize, and/or diagnose, a breast density, including but not limited to, mammography, digital mammography, magnetic resonance imagery (MRI), ultrasound, digital breast tomosynthesis (DBT), virtual touch tissue imaging quantification (VTIQ), or combinations thereof. The physician may also use other indicia, such as medical history or family history (to account for a genetic predisposition to breast density), and/or qualitative assessments of MBD, such as BI-RADS® (e.g., 5th edition, using Breast Composition categories of "a" (the breasts are almost entirely fatty), "b" (there are scattered areas of fibro-glandular density), "c" (the breasts are heterogeneously dense, which may obscure small masses), and "d" (the breasts are extremely dense, which lowers the sensitivity of mammography) (D'Orsi CJ, Sickles EA, Mendelson EB, Morris EA et al. (2013). *ACR BI-RADS® Atlas, Breast Imaging Reporting and Data System*. Reston, VA: American College of Radiology).

Breast pain is a significant problem in female health. Breast pain is also associated with increases in VBD% and/or AVBD, breast stiffness, the risk of breast cancer or combinations thereof. Certain embodiments are directed to the use of an androgen agent and an aromatase inhibitor to reduce the breast pain in a patient.

Certain embodiments are directed to the use of a subcutaneous pellet comprising: an effective amount of anastrozole and an effective amount of testosterone. In exemplary embodiments, a subject is provided between 0.5-10mg anastrozole (2,2'-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]bis(2-methylpropanenitrile)) and between 60-120mg testosterone in a pharmaceutical formulation that has been compressed into a pellet. In exemplary embodiments, a subject is provided between 4-6mg anastrozole and between 60-120mg testosterone in a pharmaceutical formulation

that has been compressed into a pellet. In exemplary embodiments, a subject is provided approximately 4mg anastrozole and approximately 80mg testosterone in a pharmaceutical formulation that has been compressed into a pellet. Other androgenic agents may also be used. The duration of treatment for administration of the subcutaneous pellet comprising: anastrozole and testosterone may vary between 2 weeks to 4 weeks, 3 months to 3 years, 6 months to 2 years, 3 months to 5 years, 1 year to 5 years, or 1 year to 3 years. In certain embodiments, the duration of treatment may be approximately 2 weeks, 3 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years. In certain embodiments, the duration of treatment may be at least 2 weeks, 3 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 3 year or 4 years. In certain embodiments, the duration of treatment may be approximately 3 years. The treatment may be applied to one or more of the following; a reduction in ABD%, a reduction in AABD, a reduction in VBD%; a reduction in AVBD; a reduction in breast pain; a reduction in breast stiffness; a reduction in breast elasticity; a reduction in macromastia; a reduction in breast cysts; an improvement in mammographic diagnostic sensitivity and a reduction in false positives; an increase in the ratio between fibro-glandular and adipose tissue; and a stabilization and/or an increase in levels of androgen receptor expression.

Certain embodiments are directed to the use of a subcutaneous pellet comprising: an effective amount of letrozole and an effective amount of testosterone. In exemplary embodiments, a patient is provided a subcutaneous pellet comprising an 0.5-20mg letrozole (4,4'-((1H-1,2,4-triazol-1 yl)methylene)dibenzonitrile) and between 40-130 mg testosterone for subcutaneous administration. In exemplary embodiments, a patient is provided a subcutaneous pellet comprising approximately 10mg letrozole (4,4'-((1H-1,2,4-triazol-1 yl)methylene)dibenzonitrile) and between 40-130 testosterone for subcutaneously administration. Other androgenic agents may also be used. The duration of treatment for administration of the subcutaneous pellet comprising letrozole and testosterone may vary between 2 weeks to 4 weeks, 3 months to 3 years, 6 months to 2 years, 3 months to 5 years, 1 year and 5 years or 1 year to 3 years. In certain embodiments, the duration of treatment may be approximately 2 weeks, 3 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years, or 3 years. In certain embodiments, the duration of treatment may be at least 2 weeks, 3 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 3 year or 4 years. In certain embodiments, the

duration of treatment may be approximately 3 years. The treatment may be applied to one or more of the following: a reduction in ABD%, a reduction in AABD, a reduction in VBD%; a reduction in AVBD; a reduction in breast pain; a reduction in breast stiffness; a reduction in breast elasticity; a reduction in macromastia; a reduction in breast cysts; an improvement in mammographic diagnostic sensitivity and a reduction in false positives; an increase in the ratio between fibro-glandular and adipose tissue; and a stabilization and/or an increase in levels of androgen receptor expression.

Certain embodiments are directed to the use of a subcutaneous pellet comprising: an effective amount of exemestane and an effective amount of testosterone. In exemplary embodiments, a patient is provided a subcutaneous pellet comprising exemestane 10-75mg 6-Methylideneandrosta-1,4-diene-3,17-dione and between 40-130mg testosterone for subcutaneously administration. Other androgenic agents may also be used. The duration of treatment for administration of the exemestane and the testosterone may vary between 2 weeks to 4 weeks, 3 months to 3 years, 6 months to 2 years, 3 months to 5 years, 1 year and 5 years, or 1 year to 3 years. In certain embodiments, the duration of treatment may be approximately 2 weeks, 3 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years. In certain embodiments, the duration of treatment may be at least 2 weeks, 3 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 3 year or 4 years. In certain embodiments, the duration of treatment may be approximately 3 years. The treatment may be applied to one or more of the following: a reduction in ABD%, a reduction in AABD, a reduction in VBD%; a reduction in AVBD; a reduction in breast pain; a reduction in breast stiffness; a reduction in breast elasticity; a reduction in macromastia; a reduction in breast cysts; an improvement in mammographic diagnostic sensitivity and a reduction in false positives; an increase in the ratio between fibro-glandular and adipose tissue; and a stabilization and/or an increase in levels of androgen receptor expression.

Preparation of esters, as noted herein, involves functionalization of hydroxyl and/or carboxyl groups that may be present, as will be appreciated by those skilled in the arts of pharmaceutical chemistry and drug delivery. For example, to prepare testosterone esters, the 17-hydroxyl group of the testosterone molecule is generally caused to react with a suitable organic acid under esterifying conditions, such conditions typically involving the use of a strong acid such as sulfuric acid, hydrochloric acid, or the like, and a temperature sufficient to allow the reaction to

proceed at reflux. Esters may be reconverted to the free acids, if desired, by using conventional hydrogenolysis or hydrolysis procedures.

An effective amount of an androgenic agent may vary among androgenic agents. In addition, the effective amount per day of testosterone may also vary. In certain aspects, an effective amount of testosterone may be delivered in the form of a subcutaneous implant. In certain embodiments, an effective amount of testosterone may be between 40 and 200mg. For example, in certain embodiments, the effective amount of testosterone may be between 40 to 120mg, for example, 20mg, 40mg, 60mg 80mg, 100mg or 120mg.

In certain embodiments, an effective amount of testosterone may be delivered in the form of a subcutaneous implant, such as a subcutaneous pellet, containing between 40 to 200mg testosterone, such as between 40 to 150mg, 40 to 100mg, 100 to 200mg, 50 to 150mg, 50 to 100mg, 40 to 100mg, 30 to 80mg, 40 to 90mg, 40 to 90mg, 40 to 80mg, 40 to 70mg, 40 to 60mg, 40 to 50mg, 40 to 100mg, 60 to 100mg, 45 to 75mg or 40 to 45mg testosterone.

An effective amount per day of methyltestosterone may vary. In exemplary embodiments, the effective amount of methyltestosterone may be between 0.1mg to 10mg, such as between 0.5mg to 9mg, 2mg to 8mg, 3mg to 7mg, or 4mg to 5mg. For example, the effective amount of methyltestosterone may be 0.5mg, 1.25mg or 2.5mg.

The effective amount of androgenic agent used in conjunction with an aromatase inhibitor may be relatively lower than a standard dose because of low levels of sex hormone binding globulin in the patient's serum which may be caused by the aromatase inhibitor.

Sex hormone binding globulin binds an androgenic agent (*e.g.*, testosterone) and transports it around the body. Its production is regulated by several mechanisms, but one of the effectors of its level is the amount of estrogen in the serum: the higher the estrogen, the higher the sex hormone binding globulin and the lower the free androgenic agent. Conversely, the lower the estrogen, the lower the sex hormone binding globulin, and the higher the free androgenic agent, which means the androgenic agent has higher bioavailability. Thus, after menopause, as the estrogen level falls, the sex hormone binding globulin level falls and the free androgenic agent such as testosterone rises. This free androgenic agent has multiple functions, as the androgen receptor is expressed in all cells of the body.

In certain embodiments, the dosage levels below the lower limit of the aforesaid range of the androgenic agent may be more than adequate, while in other cases still larger doses above the upper limit of the aforesaid range may be employed without causing any harmful side effects.

In certain embodiments, the aromatase inhibitor may be, for example, a steroidal aromatase inhibitor, a nonsteroidal aromatase inhibitor, and/or isomers thereof. Steroidal aromatase inhibitors developed to date build upon the basic androstenedione nucleus and incorporate chemical substituents at varying positions on the steroid. Examples of steroidal aromatase inhibitors include but are not limited to, exemestane (Aromasin®) and formestane. Additional examples include mechanism-based steroidal aromatase inhibitors that mimic the substrate, are converted by the enzyme to a reactive intermediate, and result in the inactivation of aromatase. In certain embodiments, the aromatase inhibitor is exemestane. Nonsteroidal aromatase inhibitors may be divided into three classes: aminoglutethimide-like molecules, imidazole/triazole derivatives, and flavonoid analogs. Examples of non-steroidal aromatase inhibitors include anastrozole, exemestane, or letrozole. In certain embodiments, the aromatase inhibitor is either anastrozole or letrozole. In certain embodiments, the aromatase inhibitor is anastrozole.

Aromatase inhibitors often include third-generation aromatase inhibitors, such as anastrozole (Arimidex®), exemestane (Aromasin®), and letrozole (Femara®). These third-generation aromatase inhibitors have brought a change in the therapeutic approach to patients with hormone-sensitive breast cancer. Such aromatase inhibitors are specific in their action in that they virtually ablate estrogen in the serum and thus lower sex hormone binding globulin, which enables the achievement of a synergistic effect.

In certain embodiments, the aromatase inhibitor may be selected from the group consisting of anastrozole, exemestane, or letrozole. In certain embodiments, the aromatase inhibitor is either anastrozole or letrozole. In certain embodiments, the aromatase inhibitor is anastrozole.

In certain embodiments, a method is provided for determining if the patient has a breast with a BI-RADS® score of 3 or 4 (or c or d); a breast with a mammographic breast density of 7.5% or greater; a mammographically dense breast; a breast with the same or more breast tissue than fat; a breast with more breast tissue than fat; breast cancer or combinations thereof.

In certain embodiments, the patient has or is diagnosed with having, a breast with a BI-RADS® score (1-4 scale) in the range of between 2 and 4, for example, between 2 and 3, or between 3 and 4. In certain embodiments, the patient has or is diagnosed with having, a breast with a BI-RADS® score of 2 or more, for example, a BI-RADS® score of 3 or 4, or a BI-RADS® score of 4.

In certain embodiments, the patient has or is diagnosed with having, a breast with a BI-RADS® score (a-d scale) in the range of between b and d, for example, between b and c, or between c and d. In certain embodiments, the patient has or is diagnosed with having, a breast with a BI-RADS® score of b or more, for example, a BI-RADS® score of c or d, or a BI-RADS® score of d.

In certain embodiments, the patient has or is diagnosed with having, a breast with a mammographic breast density of 7.5% or greater, for example, a mammographic breast density of 10% or greater, 15% or greater, 20% or greater, 30% or greater, 50% or greater, 70% or greater, or 95% or greater.

In certain embodiments, the patient has or is diagnosed with having, a breast with a VBD% of 7.5% or greater, for example, a VBD% of 10% or greater, 15% or greater, 20% or greater, 30% or greater, 50% or greater, 70% or greater, or 95% or greater.

In certain embodiments, the patient has or is diagnosed with having, a breast with an ABD% of 7.5% or greater, for example, an ABD% of 10% or greater, 15% or greater, 20% or greater, 30% or greater, 50% or greater, 70% or greater, or 95% or greater.

In certain embodiments, the patient has or is diagnosed with having, a breast with a BI-RADS® score of 3 (or c) and a mammographic breast density of 7.5% or greater, for example, a mammographic breast density of 10% or greater, 15% or greater, 20% or greater, 30% or greater, 50% or greater, 70% or greater, or 95% or greater. In certain embodiments, the patient has or is diagnosed with having, a breast with a BI-RADS® score of 4 (or d) and a mammographic breast density of 7.5% or greater, for example, a mammographic breast density of 10% or greater, 15% or greater, 20% or greater, 30% or greater, 50% or greater, or greater, or 95% or greater.

In certain embodiments, the patient has or is diagnosed with having, a breast with a VBD% in the range of between 1% to 100%, for example, a VBD% of between 1% and 24%, 5% to 100%, 5% to 95%, 5% to 90%, 5% to 80%, 5% to 70%, 5% to

60%, 5% to 50%, 5% to 40%, 5% to 30%, 5% to 25%, 5% to 20%, 10% to 100%, 10% to 95%, 10% to 90%, 10% to 80%, 10% to 70%, 10% to 60%, 10% to 50%, 10% to 40%, 10% to 30%, 10% to 25%, 10% to 20%, 25% to 100%, 25% to 75%, 25% to 50%, 25% to 49%, 30% to 100%, 30% to 95%, 30% to 90%, 30% to 80%, 30% to 70%, 30% to 60%, 30% to 50%, 30% to 40%, 40% to 100%, 40% to 95%, 40% to 90%, 40% to 80%, 40% to 70%, 40% to 60%, 40% to 50%, 50% to 100%, 50% to 95%, 50% to 90%, 50% to 80%, 50% to 75%, 50% to 74%, 50% to 70%, 50% to 60%, 75% to 100%, 75% to 95%, or a VBD% of 75% to 90%. In certain embodiments, the patient has or is diagnosed with having, a breast with a VBD% in the range of between 10% to 40%.

In certain embodiments, the patient has or is diagnosed with having, a breast with a ABD% in the range of between 1% to 100%, for example, a ABD% of between 1% and 24%, 5% to 100%, 5% to 95%, 5% to 90%, 5% to 80%, 5% to 70%, 5% to 60%, 5% to 50%, 5% to 40%, 5% to 30%, 5% to 25%, 5% to 20%, 10% to 100%, 10% to 95%, 10% to 90%, 10% to 80%, 10% to 70%, 10% to 60%, 10% to 50%, 10% to 40%, 10% to 30%, 10% to 25%, 10% to 20%, 25% to 100%, 25% to 75%, 25% to 50%, 25% to 49%, 30% to 100%, 30% to 95%, 30% to 90%, 30% to 80%, 30% to 70%, 30% to 60%, 30% to 50%, 30% to 40%, 40% to 100%, 40% to 95%, 40% to 90%, 40% to 80%, 40% to 70%, 40% to 60%, 40% to 50%, 50% to 100%, 50% to 95%, 50% to 90%, 50% to 80%, 50% to 75%, 50% to 74%, 50% to 70%, 50% to 60%, 75% to 100%, 75% to 95%, or a ABD% of 75% to 90%. In certain embodiments, the patient has, or is diagnosed with having, a breast with a ABD% in the range of between 50% to 100%. In certain embodiments, the patient has or is diagnosed with having, a mammographically dense breast, for example, a breast having about the same or more breast tissue than fat.

In certain embodiments, the patient is a peri-menopausal woman or a post-menopausal woman. In certain embodiments, the patient is a perimenopausal woman.

In certain embodiments, the pharmaceutical formulation may be used to reduce or decrease the patient's BI-RADS® score one or more annual intervening mammographic detections. For example, the pharmaceutical formulation may be used to reduce or decrease the patient's BI-RADS® score by 1 or more points one or more annual intervening mammographic detections, such as, by 2 or more, 3 or 4, or 4 points one or more annual intervening mammographic detections. In certain embodiments, the pharmaceutical formulation may be used to reduce or decrease the patient's BI-RADS® score by 1 point one or more annual intervening mammographic detections, for

example, by 2, 3, or 4 points one or more annual intervening mammographic detections. In certain embodiments, the pharmaceutical formulation maintains or stabilizes the patient's BI-RADS® score one or more annual intervening mammographic detections.

The time period the one or more annual intervening mammographic detections may be 1 to 20 years, for example, 1 year, 1.5 years, 2 years, 3 years, 4 years, 5 years, 6 years, 10 years, 15 years, or 20 years. The time period the one or more annual intervening mammographic detections may be 1 year, 2 years, 4 years, 5 years, 7 years, 10 years, 15 years, or 20 years.

In certain embodiments, the pharmaceutical formulation may be used to reduce or decrease the mammographic breast density of the patient's breast one or more annual intervening mammographic detections. In certain embodiments, the pharmaceutical formulation may be used to reduce or decrease the VBD% and/or AVBD of the patient's breast one or more annual intervening mammographic detections. In certain embodiments, the pharmaceutical formulation may be used to reduce or decrease the ABD% and/or AABD of the patient's breast between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation may be used to reduce or decrease the mammographic breast density of the patient's breast in the range of between 1% to 99% between one or more annual intervening mammographic detections, such as, in the range of between 1% to 80%, 1% to 50%, 1% to 30%, 1% to 20%, 1% to 10%, 3% to 40%, 3% to 20%, 5% to 60%, 5% to 25%, 5% to 15%, 5% to 10%, 10% to 60%, 10% to 40%, 10% to 30%, 10% to 20%, 10% to 15%, 20% to 60%, 20% to 40%, 20% to 30%, 30% to 60%, 30% to 50%, or 30% to 40% between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation may be used to reduce or decrease the VBD% and/or AVBD of the patient's breast in the range of between 1% to 99% between one or more annual intervening mammographic detections, such as, in the range of between 1% to 80%, 1% to 50%, 1% to 30%, 1% to 20%, 1% to 10%, 3% to 40%, 3% to 20%, 5% to 60%, 5% to 25%, 5% to 15%, 5% to 10%, 10% to 60%, 10% to 40%, 10% to 30%, 10% to 20%, 10% to 15%, 20% to 60%, 20% to 40%, 20% to 30%, 30% to 60%, 30% to 50%, or 30% to 40% between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation may be used to reduce or decrease the ABD% and/or AABD of the patient's breast in the range of between 1% to 99% between one or more annual intervening mammographic detections, such as, in the range of between

1% to 80%, 1% to 50%, 1% to 30%, 1% to 20%, 1% to 10%, 3% to 40%, 3% to 20%, 5% to 60%, 5% to 25%, 5% to 15%, 5% to 10%, 10% to 60%, 10% to 40%, 10% to 30%, 10% to 20%, 10% to 15%, 20% to 60%, 20% to 40%, 20% to 30%, 30% to 60%, 30% to 50%, or 30% to 40% between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation may be used to reduce or decrease the mammographic breast density of the patient's breast by at least 2% between one or more annual intervening mammographic detections, such as, by at least 5%, 10%, 20%, 30%, 40%, 50%, 75%, 85%, 95%, or 99% between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation may be used to reduce or decrease the VBD% and/or AVBD of the patient's breast by at least 2% between one or more annual intervening mammographic detections, such as, by at least 5%, 10%, 20%, 30%, 40%, 50%, 75%, 85%, 95%, or 99% between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation may be used to reduce or decrease the ABD% and/or AABD of the patient's breast by at least 2% between one or more annual intervening mammographic detections, such as, by at least 5%, 10%, 20%, 30%, 40%, 50%, 75%, 85%, 95%, or 99% between one or more annual intervening mammographic detections. In certain embodiments, the pharmaceutical formulation may be used to maintain or stabilize the mammographic breast density of the patient's breast between one or more annual intervening mammographic detections.

In certain embodiments, the pharmaceutical formulation may be used to reduce or decrease the mammographic breast density of the patient's breast by at least 2%, such as 5%, 10%, 20%, or 30%, over a 4 hour period, such as over an 8 hour, 24 hour, 1day, 3 days, 1 week, 2 weeks, 1 month, 2 month, 3 months, 6 months, 9 months, or 1 year period.

In certain embodiments, the pharmaceutical formulation may be used to reduce or decrease the mammographic breast density VBD% and/or AVBD of the patient's breast by at least 2%, such as 5%, 10%, 20%, or 30%, over a 4 hour period, such as over an 8 hour, 24 hour, 1day, 3 days, 1 week, 2 weeks, 1 month, 2 month, 3 months, 6 months, 9 months, or 1 year period.

In certain embodiments, the pharmaceutical formulation may be used to reduce or decrease the mammographic breast density ABD% and/or AABD of the patient's breast by at least 2%, such as 5%, 10%, 20%, or 30%, over a 4 hour period, such as

over an 8 hour, 24 hour, 1day, 3 days, 1 week, 2 weeks, 1 month, 2 month, 3 months, 6 months, 9 months, or 1 year period.

In certain embodiments, the pharmaceutical formulation may be used to mitigate or reduce the patient's risk of developing breast cancer. For example, in certain embodiments, the pharmaceutical formulation may be used to mitigate or reduce the patient's risk of developing breast cancer between one or more annual intervening mammographic detections. In certain embodiments, the pharmaceutical formulation may be used to mitigate or reduce the patient's risk of developing breast cancer and avoids, mitigates, reduces or reverses one or more peri-menopausal symptoms between one or more annual intervening mammographic detections. For example, the one or more peri-menopausal symptoms that may be mitigated, reduced, or avoided may include but is not limited to, menstrual irregularity; hot flashes and sleep problems; mood changes; mood swings; irritability; depression; vaginal dryness; urinary or vaginal infections; urinary incontinence; decreasing fertility; changes in sexual arousal or desire; bone loss; fragile bones; osteoporosis; or changing cholesterol levels, such as an increase in low-density lipoprotein (LDL) cholesterol, a decrease in high-density lipoprotein (HDL) cholesterol; or combinations thereof.

In certain embodiments, the pharmaceutical formulations may be used to increase or improve the patient's fat to breast tissue ratio between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation increases or improves the patient's fat to breast tissue ratio from 1:19 to 19:1 between one or more annual intervening mammographic detections, such as increases or improves the treated patient's fat to breast tissue ratio from 1:15 to 19:1, from 1:10 to 19:1, from 1:5 to 19:1, from 1:2 to 19:1, from 2:3 to 19:1, from 2:1 to 19:1, from 4:1 to 19:1, from 6:1 to 19:1, from 8:1 to 19:1, from 10:1 to 19:1, from 1:19 to 10:1, from 1:10 to 10:1, from 1:4 to 10:1, from 1:2 to 10:1, from 3:2 to 10:1, from 3:1 to 10:1, from 5:1 to 10:1, from 7:1 to 10:1, from 9:1 to 10:1, from 15:1 to 10:1, from 1:15 to 5:1, from 1:5 to 5:1, from 1:3 to 5:1, from 3:2 to 5:1, from 3:1 to 5:1, from 6:1 to 5:1, 8:1 to 5:1, from 10:1 to 5:1, from 1:19 to 3:1, from 1:10 to 3:1, from 1:4 to 3:1, from 1:2 to 3:1, from 2:1 to 3:1, from 4:1 to 3:1, from 6:1 to 3:1, from 8:1 to 3:1, from 10:1 to 3:1, or from 15:1 to 3:1 between one or more annual intervening mammographic detections.

In certain embodiments, the pharmaceutical formulation may be used to increase or improve the patient's fat to breast tissue ratio from 1:19 to 19:1, such as from 1:10 to 19:1, from 1:5 to 19:1, from 1:2 to 19:1, from 2:3 to 19:1, from 2:1 to 19:1, over a 4 hour period, over an 8 hour period, over a 24 hour period, over a 3 day period , over a 1 week period, over a 2 week period , over a 1 month period, over a 2 month period, over a 3 month period , over a 6 month period , over a 9 month period , over a 1 year period, or over a 5 year period.

In certain embodiments, the pharmaceutical formulations may be used to increase the percentage of fat in the treated patient's breast between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation increases the percentage of fat in the treated patient's breast in the range of between 1% to 99% between one or more annual intervening mammographic detections, such as, in the range of between 1% to 90%, 1% to 70%, 1% to 50%, 1% to 30%, 1% to 20%, 1% to 15%, 1% to 10%, 3% to 60%, 3% to 20%, 5% to 70%, 5% to 50%, 5% to 30%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 60%, 10% to 40%, 10% to 30%, 10% to 20%, 10% to 15%, 20% to 50%, 20% to 30%, 30% to 60%, 30% to 50%, or 30% to 40% between one or more annual intervening mammographic detections.

In certain embodiments, the pharmaceutical formulation increases the percentage of fat in the treated patient's breast by at least 2%, such as by at least 5%, by at least 10%, by at least 25%, by at least 40%, by at least 75%, by at least 95%, or by at least 99%, over a 4 hour period, such as over an 8 hour period, over a 24 hour period, over a 3 day period, over a 1 week period, over a 2 week period, over a 1 month period, over a 2 month period, over a 3 month period, over a 6 month period, over a 9 month period, over a 1 year period, or over a 5 year period.

In certain embodiments, the pharmaceutical formulation enhances, increases, or improves, breast compression during mammographic visualization or detection of the breast between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation enhances, increases, or improves, breast compression during mammographic visualization or detection of the breast in the range of between 5% to 70%, 5% to 50%, 5% to 30%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 50%, 10% to 30%, 10% to 20%, 10% to 15%, 20% to 60%, 20% to 40%, 20% to 30%, 30% to 70%, 30% to 50%, or between 30% to 40% between one or more annual intervening mammographic detections. In certain embodiments, as a result of

the enhanced, increased, or improved, breast compression during mammographic visualization or detection of the breast, the pharmaceutical formulation further mitigates or reduces the patient's pain during the breast compression. For example, the pharmaceutical formulation further mitigates, reduces or minimizes, the patient's pain during the breast compression in the range of between 5% to 80%, 5% to 50%, 5% to 30%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 80%, 10% to 60%, 10% to 40%, 10% to 20%, 10% to 15%, 20% to 70%, 20% to 50%, 20% to 30%, 30% to 70%, 30% to 50%, or between 30% to 40% less pain as a result of the enhanced, increased, or improved, breast compression during mammographic visualization or detection of the breast.

In certain embodiments, the pharmaceutical formulation mitigates or reduces the patient's pain during the breast compression. For example, the pharmaceutical formulation mitigates or reduces the patient's pain during the breast compression in the range of between 5% to 80%, 5% to 60%, 5% to 30%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 80%, 10% to 60%, 10% to 40%, 10% to 30%, 10% to 20%, 10% to 15%, 20% to 70%, 20% to 50%, 20% to 30%, 30% to 90%, 30% to 50%, or between 30% to 40% between one or more annual intervening mammographic detections. In certain embodiments, as a result of the patient's mitigated, reduced or minimized pain during the breast compression, the pharmaceutical formulation further enhances, increases, or improves, breast compression during mammographic visualization or detection of the breast between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation further enhances, increases, or improves, breast compression during mammographic visualization or detection of the breast between one or more annual intervening mammographic detections in the range of between 5% to 80%, 5% to 60%, 5% to 40%, 5% to 30%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 80%, 10% to 60%, 10% to 40%, 10% to 20%, 10% to 15%, 20% to 80%, 20% to 60%, 20% to 30%, 30% to 80%, 30% to 50%, or between 30% to 40% between one or more annual intervening mammographic detections.

In certain embodiments, the pharmaceutical formulation mitigates or reduces the patient's pain according to the visual analog scale (VAS) during the breast compression. For example, the pharmaceutical formulation mitigates or reduces the patient's pain according to the VAS during the breast compression such that the patient does not suffer from significant pain between 50-100 mm, 50-80 mm, 50-70 mm, 60-

100 mm, 70-100 mm, 80-100 mm or between 90-100 mm during one or more mammographic detections or during one or more annual intervening mammographic detections.

In certain embodiments, the pharmaceutical formulation enhances increases or improves the patient's compliance of having regular mammographic visualizations or detections, for example, compliance with mammographic visualizations or detections at every 6 months, annually, every 2 years, every 3 years, or every 5 years.

In certain embodiments, the pharmaceutical formulation mitigates or reduces the amount of radiation exposure required to visualize or detect the patient's breast during one or more subsequent mammographies, such as during one or more subsequent annual mammographies. For example, the pharmaceutical formulation mitigates or reduces the amount of radiation exposure required to visualize or detect the patient's breast in the range of between 5% to 99%, 5% to 80%, 5% to 70%, 5% to 50%, 5% to 30%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 80%, 10% to 60%, 10% to 40%, 10% to 20%, 10% to 15%, 20% to 80%, 20% to 60%, 20% to 40%, 20% to 30%, 30% to 80%, 30% to 60%, 30% to 50% or between 30% to 40% during one or more subsequent mammographies, such as during one or more subsequent annual mammographies.

In certain embodiments, the pharmaceutical formulation induces breast involution in the breast of the patient, for example, in the breast of a peri-menopausal patient.

In certain embodiments, the pharmaceutical formulation induces involution of breast cells in the breast of the patient, for example, in the breast of a peri-menopausal patient.

In certain embodiments, the pharmaceutical formulation induces net cell death over proliferation in the breast of the patient, for example, in the breast of a peri-menopausal patient.

In certain embodiments, the pharmaceutical formulation reverses cell number and mammographic breast density in the breast of the patient, for example, in the breast of a peri-menopausal patient.

In certain embodiments, the pharmaceutical formulation mitigates or reduces breast stiffness in the breast of the patient, for example, in the breast of a peri-menopausal patient. For example, the pharmaceutical formulation mitigates or reduces breast stiffness in the breast of the patient in the range of between 5% to 80%, 5% to

60%, 5% to 40%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 80%, 10% to 60%, 10% to 40%, 10% to 30%, 10% to 20%, 10% to 15%, 20% to 80%, 20% to 60%, 20% to 40%, 20% to 30%, 30% to 80%, 30% to 60%, or between 30% to 40%, between one or more annual intervening mammographic detections. For example, the pharmaceutical formulation mitigates or reduces breast stiffness in the breast of the patient by at least 5%, such as at least 8%, at least 10%, at least 15%, at least 20%, or at least 30%, per annum. In certain embodiments, the pharmaceutical formulation mitigates or reduces breast stiffness in the breast of the patient by at least 5%, such as at least 8%, at least 10%, at least 15%, at least 20%, or at least 30%, over a 4 hour period, such as over an 8 hour period, a 24 hour period, a 3 day period, a 1 week period, a 2 week period, a 1 month period, a 2 month period, a 3 month period, a 6 month period, a 9 month period, a 1 year period, or a 5 year period.

In certain embodiments, the pharmaceutical formulation enhances, increases, or improves mammographic visualization or detection of the breast of the patient, for example, the breast of a peri-menopausal patient. For example, the pharmaceutical formulation enhances, increases, or improves mammographic visualization or detection of the breast of the patient in the range of between 5% to 80%, 5% to 50%, 5% to 30%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 80%, 10% to 60%, 10% to 30%, 10% to 20%, 10% to 15%, 20% to 80%, 20% to 60%, 20% to 30%, 30% to 80%, 30% to 60%, or between 30% to 40%, between one or more annual intervening mammographic detections. In certain embodiments, the pharmaceutical formulation enhances, increases, or improves mammographic visualization or detection of the breast of the patient by at least 5%, such as at least 10%, at least 15%, at least 25%, at least 40%, at least 50%, or at least 75%, over a 4 hour period, or over other time periods, such 8 hours, 24 hours, 3 days, 1 week, 2 weeks, 1 month, 2 month, 3 months, 6 months, 9 months, 1 year, or 5 years.

In certain embodiments, the pharmaceutical formulation reduces mammographic breast density and avoids inducing masculinizing androgenic side-effects or inducing a hyper-androgenic state. For example, masculinizing androgenic side-effects may include male-type baldness, hirsutism, or increased hair in areas unwanted by the patient, voice deepening, acne, or combinations thereof. In certain embodiments, the pharmaceutical formulation reduces mammographic breast density and is exclusive of inducing masculinizing androgenic side-effects or inducing a hyper-androgenic state. In

certain embodiments, the pharmaceutical formulation reduces mammographic breast density and minimizes induction of masculinizing androgenic side-effects or induction of a hyper-androgenic state.

In certain embodiments, the pharmaceutical formulation substantially improves or improves the patient's physical functioning, such as physical functioning related to the patient's central nervous system, libido, musculoskeletal system, cardiovascular system, risk of contracting autoimmune diseases, the severity of symptoms associated with autoimmune disease, or combinations thereof. For example, as related to the patient's central nervous system, the pharmaceutical formulation may reduce depression, anxiety, general cognitive dysfunction including memory, or reduce the risk of dementia and Parkinsonism. For example, as related to the patient's libido, the pharmaceutical formulation may provide a significant improvement in global libido, including speed to sexual arousal and the ability to achieve orgasm. For example, as related to the patient's musculoskeletal system, the pharmaceutical formulation may provide for a reduction in inflammatory and degenerative arthritis, an improvement in bone mineral density, or an improvement in muscle strength. For example, as related to the patient's cardiovascular system, the pharmaceutical formulation may provide a reduction in foamy macrophage deposition in the arterial wall, a reduction in atherosclerosis, an increase in high density lipoprotein's leading to an improvement in cholesterol, or a high-density lipoprotein ratio. For example, as related to the patient's risk of contracting autoimmune diseases, the pharmaceutical formulation may substantially reduce or reduces the treated patient's risk of contracting autoimmune diseases, such as Sjogren's syndrome, lupus, and rheumatoid arthritis. For example, as related to the severity of symptoms associated with the patient's autoimmune disease, the pharmaceutical formulation may substantially reduce or reduces the severity of symptoms associated with a treated patient's autoimmune disease, such as Sjogren's syndrome, lupus, and rheumatoid arthritis. In certain embodiments, the pharmaceutical formulation substantially improves or improves the patient's physical functioning, such as cognitive function; reduction of a degenerative CNS disease, comprising dementia or parkinsonism; muscle strength; libido; energy; reduction of monoamine oxidase induced anxiety and depression; or combinations thereof.

In certain embodiments, the pharmaceutical formulation further provides one or more of the following: i) reduces mammographic breast density; ii) increases

involutionary effects on the patient's breast without conversion of testosterone to estrogen; iii) substantially reduces, reduces, or reverses peri-menopausal symptoms; or iv) substantially improves or improves the patient's physical functioning, comprising cognitive function; reduction of symptoms associated with a degenerative CNS disease, comprising dementia or parkinsonism; muscle strength; libido; energy; reduction of monoamine oxidase induced anxiety and depression; or combinations thereof. In certain embodiments, the pharmaceutical formulation further provides one or more of the following: i) reduces mammographic breast density; ii) increases involutionary effects on hormonally affected end organs, comprising breast, without conversion of testosterone to estrogen; iii) substantially reduces, reduces, or reverses peri-menopausal symptoms related to fluctuating estrogen levels; or iv) substantially improves or improves the patient's physical functioning, comprising cognitive function; reduction of symptoms associated with a degenerative CNS disease, comprising dementia or parkinsonism; muscle strength; libido; energy; reduction of monoamine oxidase induced anxiety and depression; or combinations thereof.

In certain embodiments, the patient has a high free androgenic index level, for example, 30% or greater, within their breast within four hours of the administration of the androgenic agent and the aromatase inhibitor. In certain embodiments, the patient has a supra-physiological free androgenic index level within their breast within four hours of the administration of the androgenic agent and the aromatase inhibitor.

In certain embodiments, the treatment with a pharmaceutical formulation or combination treatment of the androgenic agent and the aromatase inhibitor as described herein further comprises: a) measuring free androgenic index levels and/or aromatase inhibitor levels in serum isolated from a blood sample taken from the patient after at least 1 month of treatment; b) determining a subsequent dose, comprising a subsequent effective amount of androgenic agent and a subsequent effective amount of an aromatase inhibitor; and c) administering to the patient the subsequent dose.

In certain embodiments, the treatment with a pharmaceutical formulation or combination treatment of the androgenic agent and the aromatase inhibitor as described herein further comprises: a) measuring free androgenic index levels and/or aromatase inhibitor levels in serum isolated from a blood sample taken from the patient after at least 1 month of treatment, comprising centrifuging the patient's blood sample to isolate the serum; b) determining a subsequent dose, comprising a subsequent effective amount

of androgenic agent and a subsequent effective amount of an aromatase inhibitor; and c) administering to the patient the subsequent dose.

In certain embodiments, the measured free androgenic index serum levels of a treated patient after 1 month may be between 10-25%, such as between 10-20%, 10-15%, 15-25%, 15-20%, 12-18%, 8-15%, or between 11-14%.

In certain embodiments, the measured free androgenic index serum levels of a treated patient after 3 months may be between 2-10%, such as between 2-8%, 2-6%, 2-5%, 2-4%, 4-10%, 5-8%, 3-7%, 4-6%, 3-6%, 4-7%, 5-10% or between 2-5%.

In certain embodiments, the administration of the aromatase inhibitor reduces aromatization of testosterone to estrogen in the subcutaneous fat of the treated patient, for example, reduces aromatization by 80-95% or 100%. For example, the administered aromatase inhibitor may reduce aromatization of testosterone to estrogen in the subcutaneous fat of the patient's breast, the subcutaneous fat of the patient's pelvis, the subcutaneous fat of the patient's buttocks, the subcutaneous fat of the patient's abdomen or combinations thereof, for example, reduces aromatization by 80-95% or 100%.

In certain embodiments, the administration of the aromatase inhibitor reduces aromatization of adrenal androgens, for example, androstenedione, to estrogen in the subcutaneous fat of the treated patient, for example, reduces aromatization by 80-95% or 100%. For example, the administered aromatase inhibitor may reduce aromatization of testosterone to estrogen in the subcutaneous fat of the patient's breast, the subcutaneous fat of the patient's pelvis, the subcutaneous fat of the patient's buttocks, the subcutaneous fat of the patient's abdomen or combinations thereof, for example, reduces aromatization by 80-95% or 100%.

Annual mammographic screening of density may be undertaken to determine the reduction in breast density utilizing an appropriate mammographic algorithm that measures the volume of fibro-glandular tissue as a percentage of total breast volume (MBD). The objective is to achieve MBD of less than 10% when this is a function of the average of both breast densities. The rate of breast density reduction should be at least 2% per annum. An annual uplift factor (V1(N) in the above) may be introduced into TD and AD of 10% of dosing if 2% is not achieved in the first year. This annual uplift factor typically will only be introduced, on an annual basis, if there is less than 10% increase in androgenicity index (AI).

Annual mammographic screening of density may be undertaken to determine a reduction in breast density utilizing an appropriate mammographic algorithm that measures the volume of fibro-glandular tissue as a percentage of total breast volume (VBD%). The objective is to achieve VBD% of less than 10% when this is a function of the average of both breast densities. The rate of breast density reduction (VBD%) should be at least 2% per annum. An annual uplift factor (V1(N) in the above) may be introduced into TD and AD of 10% of dosing if 2% is not achieved in the first year. This annual uplift factor typically will only be introduced, on an annual basis, if there is less than 10% increase in androgenicity index (AI).

In certain embodiments, the administration of aromatase inhibitor in combination with testosterone results in an improvement in the bioavailability of dihydrotestosterone between 25% to 75%, 35% to 65% or 45% to 55%. In certain embodiments, the amount of increase in bioavailability of dihydrotestosterone is greater than 25%, greater than 35%, greater than 45% or greater than 55%.

The androgenic agent used in a pharmaceutical formulation or combination treatment as described herein, for example, may be selected from the group consisting of testosterone, methyltestosterone, and/or dehydroepiandrosterone. In certain embodiments, the androgenic agent may be testosterone undecanoate, such as about 40mg of testosterone undecanoate. The aromatase inhibitor, for example, may be selected from the group consisting of anastrozole, exemestane, or letrozole. In certain aspects, the aromatase inhibitor may be anastrozole, such as about 1mg of anastrozole.

In certain embodiments, the pharmaceutical formulation may include administering a pharmaceutical formulation comprising an androgenic agent linked to an aromatase inhibitor, *e.g.*, via an ester linkage, or an androgenic agent/aromatase inhibitor complex, wherein the complex is created by methods in the art.

In preferred embodiments, both the androgenic agent (*e.g.*, testosterone, methyltestosterone, and/or dehydroepiandrosterone), and the aromatase inhibitor (*e.g.*, anastrozole, exemestane, or letrozole) are administered subcutaneously, e.g., as an implant such as a pellet.

In certain embodiments, the androgenic agent (*e.g.*, testosterone, methyltestosterone, and/or dehydroepiandrosterone), and the aromatase inhibitor (*e.g.*, anastrozole, exemestane, or letrozole) are administered subcutaneously, *e.g.*, as a pellet. For example, testosterone and anastrozole may be administered subcutaneously.

The appropriate dosing regimen utilizing the androgenic agent, the aromatase inhibitor, or pharmaceutical formulations comprising the androgenic agent and the aromatase inhibitor, the amount of each dose administered, and the intervals between doses of the compounds may depend on various factors such as the particular aromatase inhibitor and androgenic agent being used in combination, the type of pharmaceutical formulation being used, the type of physiological condition being treated, the characteristics of the subject being treated (*e.g.*, species, age, weight, sex, medical condition, fed/fasted), the route of administration, and the severity of the disorder being treated or combinations thereof. A physician or diagnostician of ordinary skill may readily determine and prescribe the effective amount of the androgenic agent, the aromatase inhibitor, or pharmaceutical formulation to prevent or to treat the specific physiological condition.

The pharmaceutical formulation or formulation to be administered may contain a quantity of the compounds or pharmaceutically acceptable salts or esters thereof in an amount effective to treat the condition of the subject being treated. Because two different compounds may be used together in a combination therapy, the potency of each of the compounds and the interactive effects achieved by combining them together typically will also be taken into account. A consideration of these factors is well within the purview of the ordinarily skilled clinician for the purpose of determining the therapeutically effective or prophylactically effective dosage amounts needed to improve side effects.

Administration of the androgenic agent and the aromatase inhibitor, or the pharmaceutical formulation comprising a combination of the same, to the subject includes both self-administration and administration to the subject by another person (*e.g.*, physician, nurse, health care worker, friend, etc.).

In certain embodiments, the pharmaceutical formulation may be formulated in a manner compatible with a desired outcome. The pharmaceutical formulations may be administered in a convenient formulation. The following formulation examples only are illustrative and are not intended to limit the scope of the present disclosure.

In certain embodiments, the pharmaceutical formulation can be an implant or pellet formulated by direct compression. For example, the pellet formulations may incorporate diluents, binders, lubricants and, disintegrators as well as the active ingredients. Typical diluents include, for example, various types of starch, lactose,

mannitol, kaolin, calcium phosphate or sulfate, inorganic salts such as sodium chloride and powdered sugar. Powdered cellulose derivatives are also useful. Natural and synthetic gums are also convenient, including acacia, alginates, methylcellulose, and/or polyvinylpyrrolidine. Polyethylene glycol, ethyl cellulose and waxes may also serve as binders. A lubricant may be necessary for a pellet formulation to prevent the pellet and punches from sticking in the die. The lubricant may be chosen from such slippery solids as talc, magnesium and calcium stearate, stearic acid and/or hydrogenated vegetable oils. Typically, the implant or pellet will have an implant or pellet hardness in a range of from about 6 Kg/N to about 10Kg/N, preferably from about 7Kg/N to about 9Kg/N, most preferable about 8Kg/N and all such dosage forms within this range are expressly provided for herein.

By compressing the formulation into a solid dosage form such as an implant in the form of pellet for subcutaneous administration, a sustained release multi-phasic concentration pattern as described herein may be obtained. Knowing that such a release pattern of an androgenic agent and aromatase inhibitor may be obtained, a person of ordinary skill in the art will be able to utilise different ingredients in the formulation in view of the disclosures provided herein and provide such pharmaceutical formulations in accordance with the present disclosure.

In certain embodiments, the implant or pellet may be inserted into the subcutaneous fat of the subject's pelvis, the subcutaneous fat of the subject's breast, the subcutaneous fat of the subject's buttocks, the subcutaneous fat of the subject's abdomen or combinations thereof. In certain emboidments, the implant or pellet may be inserted into the subcutaneous fat of the subject's lower abdominal wall. In certain emboidments, the implant or pellet may be inserted into the subcutaneous fat of the subject's upper gluteal region.

As will be understood, the androgenic agent and aromatase inhibitor may be provided in pharmaceutical formulations other than for subcutaneous administration either together in the same formulation or in separate formulationsfor use in combination treatments as described herein, such formulations comprising one or more of suitable filler(s), lubricants, binders, disintergrants and/or pharmaceutically acceptable carriers or excipients as described above or used in the provision of pharmacutical formulations for the desired route of administration and/or for obtaining

the desired release pattern (e.g., a sustained release multi-phasic release pattern) of the active(s).

In certain embodiments, the androgenic agent, the aromatase inhibitor, or pharmaceutical formulation comprising a combination of the same, may be provided in the form of an article of manufacture, such as a kit, which includes the active ingredients therein, or the active ingredients in suitable pharmaceutical formulations, packaged for distribution. Kits may additionally include instructions for using the kit components in one or more of the disclosed methods. Instructions may include instructions for practicing one or more of the disclosed methods. Thus, for example, a kit may include an androgenic agent or an aromatase inhibitor in a pharmaceutical formulation in a container, pack, or dispenser together with instructions for administration to a human subject. Instructions may additionally include indications of a satisfactory clinical endpoint or any adverse symptoms that may occur or any additional information required by the Food and Drug Administration for use in humans.

The instructions may be on "printed matter," *e.g.*, on paper or cardboard within the kit, or on a label affixed to the kit or packaging material, or attached to a vial or tube containing a component of the kit. Instructions may additionally be included on a computer readable medium, such as a disk (floppy diskette or hard disk), optical CD such as CD- or DVD-ROM/RAM, magnetic tape, electrical storage media such as RAM and ROM, and hybrids of these such as magnetic/optical storage media.

While the present disclosure has been described in terms of certain exemplary embodiments in order to facilitate a better understanding of the present disclosure, it should be appreciated that various modifications may be made without departing from the principles of the disclosed herein. Therefore, the inventions should be understood to include such modifications within its scope.

EXAMPLES

Example 1: A Single Dose Trial to Evaluate the Pharmacokinetics of Testosterone and Anastrozole from a Subcutaneous Testosterone and Anastrozole Implant, 80 mg/4 mg (T+Ai) in Women

Pharmacokinetic analysis was undertaken on a subcutaneous testosterone 80 g and anastrozole 4 mg implant in 11 healthy volunteers with high mammographic breast density. This study evaluated the patterns for both anastrozole and testosterone and permitted modeling of the pharmacokentics of this exemplary implant in order to demonstrate a pattern and end organ pharmacodynamic response where it is known that there is an excess of aromatase activity, i.e., in the tissue of women with high mammographic breast density. This is evaluated by direct measurement of breast tissue elasticity which is related to mammographic breast density.

Ultrasound evaluation of pellet dissolution was undertaken to demonstrate how input dissolution comprehensive analysis of reproductive hormones were undertaken to evaluate the potential impact on hypothalamic-pituitary function.

Primary objectives:

To evaluate the pharmacokinetic characteristics of testosterone following a single dose of T+Ai by subcutaneous implantation.

To evaluate the pharmacokinetic characteristics of anastrozole following a single dose of T+Ai by subcutaneous implantation.

Secondary Objective

To explore the potential effect on breast tissue elasticity as assessed by shear wave ultrasound.

Implant Preparation: Testosterone-Anastrozole Pellets

Pellets for subcutaneous insertion were prepared by compressing sterile non-micronized testosterone, anastrozole and stearic acid in an 80:4:2 ratio into a 5mm diameter cylindrical die. The pellets are transferred to a sterile 5ml amber vial covered with sterile cotton wool and sealed with a rubber stopper and aluminium rip seal which is crimped. The vials are sterilized by gamma irradiation at 25KGy. Testosterone non-micronized and anastrozole, USP grade, were obtained from Azelis (Brookvale, NSW). The stearic acid, NF quality, was obtained from Medisca (Mascot, NSW). The steps for producing these pellets are outlined below:

1. Blend titration of testosterone (non-micronized), anastrozole, and stearic acid by mechanical means for 2 hours.
2. Mix titration thoroughly.
3. Compress pellets with a press that produces pellet hardness of 8Kg/N
4. The pellet is stored in amber glass 5 ml vial, pellet cushioned with sterile cotton wool.
5. The stored pellet is gamma sterilize at 25KyG.
6. Properly label pellets.
7. Store in a cool, dry place (room temperature; 20-25° C, 66-78° F).

Methodology

Pre-menopausal women with high Mammographic Breast Density (MBD) was selected for the study. Following the successful screening, participants came to the Wellend Clinic South Australia for dosing. The participants were to be dosed while in the luteal phase of their menstrual cycle to avoid the highly changing hormonal environment of the follicular phase. Following insertion of the implant, PK blood sampling was carried out for a duration of three months. Briefly, samples were taken repeatedly on the day of dosing, daily during the first week, weekly for the next four weeks and every second week during the last eight weeks, for a total sampling period of three months. After assessments had been completed on the last day of assessments, Day 85, the trial concluded.

To determine whether the selected pharmacokinetic (PK) sampling schedule provided an adequate description of the serum/plasma concentration time profile of T+Ai, after the Day 29 blood samples had been collected from the first two participants, the samples were analysed for testosterone, dihydrotestosterone, and anastrozole. The sampling schedule was revised with the addition of one blood sample (at 6 hours post-dose), and no adjustment of the timing of existing time points. The duration of sampling was not extended.

In summary, following dosing on Day 1, blood samples were taken pre-dose (within one hour of dosing) and at 1, 2, 4, 6, 8 and 12 hours. Participants returned for a blood sample to be taken at 9 am in the morning on Days 2, 3, 4, 5, 8, 15, 22, 29, 43, 57, 71 and 85.

A total of 11 subjects were dosed and included in the safety analysis set. Eleven subjects were included in the PK analysis set. Women were aged between 35 and 55 years of age, Body Mass Index (BMI) range of 20-30 kg/m². The demographics of the participants are shown in Table 1.

Table 1: Summary of Participant Demographic Data (Safety Analysis Set)

Demographic Parameter (Units)	Number of Participants	Average	Standard Deviation	Median	Minimum	Maximum
Gender (Female)	11					
Age (years)	11	41.5	3.7	40	37	49
Weight (kg)	11	67.87	10.27	64.9	50	83.4
Height (m)	11	1.673	0.066	1.67	1.54	1.79
BMI (kg/m^2)	11	24.14	2.26	23.4	20.8	27.4
Race (White)	10					
Race (White/Asian)	1					
Ethnicity (Not Hispanic or Latino)	11					

PK Results

A summary of the PK parameters for plasma anastrozole and serum testosterone is presented in Table 2 and illustrated in Figures 1 to 6.

Table 2: Summary of Plasma Anastrozole and Serum Testosterone PK Parameters

	Median (Range)	Arithmetic Mean (CV%)					
Analyte	T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (hr*ng/mL)	$t_{½}$ (days)	AUC_{0-inf} (hr*ng/mL)	CL/F (L/hr)	AUC_{84d} (hr*ng/mL)
Plasma Anastrozole (n=11)	48.6 (23.6-72.5)	5.30 (14%)	88.0 (25%)	13.6 (31%)	93.2 (25%)	1.88 (22%)	-
Serum Testosterone (n=11)	8.0 (5.9-12.0)	4.08 (21%)		42.8 (34%)	-	-	127.2 (23%)

With regard to pharmacokinetics, median T_{max} for plasma anastrozole was 2 days post-dose (range 1-3 days), with average C_{max} = 5.30 ng/mL, and an average terminal half-life of 14 days. Concentrations were below the limit of quantitation (0.1

ng/mL) in most participants after 12 weeks. The half-life of 14 days observed from T+Ai is considerably longer than the 2 days half-life stated for oral anastrozole, confirming that the T+Ai product acted as a sustained-release formulation. As the objective of this therapy is to reduce aromatisation of T at the insertion site (i.e., subcutaneous adipose where the aromatase level is average and in tissues where the aromatase is high) the minimal effective dose is given compared to when treating breast cancer where total body aromatase inhibition is sought to maximise anti-neoplastic effect. The IC50 for anastrozole in tissue overexpressing aromatase (such MCF7 breast cancer cell line and JEG3 human choriocarcinoma cell line) is 3.62 and 5.66 nM respectively. The C_{max} = 5.3 ng/mL (18.07 nM) would, therefore, cover the initial higher C_{max} = 4.1 ng/mL of T with a sustained lower level of anastrozole with the decline in T level. Accordingly, the use of 4mg of anastrozole in the pellet achieved a concentration required for an IC50 of aromatase sufficient to inhibit the aromatase enzyme in the target tissue. In contrast, a dose of 2mg would not have been sufficient.

For serum testosterone, the median T_{max} was 8 hours post-dose (range 6-12 hours), with average C_{max} = 4.1 ng/mL, compared with average at baseline of 0.2 ng/mL. There was a second lower peak at 2-3 weeks. Increase in testosterone compared with baseline was sustained in participants at 12 weeks, with an average concentration of 0.8 ng/mL. Serum dihydrotestosterone was not measurable in most samples, with T_{max} at 2-3 days post-dose in participants with measurable maximal values. This example shows that the pellets after subcutaneous insertion exhibited a pattern.

Example 2: Testosterone and Anastrozole Combination Therapy for MBD reduction in Woman

This example provides an analysis of the used testosterone and anastrozole combination therapy for mammographic breast density (MBD) reduction in woman. The study was conducted at the Wellend Clinic (Burnside War Memorial Hospital, Adelaide, South Australia). The major indication for therapeutic intervention was one or more of the following: perimenopausal hormone dysfunction, high MBD, which is considered a factor in reducing breast cancer (BC) risk.

All 652 patients were female, with an average age at the time of first T+Ai implant of 52 years (ranging from 23 to 79 years).

Reduction of MBD/BC risk was the primary indication for treatment in 89 patients (14%), assistance with hormonal dysfunction in 334 patients (51%) and both indications in 177 patients (27%). The primary indication was not provided for 52 patients.

A history of BC was noted in 90 patients (14% of patients with non-missing responses for BC history). The type of BC was noted for 20 patients – 14 had in-situ type BC, and six had invasive type BC.

Estrogen based concomitant medication use was noted for 222 patients (34%), of which the majority was in the form of topical medications (n=192).

At the time of the data cut in July 2017, 365 patients (56%) were continuing to receive T+Ai therapy. One patient did not have disposition information recorded, while the remaining 286 patients had stopped T+Ai therapy. The most frequent reason for stopping T+Ai therapy was lost-to-follow-up (n=123), followed by subject decision to cease therapy (n=46), cost of therapy (n=30), subject feeling T+Ai therapy was not working (n=28) and primary care physician advising the patient that T+Ai therapy was complete (n=25).

Measurement of Efficacy

Summary statistics for continuous variable measurements (including %VBD and AVBD measures of MBD) obtained from mammograms. As mammograms were not scheduled for specific time-points, a visit windowing system was applied to the data in order to provide a specific data value to a specific time-point. The windows chosen were as follows:

Six months (180 days after first T+Ai implant +/- 60 days)
One year (365 days after first T+AI implant +/- 180 days)
Two years (730 days after first T+AI implant +/- 180 days)
Three years (1095 days after first T+AI implant +/- 180 days)
Four years (1460 days after first T+AI implant +/- 180 days)

Drug Dose and Relationship to %VBD Response

To examine the impact of T+Ai intervention on MBD as measured by %VBD, a subset of patients who had mammograms both before and subsequent to the

commencement of treatment was considered. For this subset of patients (n=142), the change from baseline MBD was used as the outcome measure in a mixed model analysis using SAS® PROC MIXED. This procedure allows for the potential of individual patients to contribute more than one mammogram following commencement of T+Ai treatment (repeated measures analysis), as well as examining different covariance patterns amongst the data.

From the data set provided for analysis, the following independent (explanatory) variables were used to examine their impact on the change in MBD:

i. Days since first implant
ii. Baseline %VBD MBD measurement (the value closest to, but not later
iii. than, the first T+Ai implant)
iii. Cumulative testosterone dose (mg) across the entire study (stratified into <500mg, 500mg to <700mg, and 700mg+).
iv. Cumulative anastrozole dose (mg) across the entire study (as a continuous covariate)
v. Age (in years) at first implant
vi. Machine type (GE or Hologic machine)
vii. Radiation dose at the mammogram
viii. Compression pressure at the mammogram
ix. History of breast cancer (yes or no)
x. Use of concomitant estrogen medications (yes or no)
xi. Interaction term between days since first implant and cumulative dose of testosterone strata.

The last interaction term listed above allows for the fitting of different slopes to each of the testosterone dose strata to see if potential differences exist across the strata.

The following syntax was used in SAS to fit the model:

```
proc mixed data=mmg3;
class TESTO_GP SUBJIDC MMG_MACH_N BCA_HIST_N E2_USED_N ;
model CHANGE_MMG_RESULT=MMG_DAY BASE_MMG_RESULT
TESTO_GP MMG_MACH_N BCA_HIST_N E2_USED_N SUM_ANAST
MMG_RAD_DOSE_N MMG_COMP_PRES_N
MMG_DAY*TESTO_GP /ddfm=kr cl s;
random INT MMG_DAY /subject=SUBJIDC type=un;
lsmeans TESTO_GP / at MMG_DAY=365 cl;
lsmeans TESTO_GP / at MMG_DAY=730 cl;
lsmeans TESTO_GP / at MMG_DAY=1095 cl;
lsmestimate TESTO_GP 'Across all dose levels at 1 yr' 1 1 1 /divisor=3 at
MMG_DAY=365 cl;
lsmestimate TESTO_GP 'Across all dose levels at 2 yr' 1 1 1 /divisor=3 at
MMG_DAY=730 cl;
lsmestimate TESTO_GP 'Across all dose levels at 3 yr' 1 1 1 /divisor=3 at
MMG_DAY=1095 cl;
format TESTO_GP testo. BCA_HIST_N E2_USED_N yesno.
MMG_MACH_N machine;
run.
```

Each of the terms in the model statement represents an item from the previous bullet list. The random statement is used to account for repeated measurements taken over time within each patient. An unstructured covariance structure was used as it provided a slightly better fit when compared to compound symmetry (CS) and autoregressive (AR1) options in SAS®. LSMEANS and LSMESTIMATE statements were used to estimate the change from baseline in MBD by testosterone dose strata, at 1-year, 2-years, and 3-years. Table 3 presents parameter estimates obtained from the model.

Statistically significant findings were noted for days since first T+Ai implant, and the interaction between days since first T+Ai implant and cumulative testosterone strata. Specifically, larger decreases in MBD were noted over time for patients with accumulated testosterone dosing of over 500mg compared with patients with <500mg. Cumulative anastrozole dosing also approached significance levels of 0.05 (p=0.06),

with estimates also indicating a larger reduction in MBD for higher accumulated anastrozole doses. Baseline %VBD was also statistically significant, with higher baseline scores having larger observed changes (reduction in %VBD).

Table 3: SAS PROC MIXED model estimates for change from baseline in MBD

Explanatory variable	Category comparison	Estimate	p-value	Lower 95% CI	Upper 95% CI
Intercept	N/A	2.4813	0.1799	-1.1552	6.1178
Days since first T+Ai implant	N/A	0.001084	0.3692	-0.00131	0.00348
Baseline %VBD MBD measurement	N/A	-0.1527	<.0001	-0.2248	-0.08049
Cumulative testosterone strata	500 to <700mg versus <500mg	1.446	0.2331	-0.9479	3.8399
Cumulative testosterone strata	700+mg versus <500mg	0.6022	0.683	-2.3086	3.513
Mammogram machine type (broad category)	GE versus Hologic	0.07725	0.8821	-0.9491	1.1036
BC history	No versus Yes	0.1302	0.8717	-1.4632	1.7236
Concomitant estrogen medication use	No versus Yes	-0.2762	0.6333	-1.4196	0.8673
Cumulative anastrozole dose (mg)	N/A	-0.07478	0.3034	-0.218	0.06846
Mammogram radiation dose	N/A	-0.2979	0.3998	-0.9944	0.3985
Mammogram compression pressure	N/A	-0.02976	0.6683	-0.1666	0.1071
Interaction: Days since first T+Ai implant by Cumulative testosterone strata	500 to <700mg versus <500mg	-0.00264	0.1515	-0.00628	0.001006
Interaction: Days since first T+Ai implant by Cumulative testosterone strata	700+mg versus <500mg	-0.00236	0.1764	-0.00585	0.00112

Least squares mean of the change from baseline in %VBD measure of MBD, by cumulative testosterone strata, were estimated at 1 year, 2 years, and 3 years. Table 4 below presents the results from the model. Rows highlighted show estimated changes from baseline that are statistically significantly different from zero ($p<0.05$). An analysis of change from baseline in %VBD least squares mean estimates at one, two and three years.

Table 4: Least squares estimated change from baseline in MBD, by cumulative testosterone dose and time since first implant

Cumulative testosterone dose group	Time since first implant	Least squares mean estimate	Lower 95% CI	Upper 95% CI	p-value
<500mg	1 year	-1.6085	-2.8628	-0.3542	0.0123
<500mg	2 years	-1.213	-2.6838	0.2579	0.1053
<500mg	3 years	-0.8174	-2.8829	1.248	0.4349
500 to <700mg	1 year	-1.1249	-2.4165	0.1668	0.0872
500 to <700mg	2 years	-1.6916	-3.0057	-0.3775	0.0121
500 to <700mg	3 years	-2.2584	-4.2275	-0.2893	0.0251
700+mg	1 year	-1.8688	-3.6155	-0.1222	0.0362
700+mg	2 years	-2.3358	-3.8817	-0.7899	0.0034
700+mg	3 years	-2.8028	-4.6566	-0.949	0.0035

Efficacy of T+Ai in Reduction of MBD as Measured by AVBD

In addition to the analysis conducted using %VBD MBD measurement, a complementary analysis was undertaken using the same model discussed above in this example, with a change from baseline Absolute Volumetric Breast Density (AVBD) as the dependent variable. The only other change to the list of dependent variables used was to replace baseline %VBD with baseline AVBD. Least squares mean estimates from the AVBD model are displayed in Table 5 below. Again, statistically significant values are shown in the highlighted rows. The only values of significance were those in the 700mg+ cumulative testosterone group, at two and three years post-first T+Ai implant. The change from baseline AVBD estimates was -22cm^3 and -60cm^3, at two years and three years respectively.

Table 5: Least squares estimated change from baseline in AVBD, by cumulative testosterone dose and time since first implant

Cumulative testosterone dose group	Time since first implant	Least squares mean estimate	Lower 95% CI	Upper 95% CI	p-value
<500mg	1 year	-12.6001	-25.7233	0.523	0.0597
<500mg	2 years	-11.2073	-28.3175	5.9029	0.1975
<500mg	3 years	-9.8144	-35.6603	16.0314	0.4537
500 to <700mg	1 year	0.2747	-12.2756	12.8251	0.9652
500 to <700mg	2 years	-4.1993	-19.8209	11.4223	0.595
500 to <700mg	3 years	-8.6734	-34.0633	16.7166	0.4985
700+mg	1 year	-7.7803	-26.2516	10.691	0.4052
700+mg	2 years	-21.9964	-39.4828	-4.5101	0.0142
700+mg	3 years	-36.2126	-59.7196	-12.7056	0.0029

Efficacy Conclusions

Using mammogram information obtained from 142 patients with mammograms taken both pre- and post-commencement of T+Ai therapy, there were statistically significant changes from baseline MBD measurement of both %VBD and AVBD following intervention with T+Ai therapy.

When MBD was measured by %VBD, patients with cumulative testosterone dosing of 700mg+, the 1-year post-commencement of therapy estimated change was -1.87, the 2-year post-commencement of therapy estimated change was -2.34, and the 3-year post-commencement of therapy estimated change was -2.82. For patients with cumulative testosterone dosing of 500-<700mg, the 1-year post-therapy commencement estimated change was -1.12 (p=0.09), the 2-year post-therapy commencement estimated change was -1.69, and the 3-year post-therapy commencement estimated change was -2.26.

When MBD was measured by AVBD, the values of significance were those in the 700mg+ cumulative testosterone group, at 2 and 3 years post-first T+Ai implant. The change from baseline AVBD estimates was -22cm^3 and -60cm^3, at 2 years and 3 years respectively.

The majority of women in example 2 received a dose of anastrozole of 2mg per implant. This dose was chosen in order to attempt to maintain as low a dose as possible in order to avoid side-effects. Although efficacy at this dosing was effective, Example 2 shows that cumulative anastrozole dosing is a variable of interest when assessing change in MBD (p=0.06). Larger values of cumulative anastrozole dose were associated with larger changes from baseline (decreases from baseline) in MBD. Surprisingly and unexpectedly the PK and PD of anastrozole shown in Example 1 shows that a larger dose of anastrozole (4mg per implant) may be used that achieves efficacy and also avoids, or minimizes, side effects associated with the aromatase inhibitors disclosed herein.

Example 3: Pharmacokinetics Profile of Anastrozole Using a Modelling Approach

This example provides an analysis of a pharmacokinetic profile of anastrozole using a modelling approach in order to evalute an optimal input function to describe absorption of the trial set forth in Example 1. Table 6 sets forth the sampling schedule for anastrozole and testosterone. Blood samples of at least 8 mL were collected for serum testosterone/DHT and plasma anastrozole by venepuncture at each time point specified in table 6. Figures 7A-B illustrate the anastrozole absorption rates. Data in these figures are presented as mean +/- standard deviation. Figure 7A shows the Y axis on the log scale. Figure 7B shows the Y axis on a linear scale. The figures were produced using the pellet absorption data summarized in Table 2 of Example 1. The percentage change from baseline was calculated for the average volume and plotted against time in months. Figures 8A-B plot the plasma anastrozole concentration post implantation. In Figure 8A, the data on the X axis is plotted in time (hours) after transplantation. In Figure 8B the X axis is on a log scale. In Figures 8A-B, the open circles equal observed data, the black line equals median PK profile, and the dashed dark grey line equals lower limit of quantification (0.1 ng/mL). Figure 9 shows the frequency of observation post implantation. Approximately fifteen percent of the data was below the lower limit of quantification (BLQ). The BLQ concentrations were observed either pre-dose or after 1000 hours post implantation.

Table 6

Day 1	Other Days
1, 2, 4, 6, 8 and 12 hours post implantation	Days 2, 3, 4, 5, 8, 15, 22, 29, 43, 57, 71 and 85 post implantation

In this example, a population modelling approach was used to characterise the PK of anastrozole. One and two compartment models were fitted to the data. Several absorption models were tested such as first and zero order, mixed order, as well as transit compartments. Model selection was performed on the basis of statistical criteria (objective function) as well as visual inspection of diagnostic plots. Figure 10 shows a flow chart of the models tested. Figure 11 shows the final structure model used in this example. This example illustrates that the best model fitting the data was a two-compartment disposition model with absorption described by a dual input. Between-subject variability (BSV) was included on apparent clearance (CL/F), apparent volume of distribution for the central compartment (V_C/F) and the first-order absorption rate constant (KA). A proportional error model was used to describe residual unexplained variability (RUV).

Below Table 7 provides the parameter estimates of Anastrozole. Parameters were estimated with good precision (RSE <30%) with the exception of the between subject variability term on Vc/F, and apparent inter-compartmental clearance (Q/F), however, was considered acceptable given the data available. The model estimated value of CL/F was 1.77 L/h, which is comparable to the estimate from the NCA of 1.88 L/h demonstrated in Table 2 of Example 1.

Table 7

Parameter Name	Estimated Value (%RSE)	BSV CV% (%RSE)
Apparent clearance (CL/F, L/hr)	1.77 (6.9)	21.9 (23.3)
Apparent central volume of distribution (V_c/F, L)	872 (3.0)	17.7 (30.9)
Inter-compartmental clearance (Q/F, L/hr)	0.97 (57.5)	-
Apparent peripheral volume of distribution (V_p/F, L)	106 (29.8)	-
Absorption rate constant (ka, /hr) first input	0.119 (20.6)	66.5 (13.9)
Absorption rate constant (ka, /hr) second input	0.148 (20.5)	-
Fraction absorbed via second input	0.8 FIX	-
Lagged time for second input (h)	3.77 (9.7)	-
Residual unexplained variability (Proportional)	16.8 (8.3)	-

%RSE = %relative standard error, BSV = between subject variability, CV% = %coefficient of variation

Figures 12A-B shows the population and individual predictions. Figure 12A shows the population prediction. Figure 12B shows the individual prediction. The light grey lines represent the line of identity and the dark grey lines show the trend in the data (Loess smooth). The tailing in the trend line for Fig 12B is expected since the BLQ data are censored. Overall the model predicts the data well with minimal bias. Population predicted concentration is the representation of a typical patient. The individual predicted concentration is a post-Bayes/individualized prediction. Figure 13(ID:1-11) shows the individual plots of observed and predicted plasma concentrations of anastrozole. The data was plotted at nominal times. The dashed black lines equal population model prediction, the solid grey lines equals individual model prediction, and the circles equals individual observed data. The final model was used to simulate 1000 replicates of the dataset to validate the model simulation characteristics. From the simulated data, the 95% CI around the median, and 10^{th} and 90^{th} prediction intervals were calculated. The median, 10^{th} and 90^{th} percentiles of the observed data was then overlaid on the simulated data to validate the model.

Figure 14 shows a visual predictive check (VPC) of anastrozole concentration post implantation. The VPC shows that the observed data was captured well by the simulated data from the model. X-axis and Y-axis are on a log scale. Dashed black lines = observed 10^{th} and 90^{th} percentiles, solid black line = observed median, shaded grey area = 80% prediction interval, light grey dotted line = lower limit of quantification.

Figures 15A-B shows one versus two compartment model. Figure 15A represents a one compartment disposition with first order. Figure 15B represents a two-compartment disposition with a dual input. The distribution of conditional weighted residuals (CWRES) against time for the one compartment model (Figure 15A) shows

that there was a misspecification in the elimination phase. In contrast for the two-compartment model (Figure 15B) shows the CWRES distribution over time is evenly distributed.

Figure 16 displays predictions for patient 10 from several different input models including: A single first order input (Model 9), a simultaneous first and zero order input (Model 10), a single zero order input (Model 11) and a dual first order input (Model 15 1). From the plots it was evident that models other than the dual first order input fails to properly capture the early phase of absorption.

Summary

The present analysis shows that anastrozole PK follows a two-compartment disposition profile. The absorption profile of anastrozole was well described by a dual input. The other input models failed to properly capture the absorption phase.

Example 4: Breast Tissue Elasticity

This example looks at the potential effect on breast tissue elasticity as assessed by shear wave ultrasound in the subjects that took part in the trial set forth in Example 1. Breast elasticity was measured by shear wave ultrasound measure on Days 1, 29, 57 and 85. Four measurements of glandular tissue elasticity and fatty tissue elasticity were taken for each breast. The calculated average of the eight measurements for each tissue type was the value used in summaries and analyses of breast elasticity. A summary of breast elasticity at each timepoint, and of the change from baseline is provided in table 8. Table 8 demonstrated breast tissue elasticity reduced progressively over time, with reductions from baseline of 28% for glandular tissues and 32% for fatty tissue.

Table 8

(n=11)	Breast Tissue Elasticity (kPa)			
	Measured Value Average (±Standard Deviation)		Percent of Baseline Average (±Standard Deviation)	
Visit	Fatty Tissue	Glandular Tissue	Fatty Tissue	Glandular Tissue
Week 1, Day 1	9.98 (±3.38)	16.49 (±4.79)	-	-
Week 5, Day 29	8.69 (±3.10)	14.49 (±4.34)	95.3% (±39.1)	94.7% (±40.5)
Week 9, Day 57	7.17 (±2.63)	12.43 (±5.02)	86.3% (±53.0)	86.4% (±49.5)
Week 13, Day 85 (EOS)	5.74 (±1.68)	10.65 (±2.74)	67.8% (±45.7)	71.8% (±35.4)

Example 6: Expression of CD36

The breast tissue of women with autoimmune inflammatory mastitis (AIM) is high in aromatase and estradiol and thus Treg expression are heavily suppressed. Induction of CD36 in the breast tissue of women with AIM may result in tissue specific immunosuppression without systemic immunosuppression. This example shows that CD36 can be expressed in normal breast tissue by treatment with an androgenic agent in combination with an aromatase inhibitor as described herein.

Normal breast tissue was excised from 3 peri-menopausal women taken during surgery. The tissue samples were transported to the laboratory on ice, with a maximum time of one hour between excision and tissue processing. The breast tissue samples were washed in media (phenol red-free RPMI; SAFC biosciences, Kansas, USA), supplemented with 5ml of 200mM glutamine (SAFC biosciences, Kansas, USA), 5ml of 100x anti-biotic/anti-mycotic (Sigma, St Louis, MO, USA), 10µg/ml insulin (Sigma, St Louis, MO, USA), and 10µg/ml hydrocortisone (Sigma, St Louis, MO, USA) to remove excess blood.

A representative piece of each tissue sample was immediately fixed in 4% formalin in phosphate-buffered saline (PBS) overnight at 4°C, followed by dehydration using an automatic tissue processor (Sakura Tissue-Tek VIP, USA) and paraffin wax embedding. A haematoxylin and eosin (H&E) stained section was used to assess tissue pathology. The remaining fresh tissue was cut into small (~3x3xlmm) pieces, and placed as triplicates on $1cm^3$ gelatin sponges (Spongostan; Johnson & Johnson, Skipton, UK) that were pre-soaked and then half submerged in treatment

media containing 10% dextran-coated charcoal stripped fetal calf serum (DCC-FCS) (SAFC biosciences, Kansas, USA) in 24-well tissue culture plates (BD Biosciences, NJ, USA). The tissues samples were then cultured for 24 hours in a vehicle of 0.1% ethanol, 5 nM of testosterone and 25ng/ml of anastrazole.

Western blotting whole cell lysates of and control adipose tissue were prepared by sonication at 48C in lysis buffer (1% Triton X-100, 50 mM KCl, 25 mM HEPES, pH 7.8, 10mg/ml of leupeptin, 20mg/ml of aprotinin, 125 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride) and analyzed on the same western blot. 50mg samples of total protein were mixed with 50 ml of sodium dodecyl sulfate (SDS)-mercaptoethanol sample buffer and boiled for 10 min, then the proteins were separated on 7.5% SDS gels and transferred to a polyvinylidene fluoride membrane. The membrane was then blocked for 1h at room temperature using 5% skimmed milk in phosphate-buffered saline (PBS) containing 0.5% Tween-20, immunoblotted with antibodies against human CD36 diluted in PBS and horseradish peroxidase-conjugated secondary antibodies (Jackson Immunoresearch) diluted in PBS, followed by detection with Chemiluminescence Reagent (Amersham Bioscience, Buckinghamshire, England). The band density was measured by densitometry, using Image Master VDS and Image Quant Analysis Software (Amersham Pharmacia Biotech, Hong Kong). The relative protein levels of CD36 and beta-actin in the original total protein lysate from the breast preparations were obtained. CD36 protein expression was normalized to beta-actin protein expression. Immunizing host produced the antibodies with a synthetic peptide derived from the sequence of human CD36, purified by peptide affinity chromatography and confirmed using control peptides. The results of the western blot analysis CD36 protein in 3 explant samples are shown in Figure 17 at baseline and after 24 hours of cultivation. Cell lysates were immunoblotted with antibodies to CD36. The experiment was performed twice with similar results. Data are expressed relative to actin. A 2-tailed student t-test revealed significance at p=0.00757. The results are set forth in Table 9 below.

Table 9: Western blot band density measurements

Patient number	Pre-treatment	Post-treatment
1	0.3235	0.9845
2	0.2135	1.0156
3	0.1478	0.4875
Mean	0.2283	0.8292
SC	0.0888	0.29634

Increasing CD36 increases conversion of fibroblasts to adipocytes (fat). The adipocytes (fat) is a semi-fluid and therefore has greater elasticity. The present example shows a significant increase in CD36 in normal breast tissue following treatment with the androgenic agent testosterone in combination with the aromatase inhibitor anastrozole. Increased CD36 expression is associated with a reduction in breast stiffness. As described herein, certain embodiments of the present disclosure are directed to use of a combination therapy of an androgenic agent and an aromatase inhibitor to increase CD36 and a reduction in breast stiffness.

The above demonstrates in normal breast tissue that the combination of testosterone and an aromatase inhibitor is highly effective in the inducing CD36 as demonstrated in the Western protein blot below which shows CD36 is heavily induced by the treatment of normal breast tissue harvested at surgery and grown in explant.

Example 7: Idiopathic inflammatory macromastia

Four patients with idiopathic inflammatory mastistis associated with mild to moderate macromastia were treated in accordance with the present disclosure, as discussed below.

Patient 1: 42.03416971

Presentation

A 38 year old chiropractor with rapid breast enlargement-"doubled in size", peri-areolar inflammation and with severe constant pain over 2 months unresponsive to over the counter analgesia and NSAIDs which impacted on the patient's ability to work.

Bra cup size pre-morbid

The patient had a bra cup size of 32A, and was unable to wear a bra post-morbid.

Past medical history

Included the patient having severe pre-menstrual dysphoria partly controlled by oral contraceptive pill, had ceased taking oral contraceptive pill 2 years prior to presentation, regular 28 day cycle with 2 preganancies and 2 live births. Severe post-partum arthritis -undiagnosed -settled after 1 year, no abnormal blood parameters noted.

VAS pain scale pre-morbid: 95mm

Treatment

Subcutaneous implant pellet of 80mg testosterone and 4mg anastrozole as described in Example 1 for 3 months, repeated for two further 3 month periods

Results

i. VAS pain scale at 4 weeks 10mm
ii. Inflammation undetectable at 4 weeks
iii. Fibro-glandular tissue reduction 71%
iv. Breast Volume reduction 58% back to pre-morbid size
v. Complete reversal of extreme background parenchymal enhancement on MRI which persisted over the subsequent 3 years after therapy ceased
vi. No SAEs
vii. No change in BMI

Mammograms of a breast of the patient before and after treatment are shown in Fig. 19A, and MRI images before treatment for the subsequent 3 years following treatment are shown in Fig. 19C. Breast volume and density measurements are shown in Fig. 19B.

Patient 2: 42.09761311

Presentation

A 42 year old nurse rapid breast enlargement-"very hard", diffuse inflammation and severe constant pain over 4 months which was unresponsive to over the counter analgesia and NSAIDs, impacting work and home life-could not ride her horse. Left breasts was much larger than the right breast.

Bra cup size:-pre-morbid

The patient had a bra cup size of 36C and was unable to wear bra post morbid-she wore a sports bra all day and to bed.

Past medical history

Regular 28 day cycle, 2 preganacies and 2 live births with severe pre-eclampsia, post partum depression including hospitalization, no abnormalities in bloodparameters noted.

VAS pain scale pre-morbid: 100 mm

Treatment

Subcutaneous implant pellet of 80mg Testosterone and 4mg anastrozole for 3 months, repeated for two further 3 month period.

Results

i. VAS pain scale at 4 weeks 15mm
ii. Inflammation undetectable at 4 weeks
iii. Fibro-glandular tissue reduction 41%
iv. Breast Volume reduction 10% back to pre-morbid size
v. No SAEs
vi. No change in BMI
vii. No mammaplasty

Mammograms of a breast of the patient before and after treatment are shown in Fig. 20A. Breast volume and density measurements are shown in Fig. 20B.

Patient 3: 42.09761311

Presentation

A 37 year old lawyer with rapid breast enlargement - inflammation especially around the nipples and severe constant pain over 2 months which was unresponsive to over the counter analgesia and NSAIDs-impacting work and home life.

Bra cup size

Pre-morbid 34C unable to wear bra post morbid-wore sports bra all day and to bed.

Past Medical History

Regular 28day cycles with no preganacies, moderate endometriosis, no abnormalities in blood parameters noted.

VAS pain scale pre-morbid: 100 mm

Treatment

Subcutaneous implant pellet of 80mg Testosterone and 4mg anastrozole as described in Example 1 for 3 months, repeated for two further 3 month period.

Results

i. VAS pain scale at 4 weeks 0mm
ii. Inflammation undetectable at 4 weeks
iii. Fibro-glandular tissue reduction 52%
iv. Breast Volume reduction 32% back to pre-morbid size
v. No SAEs
vi. No change in BMI
vii. No mammaplasty

Mammograms of a breast of the patient before and after treatment are shown in Fig. 21A. Breast volume and density measurements are shown in Fig. 21B.

Patient 4: 42.83593371

Presentation

A 41 year old police officer with rapid breast enlargement-"very hard", diffuse inflammation and severe constant pain over 1 month which was unresponsive to over the counter analgesia and NSAIDs impacting on the patients work and home life-could not wear Kevlar protective kit.

Bra cup size

Pre-morbid 36B unable to wear bra post morbid-wore sports bra all day and to bed.

Past medical history

Regular 28 day cycles with no preganacies, severe endometriosis with multiple surgeries, no abnormalities in blood parameters noted.

VAS pain scale pre-morbid: 100 mm

Treatment

Subcutaneous implant pellet of 80mg testosterone and 4mg anastrozole as described in Example 1 for 3 months, repeated for two further 3 month period, mammaplasty then 2 further post operative treatments for 3 months as above.

Results

i. VAS pain scale at 4 weeks 0mm
ii. Inflammation undetectable at 4 weeks
iii. Fibro-glandular tissue reduction 36%
iv. Breast Volume reduction 23% back to pre-morbid size
v. Complete reversal of MRI extreme background parenchymal enhancement
vi. No SAEs
vii. No change in BMI

MRI images of a breast of the patient before and after treatment are shown in Fig. 22A, and mammographic images before and after treatment are shown in Fig. 22B. Breast volume and density measurements are shown in Fig. 22C.

Example 8: Treatment of autoimmune inflammatory mastitis

Patient: 42.04033771

Presentation

A 24 year old physical therapist with rapid breast enlargement-"extremely painful", diffuse inflammation and severe constant pain over 4 months - could not work and unresponsive to:

i. over the counter analgesia and NSAIDs
ii. OCP
iii. Oral progesterone
iv. Danazol
v. LhRh agonist

Bra cup size

Pre-morbid 36B enlarged to 36EE.

Past medical history

Obstetric/Gynological history included an irregular 28 day cycle- amenorrhoeic due to Zoladex, with no pregnancies. No abnormalities in blood parameters were noted.

VAS pain scale pre-morbid: 100 mm

Treatment

Subcutaneous implant pellet of 100mg Testosterone and 3mg anastrozole for 3 months, reduction mammaplasty was conducted then 2 further post operative treatments for 3 months as above

Results

i. VAS pain scale at 4 weeks 50mm
ii. Inflammation significantly down at 4 weeks
iii. Mammogram intense density and MRI extreme background parenchymal enhancement reversed
iv. No SAEs
v. No change in BMI
vi. Subsequently diagnosed with myasthenia gravis

A mammographic image of a breast of the patient before treatment is shown in Fig. 23A. An MRI image after the treatment is shown in Fig. 23B.

Example 9: Treatmeng of Plasma Cell Mastitis

Plasma cell mastitis is an autoimmune inflammatory destructive process of the retro-areolar milk ducts which results in multiple fistulae and inevitably results in disfiguring surgery and a high risk of recurrence. There is no known treatment for this condition apart from surgery which has significant limitations. Plasma cell mastitis is exemplified by the invasion of the milk duct system with inflammatory cells that secrete pro-inflammatory cytokines. Recently it has been demonstrated that the IL-6 inflammatory pathways critical in this inflammatory process and has been targeted as a potential mechanism to be targeted for treatment of plasma cell mastitis (Liu, 2020). It has been demonstrated that the female breast has cells which are extremely responsive (a reduction of 53%) to treatment with testosterone in reducing IL-6 levels (Guhl, 2012).

A 43-year-old otherwise fit and well woman presented with multiple fistulae around the right nipple area of the complex which had not responded to antibiotic therapy in the first instance then high dose corticosteroids and a total duct excision as a surgical procedure only resulted in a short-term remission. There was significant inflammation around the right nipple areolar complex with four fistulae at the 4 o'clock position on the edge of the areolar. There was significant pain which measured 8 on a 0 to 10 cm visual analogue pain scale. She was commenced on T +AI therapy and received an 80 mg testosterone 4 mg anastrozole subcutaneous implant. Within three weeks her visual analogue pain scale dropped back to 3 and there was some resolution

of the peri- areolar redness. After 11 months with three implants of the same strength being inserted, there was complete resolution of the fistula formation and no associated pain. A follow-up 12 months later revealed no evidence of disease recurrence.

Example 10: Granulomatous mastitis

Granulomatous mastitis is also known as granulomatous lobular mastitis due to the inflammation occurring around the breast lobules. It is a manifestation of autoimmune inflammatory mastitis in the breast tissue which results in granuloma formation, inflammation and fistula formation. There is no known cure for this condition and women frequently undergo multiple operations and/or receive immunosuppressive treatment.

A 32-year-old woman presented for a second opinion following six months of treatment for histologically proven granulomatous mastitis. She had been given antibiotics, corticosteroids, methotrexate and had undergone surgery to remove a fistula. All of these had failed as demonstrated by an MRI taken on 23 July 2017 which demonstrateds multiple areas of granuloma formation with a large reactive axilla lymphnode.

Between the first MRI and a second MRI taken on 23 May 2018 she had three testosterone 80 mg anastrozole 4 mg implants as described in Example 1 inserted. The inflammation reduced rapidly and the one remaining fistula healed up over a three month period. The tenderness and breast discomfort persisted for five months and then slowly subsided. Clinical examination on 23 May 2018 revealed only minor scarring in the breast from previous surgery but no other abnormalities. She remained disease-free when reviewed in February 2020.

A mammographic image of a breast of the patient before treatment is shown in Fig. 24A. An MRI image obtained after treatment is shown in Fig. 23B.

Other exemplary non-limiting embodiments.

Further advantages of the claimed subject matter will become apparent from the following examples describing certain embodiments of the claimed subject matter.

A Examples

1A. A pharmaceutical formulation comprising:

an effective amount of an androgen, an effective amount of an aromatase inhibitor and a binding agent;

the formulation upon administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgen and plasma concentration for the aromatase inhibitor; and

the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising:

a first time period in which the androgen, has a first peak in concentration (Tmax) in the serum and the aromatase inhibit is increasing in concentration in the plasma but is below its Tmax concentration in the plasma; and

a second time period in which the androgen, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma.

2A. A pharmaceutical formulation comprising:

an effective amount of an androgen, an effective amount of an aromatase inhibitor and a binding agent;

the pharmaceutical formulation is compressed into a pellet;

the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgen and plasma concentration for the aromatase inhibitor; and

the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising:

a first time period in which the androgen, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in

concentration in the plasma but is below its Tmax concentration in the plasma; and

a second time period in which the androgen, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma.

3A. A pharmaceutical formulation comprising:

an effective amount of an androgen, an effective amount of an aromatase inhibitor and a binding agent;

the formulation upon administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgen and plasma concentration for the aromatase inhibitor; and

the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising:

a first time period in which the androgen, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma;

a second time period in which the androgen, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma;

a third time period in which the androgen, has a second peak concentration in the serum that is less than the Tmax and the aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the androgen; and

a fourth time period in which the androgen has a gradually decreasing serum level concentration and the aromatase inhibitor has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.

4A. A pharmaceutical formulation comprising:

an effective amount of an androgen, an effective amount of an aromatase inhibitor and a binding agent;

the pharmaceutical formulation is compressed into a pellet;

the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgen and plasma concentration for the aromatase inhibitor; and

the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising:

a first time period in which the androgen, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma;

a second time period in which the androgen, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma;

a third time period in which the androgen, has a second peak concentration in the serum that is less than the Tmax and the aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the androgen; and

a fourth time period in which the androgen has a gradually decreasing serum level concentration and the aromatase inhibitor has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.

5A. A pharmaceutical formulation comprising:

60 mg to 120 mg of a testosterone, or an ester thereof, 4 mg to 6 mg of an aromatase inhibitor and stearic acid;

the pharmaceutical formulation is compressed into a pellet that has a diameter of between 4.25 mm to 4.75 mm and a length of between 4 mm to 7 mm;

the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the testosterone or an ester thereof, and plasma concentration for the aromatase inhibitor; and

the sustained release multi-phasic concentration pattern comprising:

a first time period in which the testosterone or an ester thereof, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is

increasing in concentration in the plasma but is below its Tmax concentration in the plasma; and

a second time period in which the testosterone or an ester thereof, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma.

6A. A pharmaceutical formulation comprising:

60 mg to 120 mg of a testosterone, or an ester thereof, 4 mg to 6 mg of an aromatase inhibitor and stearic acid;

the pharmaceutical formulation is compressed into a pellet that has a diameter of between 4.25 mm to 4.75 mm and a length of between 4 mm to 7 mm;

the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the testosterone or an ester thereof, and plasma concentration for the aromatase inhibitor; and

the sustained release multi-phasic concentration pattern comprising:

a first time period in which the testosterone or an ester thereof, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma;

a second time period in which the testosterone or an ester thereof, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma;

a third time period in which the testosterone or an ester thereof, has a second peak concentration in the serum that is less than the Tmax and the aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the testosterone or an ester thereof; and

a fourth time period in which the testosterone or an ester thereof, has a gradually decreasing serum level concentration and the aromatase inhibitor has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.

7A. The pharmaceutical formulation of examples 1A, 2A, or 5A, wherein the sustained release multi-phasic concentration pattern further comprises:

a third time period in which the testosterone or an ester thereof, has a second peak concentration in the serum that is less than the Tmax and the aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the testosterone or an ester thereof; and

a fourth time period in which the testosterone or an ester thereof, has a gradually decreasing serum level concentration and the aromatase inhibitor has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.,

8A. The pharmaceutical formulation of one or more of examples 1A to 7A, wherein during the first time period the aromatase inhibitor exhibits first order release.

9A. The pharmaceutical formulation of one or more of examples 1A to 8A, wherein during the first time period the aromatase inhibitor does not exhibit zero order release.

10A. The pharmaceutical formulation of one or more of examples 1A to 9A, wherein during the second time period the aromatase inhibitor does not exhibit zero order release.

11A. The pharmaceutical formulation of one or more of examples 1A to 10A, wherein during the second time period the testosterone or an ester thereof, does not exhibit zero order release.

12A. The pharmaceutical formulation of one or more of examples 1A to 11A, wherein during the third time period the aromatase inhibitor does not exhibit zero order release.

13A. The pharmaceutical formulation of one or more of examples 1A to 12A, wherein during the third time period the testosterone or an ester thereof, does not exhibit zero order release.

14A. The pharmaceutical formulation of one or more of examples 1A to 13A, wherein during the third time period the aromatase inhibitor exhibit first order release.

15A. The pharmaceutical formulation of one or more of examples 1A to 14A, wherein during the third time period the testosterone or an ester thereof, exhibit first order release.

16A. The pharmaceutical formulation of one or more of examples 1A to 15A, wherein during the fourth time period the aromatase inhibitor does not exhibit zero order release.

17A. The pharmaceutical formulation of one or more of examples 1A to 16A, wherein during the fourth time period the testosterone or an ester thereof, does not exhibit zero order release.

18A. The pharmaceutical formulation of one or more of examples 1A to 17A, wherein during the fourth time period the aromatase inhibitor exhibit first order release.

19A. The pharmaceutical formulation of one or more of examples 1A to 18A, wherein during the fourth time period the testosterone or an ester thereof, exhibit first order release.

20A. The pharmaceutical formulation of one or more of examples 1A to 19A, wherein the first time period ends right after the androgen, has a first peak in concentration (Tmax) in the serum.

21A. The pharmaceutical formulation of one or more of examples 1A to 20A, wherein the first time period ends between 5 hours to 14 hours.

22A. The pharmaceutical formulation of one or more of examples 1A to 21A, wherein the first time period ends between 5.5 hours to 13 hours.

23A. The pharmaceutical formulation of one or more of examples 1A to 22A, wherein the second time period ends right after the aromatase inhibitor has its Tmax.

24A. The pharmaceutical formulation of one or more of examples 1A to 23A, wherein the second time period ends between 23 hours to 80 hours.

25A. The pharmaceutical formulation of one or more of examples 1A to 24A, wherein the pharmaceutical formulation is an implant.

26A. The pharmaceutical formulation of one or more of examples 1A to 25A, wherein the the implant is a compressed pellet.

27A. The pharmaceutical formulation of one or more of examples 1A to 24A, wherein the pharmaceutical formulation is transdermal patch.

28A. The pharmaceutical formulation of one or more of examples 1A to 25A, wherein the implant is subcutaneously administered to a subject.

28A. The pharmaceutical formulation of one or more of examples 1A to 26A, wherein the compressed pellet is subcutaneously administered to a subject.

B Examples

1B. A pharmaceutical formulation comprising:

approximately 80 mg of a testosterone or an ester thereof, approximately 4 mg anastrozole and approximately 2 mg of a stearic acid;

the pharmaceutical formulation is compressed into a pellet that has a diameter of between 4.4 mm to 4.6 mm and a length of between 4 mm to 7 mm;

the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the testosterone or an ester thereof, and plasma concentration for the anastrozole;

the sustained release multi-phasic concentration pattern comprising:

a first time period of in which the testosterone or an ester thereof, has a first peak in concentration (Tmax) in the serum and the anastrozole is increasing in concentration in the plasma but is below its Tmax concentration in the plasma;

a second time period in which the testosterone or an ester thereof, has initially a decreasing serum level concentration and then an increasing serum level concentration and the anastrozole has its Tmax concentration in the plasma;

a third time period in which the testosterone or an ester thereof, has a second peak concentration in the serum that is less than the Tmax and the anastrozole is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the testosterone, or an ester thereof; and

a fourth time period in which the testosterone or an ester thereof, has a gradually decreasing serum level concentration and the anastrozole has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.

While certain embodiments have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is intended that the following claims define the scope of the inventions and that methods and structures within the scope of these claims and their equivalents be covered thereby.

In the foregoing description of certain embodiments, specific terminology has been resorted to for the sake of clarity. However, the disclosure is not intended to be limited to the specific terms so selected, and it is to be understood that each specific term includes other technical equivalents which operate in a similar manner to accomplish a similar technical purpose. Terms such as "left" and right", "front" and "rear", "above" and "below" and the like are used as words of convenience to provide reference points and are not to be construed as limiting terms.

In this specification, the word "comprising" is to be understood in its "open" sense, that is, in the sense of "including", and thus not limited to its "closed" sense, that is the sense of "consisting only of". A corresponding meaning is to be attributed to the corresponding words "comprise", "comprised" and "comprises" where they appear.

In addition, the foregoing describes only some embodiments of the inventions, and alterations, modifications, additions and/or changes may be made thereto without departing from the scope and spirit of the disclosed embodiments, the embodiments being illustrative and not restrictive.

It is to be understood that the inventions are not to be limited to the disclosed embodiments, but on the contrary, is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the inventions. Also, the various embodiments described above may be implemented in conjunction with other embodiments, e.g., aspects of one embodiment may be combined with aspects of another embodiment to realize yet other embodiments. Further, each independent feature or component of any given assembly may constitute an additional embodiment.

CLAIMS

1. A pharmaceutical formulation comprising:

an effective amount of an androgenic agent, an effective amount of an aromatase inhibitor, and a binding agent;

the pharmaceutical formulation is compressed into a pellet;

the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgenic agent and plasma concentration for the aromatase inhibitor; and

the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising:

a first time period in which the androgenic agent has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma; and

a second time period in which the androgenic agent has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma.

2. A pharmaceutical formulation comprising:

an effective amount of an androgenic agent, an effective amount of an aromatase inhibitor, and a binding agent;

the pharmaceutical formulation is compressed into a pellet;

the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the androgenic agent and plasma concentration for the aromatase inhibitor; and

the sustained release multi-phasic concentration pattern in the serum or plasma of the subject comprising:

a first time period in which the androgenic agent, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma;

a second time period in which the androgenic agent has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma;

a third time period in which the androgenic agent has a second peak concentration in the serum that is less than the Tmax and the aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the androgen; and

a fourth time period in which the androgenic agent has a gradually decreasing serum level concentration and the aromatase inhibitor has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.

3. The pharmaceutical formulation of claim 1 or 2, wherein during the first time period the aromatase inhibitor exhibits first order release.

4. The pharmaceutical formulation of claim 1 or 2, wherein during the first time period the aromatase inhibitor does not exhibit zero order release.

5. The pharmaceutical formulation of any one of claims 1 to 4, wherein during the second time period the aromatase inhibitor does not exhibit zero order release.

6. The pharmaceutical formulation of any one of claims 1 to 5, wherein during the second time period the androgenic agent does not exhibit zero order release.

7. The pharmaceutical formulation of any one of claims 1 to 6, wherein during the third time period the aromatase inhibitor does not exhibit zero order release.

8. The pharmaceutical formulation of any one of claims 1 to 7, wherein during the third time period the androgenic agent does not exhibit zero order release.

9. The pharmaceutical formulation of any one of claims 1 to 8, wherein the sustained release multi-phasic concentration pattern further comprises:

a third time period in which the androgenic agent has a second peak concentration in the serum that is less than the Tmax and the aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the testosterone or an ester thereof; and

a fourth time period in which the androgenic agent has a gradually decreasing serum level concentration and the aromatase inhibitor has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.

10. The pharmaceutical formulation of claim 9, wherein during the third time period the aromatase inhibitor exhibits first order release.

11. The pharmaceutical formulation of any one of claim 9 or 10, wherein during the third time period the androgenic agent exhibits first order release.

12. The pharmaceutical formulation of any one of claims 9 to 11, wherein during the fourth time period the aromatase inhibitor does not exhibit zero order release.

13. The pharmaceutical formulation of any one of claims 9 to 12, wherein during the fourth time period the androgenic agent does not exhibit zero order release.

14. The pharmaceutical formulation of any one of claims 9 to 13, wherein during the fourth time period the aromatase inhibitor exhibits first order release.

15. The pharmaceutical formulation of any one of claims 9 to 14, wherein during the fourth time period the androgenic agent exhibits first order release.

16. The pharmaceutical formulation of any one of claims 1 to 15, wherein the first time period ends right after the androgenic agent has a first peak in concentration (Tmax) in the serum.

17. The pharmaceutical formulation of any one of claims 1 to 16, wherein the first time period ends between 5 hours to 14 hours.

18. The pharmaceutical formulation of any one of claims 1 to 17, wherein the first time period ends between 5.5 hours to 13 hours.

19. The pharmaceutical formulation of any one of claims 1 to 18, wherein the second time period ends right after the aromatase inhibitor has its Tmax.

20. The pharmaceutical formulation of one or more of claims 1 to 19, wherein the second time period ends between 23 hours to 80 hours.

21. The pharmaceutical formulation of claim 1 or 2, wherein the androgenic agent is a testosterone or pharmaceutically acceptable salt or ester thereof.

22. The pharmaceutical formulation of claim 21, wherein the androgenic agent is selected from the group consisting of testosterone, methyltestosterone, dehydroepiandrostrone, and testosterone undecanoate.

23. The pharmaceutical formulation of claim 22, wherein the androgenic agent is testosterone or a pharmaceutically acceptable salt thereof.

24. The pharmaceutical formulation of claim 1 or 2 being for subcutaneous delivery of the androgenic agent and the aromatase inhibitor.

25. A pharmaceutical formulation comprising:

60 mg to 120 mg of a testosterone, or an ester thereof, 2 mg to 6 mg of an aromatase inhibitor and more preferably 4 mg to 6 mg of an aromatase inhibitor, and stearic acid;

the pharmaceutical formulation is compressed into a pellet that has a diameter of between 4.25 mm to 4.75 mm and a length of between 4 mm to 7 mm;

the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as

measured by serum concentration for the testosterone or an ester thereof, and plasma concentration for the aromatase inhibitor; and

the sustained release multi-phasic concentration pattern comprising:

a first time period in which the testosterone or an ester thereof, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma; and

a second time period in which the testosterone or an ester thereof, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma.

26. A pharmaceutical formulation comprising:

60 mg to 120 mg of a testosterone, or an ester thereof, 2 mg to 6 mg of an aromatase inhibitor and more preferably 4 mg to 6 mg of an aromatase inhibitor, and stearic acid;

the pharmaceutical formulation is compressed into a pellet that has a diameter of between 4.25 mm to 4.75 mm and a length of between 4 mm to 7 mm;

the pellet upon subcutaneous administration to a subject provides a sustained release multi-phasic concentration pattern in the blood of the subject over time as measured by serum concentration for the testosterone or an ester thereof, and plasma concentration for the aromatase inhibitor; and

the sustained release multi-phasic concentration pattern comprising:

a first time period in which the testosterone or an ester thereof, has a first peak in concentration (Tmax) in the serum and the aromatase inhibitor is increasing in concentration in the plasma but is below its Tmax concentration in the plasma;

a second time period in which the testosterone or an ester thereof, has initially a decreasing serum level concentration and then an increasing serum level concentration and the aromatase inhibitor has its Tmax concentration in the plasma;

a third time period in which the testosterone or an ester thereof, has a second peak concentration in the serum that is less than the Tmax and the

aromatase inhibitor is gradually decreasing in concentration in the plasma and in the third time period falls below the concentration of the testosterone or an ester thereof; and

a fourth time period in which the testosterone or an ester thereof, has a gradually decreasing serum level concentration and the aromatase inhibitor has a gradually decreasing concentration in the plasma and both decreasing levels approximately parallel each other.

27. The pharmaceutical formulation of any one of claims 1 to 26, wherein the aromatase inhibitor is selected from the group consisting of anastrozole, exemestane, and letrozole.

28. The pharmaceutical formulation of any one of claims 1 to 27, wherein the aromatase inhibitor is anastrozole.

29. A method for prophylaxis or treatment of a patient for a condition selected from the group consisting of high mammographic breast density, breast pain, breast stiffness, macromastia, endometriosis, breast inflammation, male gynaecomastia and autoimmune inflammatory mastitis, and breast cysts, the method comprising administering to the patient a pharmaceutical formulation as defined in any one of claims 1 to 28.

30. The use of a pharmaceutical formulation of any one of claims 1 to 28 in the treatment of a condition in a patient selected from the group selected from high mammographic breast density, breast pain, breast stiffness, macromastia endometriosis, breast inflammation, male gynaecomastia, autoimmune inflammatory mastitis, and breast cysts.

31. The method of claim 29 or the use of claim 30, wherein the patient is selected from the group consisting of a peri-menopausal woman, menopausal woman or a post-menopausal woman.

32. A method for the prophylaxis or treatment of autoimmune inflammatory mastitis in a patient in need thereof, comprising administering to the patient i) an effective amount of an androgenic agent in combination with ii) an effective amount of an aromatase inhibitor.

33. The method of claim 32, wherein the androgenic agent and the aromatase inhibitor are administered to the patient in the same pharmaceutical formulation or in different pharmaceutical formulations to one another.

34. The method of claim 33, wherein the androgenic agent and the aromatase inhibitor are administered to the patient in the same pharmaceutical formulation.

35. The method of claim 33, wherein the androgenic agent and the aromatase inhibitor are administered to the patient in a separate pharmaceutical formulation to one another.

36. The method of claim 34 or 35, wherein the pharmaceutical formulation is a sustained-release pharmaceutical formulation.

37. The method of any one of claims 34 to 36, wherein the pharmaceutical formulation is in a solid dosage form and is delivered to the patient subcutaneously.

38. The method of claim 37, wherein the pharmaceutical formulation is in the form of a pellet.

39. The method of any one of claims 32 to 36, wherein the androgenic agent and the aromatase inhibitor are delivered to the patient subcutaneously.

40. The method of claim 34, wherein the pharmaceutical formulation provides a sustained release multi-phasic concentration pattern in the blood of the patient over time as measured by serum concentration for the androgen and plasma concentration for the aromatase inhibitor.

41. The method of claim 40, wherein the pharmaceutical formulation is a pharmaceutical formulation as defined in any one of claims 1 to 40.

42. The method of any one of claims 32 to 41, wherein the autoimmune inflammatory mastitis is selected from the group consisting of idiopathic inflammatory macromastia, plasma cell mastitis, granulomatous mastitis, and combinations of the foregoing.

43. Use of an androgenic agent in the manufacture of a medicament for the prophylaxis or treatment of autoimmune inflammatory mastitis in a patient in need thereof in combination with an effective amount of an aromatase inhibitor.

44. Use of an aromatase inhibitor in the manufacture of a medicament for the prophylaxis or treatment of autoimmune inflammatory mastitis in a patient in need thereof in combination with an effective amount of an androgenic agent.

45. Use of a pharmaceutical formulation in a method of treating autoimmune inflammatory mastitis to a patient in need thereof, comprising administering the pharmaceutical formulation to the patient in the form of a subcutaneous pellet comprising: i) an effective amount of an androgenic agent; and ii) an effective amount of an aromatase inhibitor.

46. The method of any one of claims 32 to 42 or the use of any one of claims 43 to 45, wherein the androgenic agent is a testosterone or a pharmaceutically acceptable salt or ester thereof.

47. The method or use of claim 46, wherein the androgenic agent is selected from the group consisting of testosterone, methyltestosterone, dehydroepiandrostrone, and testosterone undecanoate.

48. The method or use of claim 47, wherein the androgenic agent is testosterone or a pharmaceutically acceptable salt or ester thereof.

49. The method of any one of claims 32 to 42 or the use any one of claims 43 to 45, wherein the aromatase inhibitor is selected from the group consisting of anastrozole, exemestane, and letrozole.

50. The method or use of any one of claims 46 to 49, wherein the aromatase inhibitor is anastrozole.

51. An androgenic agent for use in a method of treating autoimmune inflammatory mastitis in a patient, wherein the method comprises administering an effective amount of the androgenic agent to the patient in combination with an effective amount of an aromatase inhibitor.

52. An androgenic agent of claim 51, wherein the androgenic agent is a testosterone or a pharmaceutically acceptable salt or ester thereof.

53. The androgenic agent of claim 52, wherein the androgenic agent is selected from the group consisting of testosterone, methyltestosterone, dehydroepiandrostrone, and testosterone undecanoate.

54. The androgenic agent or use of claim 52, wherein the androgenic agent is testosterone or a pharmaceutically acceptable salt or ester thereof.

55. The androgenic agent of any one of claims 51 to 54, wherein the aromatase inhibitor is selected from the group consisting of anastrozole, exemestane, and letrozole.

56. The androgenic agent of claim 55, wherein the aromatase inhibitor is anastrozole.

57. An aromatase inhibitor for use in a method of treating autoimmune inflammatory mastitis in a patient, wherein the method comprises administering an effective amount of the aromatase inhibitor to the patient in combination with an effective amount of an androgenic agent.

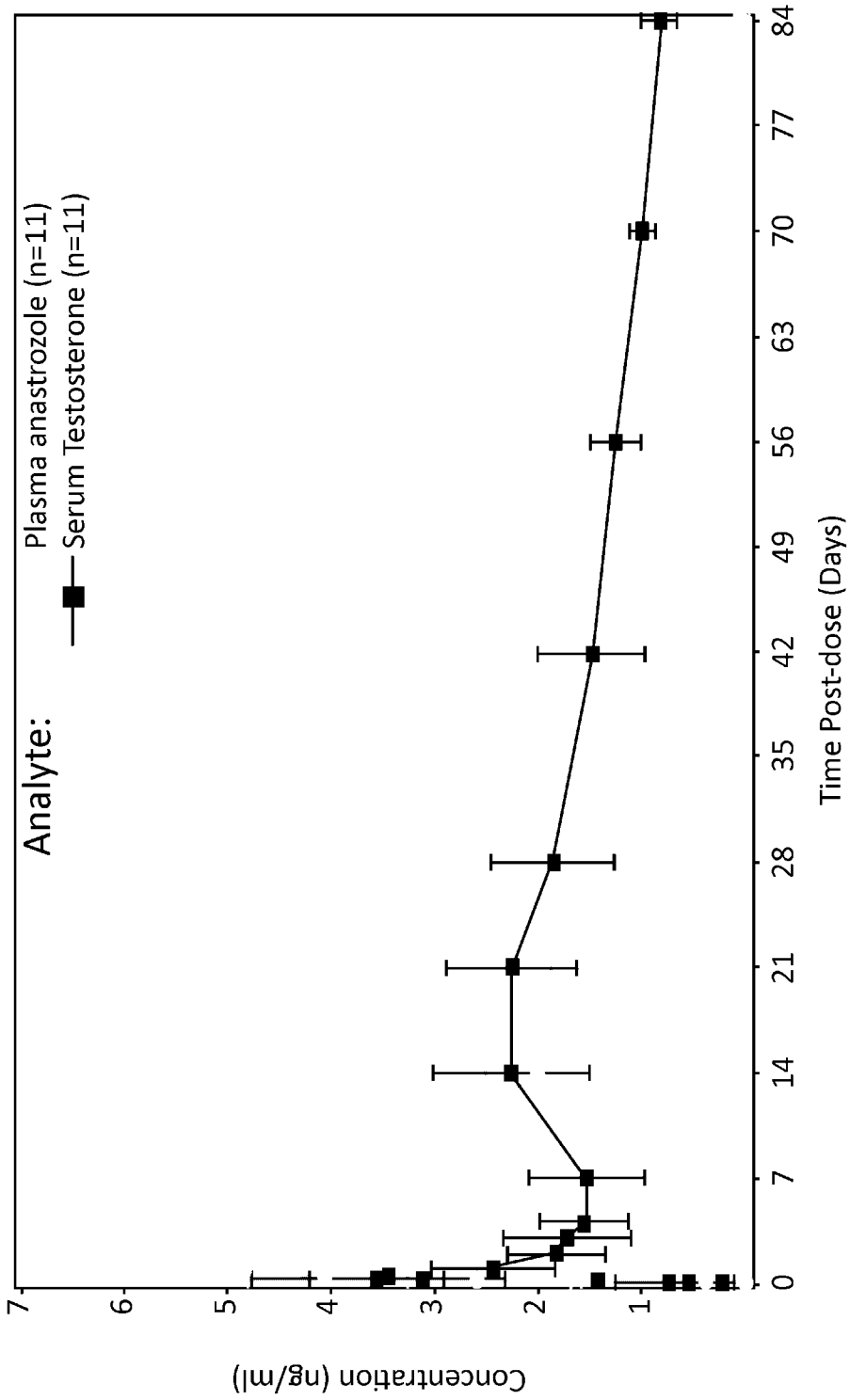

Analyte:
Plasma anastrozole (n=11)
Serum Testosterone (n=11)
Concentration (ng/ml)
Time Post-dose (Days)
0
7
14
21
28
35
42
49
56
63
70
77
84
1
2
3
4
5
6
7

Figure 1

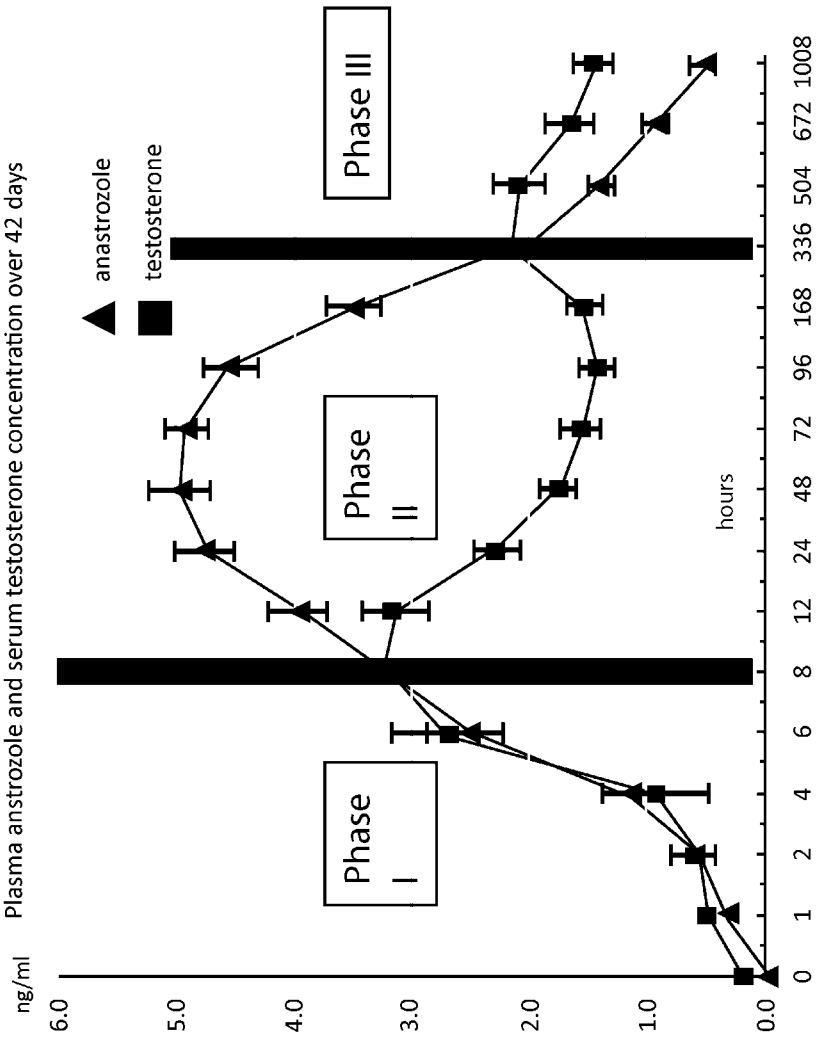

Plasma anstrozole and serum testosterone concentration over 42 days
ng/ml
6.0
5.0
4.0
3.0
2.0
1.0
0.0
anastrozole
testosterone
Phase I
Phase II
Phase III
0
1
2
4
6
8
12
24
48
72
96
168
336
504
672
1008
hours

Figure 2

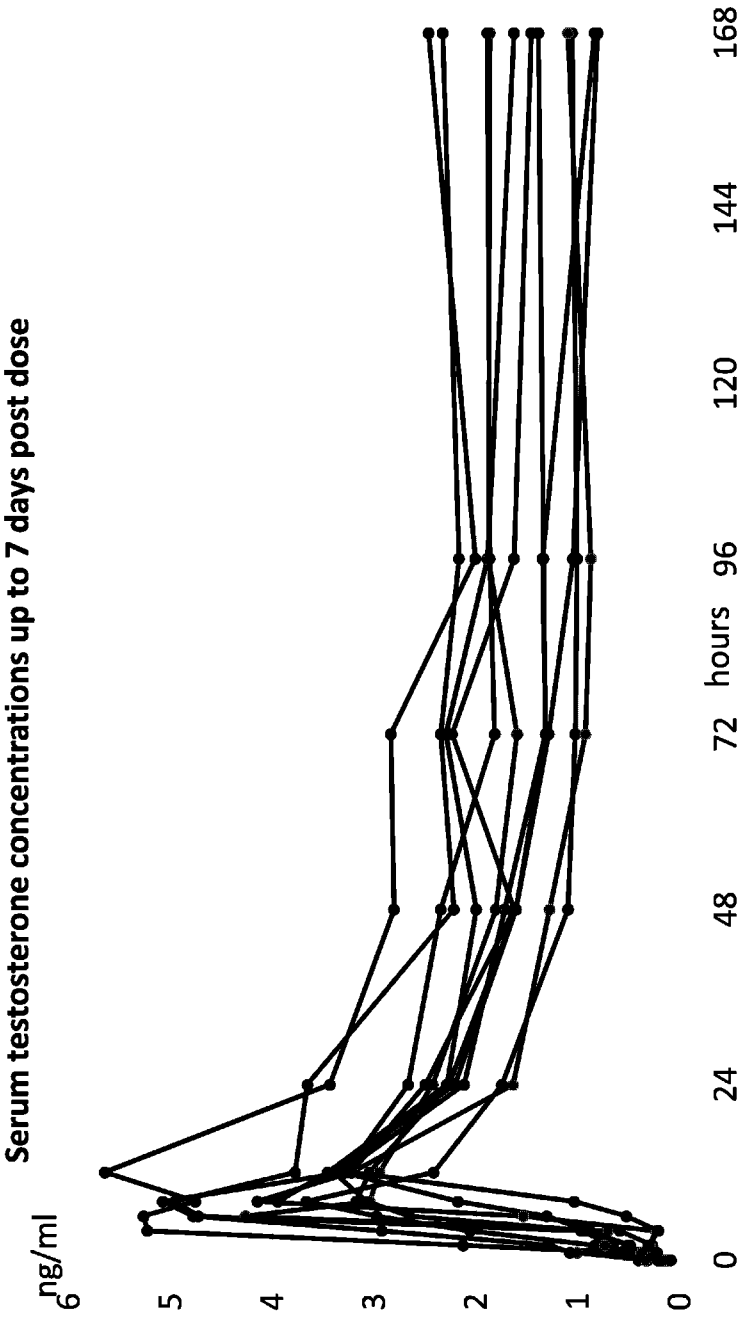

Serum testosterone concentrations up to 7 days post dose
ng/ml
6
5
4
3
2
1
0
0
24
48
72
96
120
144
168
hours

Figure 3

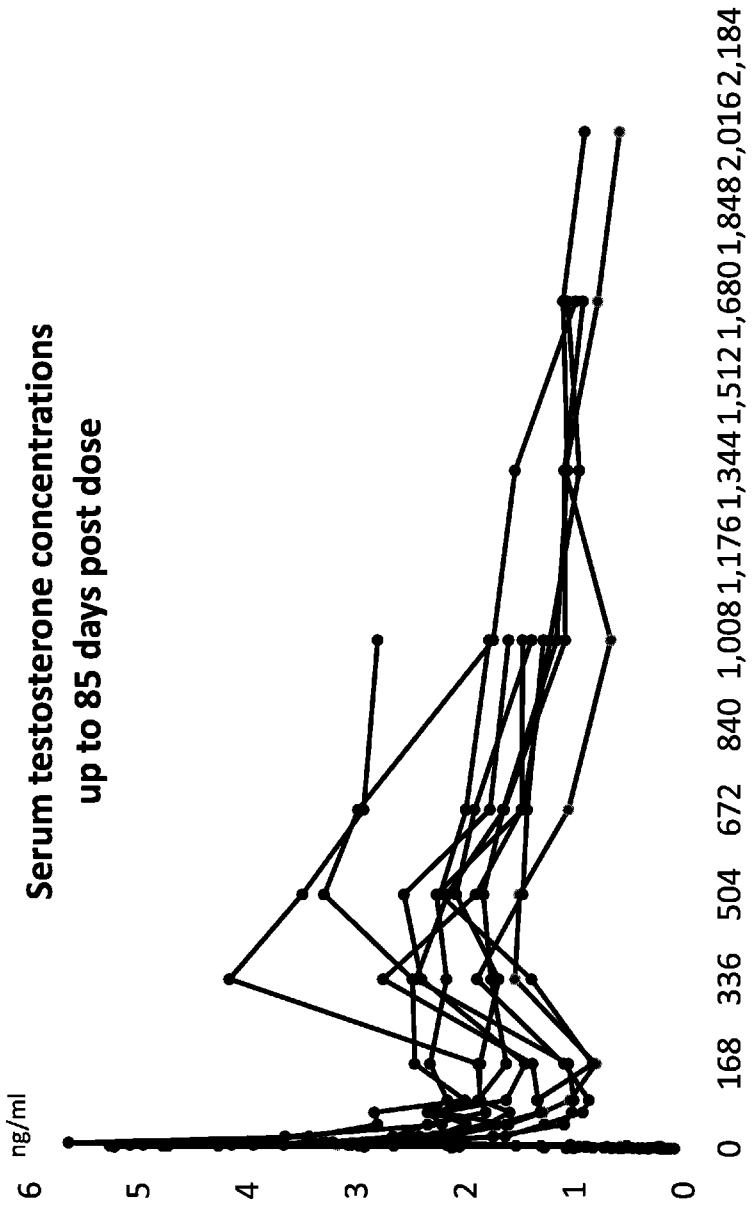

Serum testosterone concentrations
up to 85 days post dose
ng/ml
6
5
4
3
2
1
0
0
168
336
504
672
840
1,008
1,176
1,344
1,512
1,680
1,848
2,016
2,184
hours

Figure 4

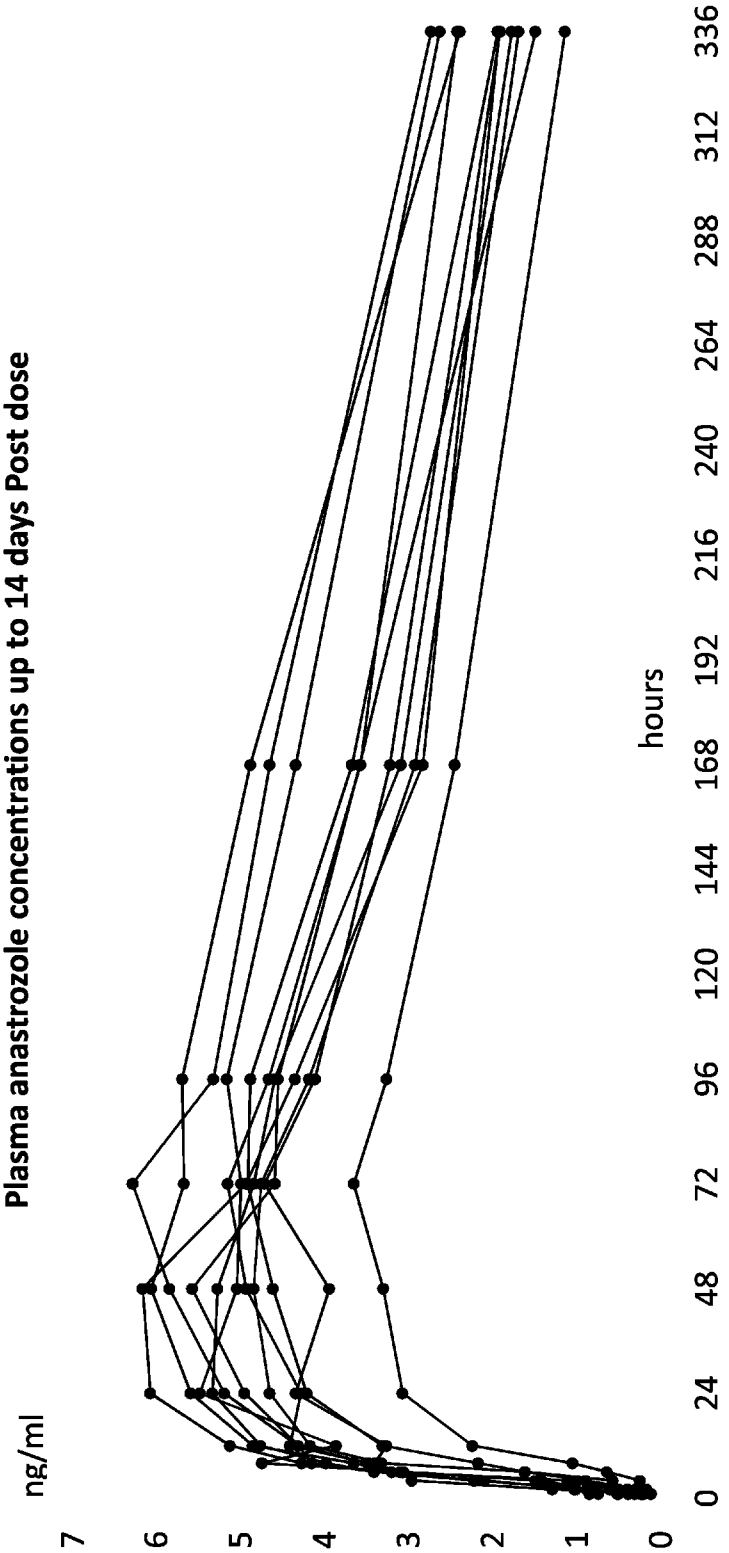

Plasma anastrozole concentrations up to 14 days Post dose
ng/ml
0
1
2
3
4
5
6
7
hours
0
24
48
72
96
120
144
168
192
216
240
264
288
312
336

Figure 5

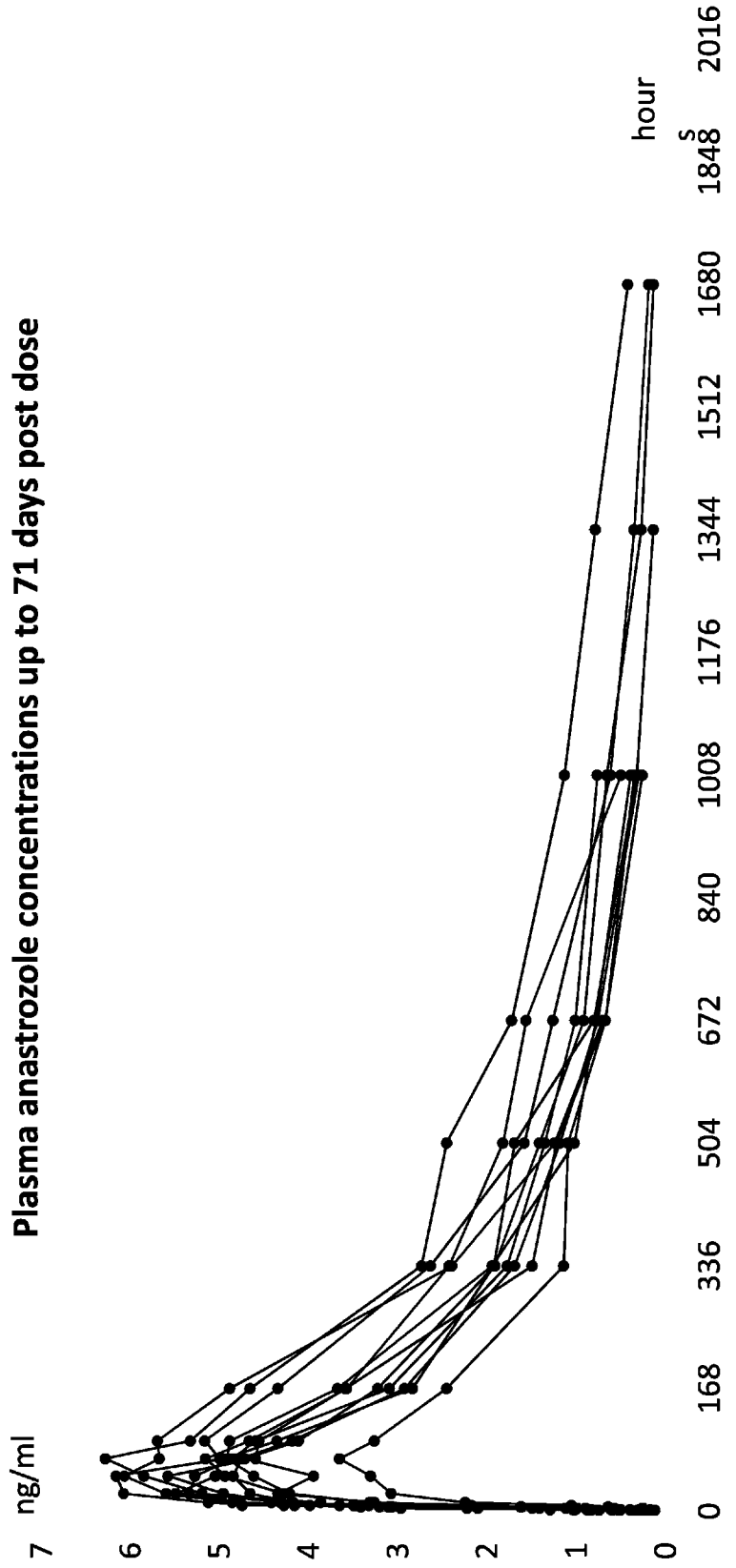
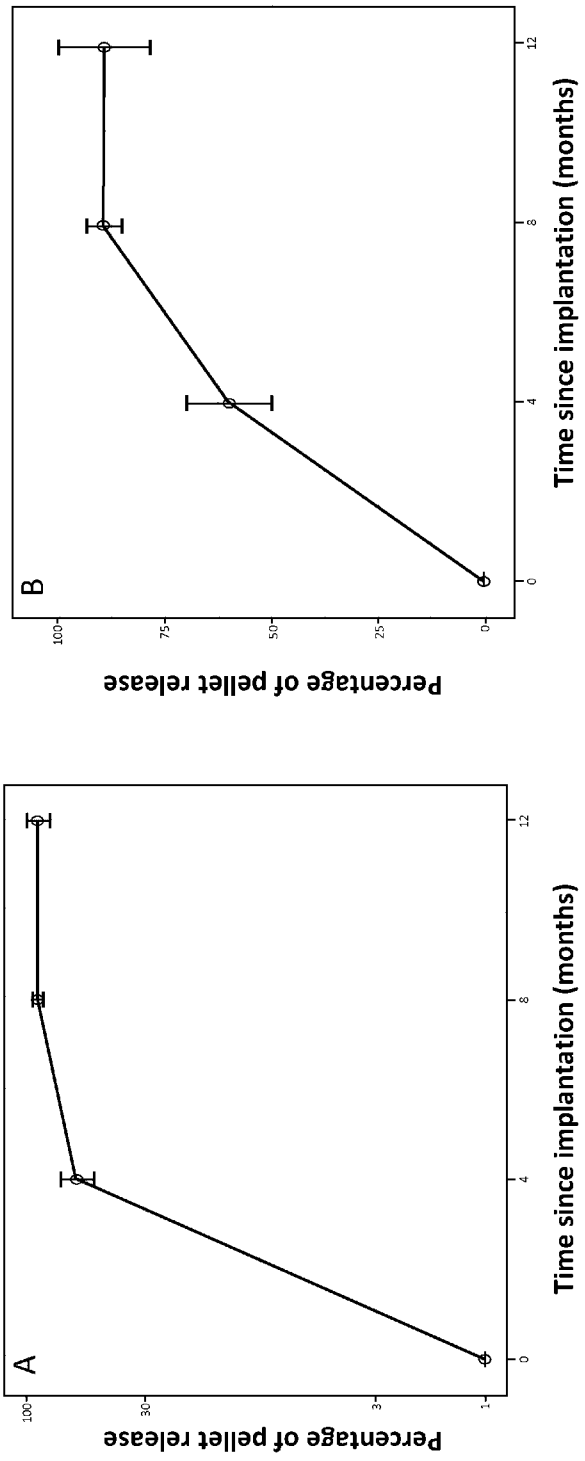

Plasma anastrozole concentrations up to 71 days post dose
ng/ml
7
6
5
4
3
2
1
0
0
168
336
504
672
840
1008
1176
1344
1512
1680
1848
2016
hours

Figure 6


A
B
Percentage of pellet release
Time since implantation (months)
100
30
3
1
75
50
25
0
4
8
12

Figures 7A-B

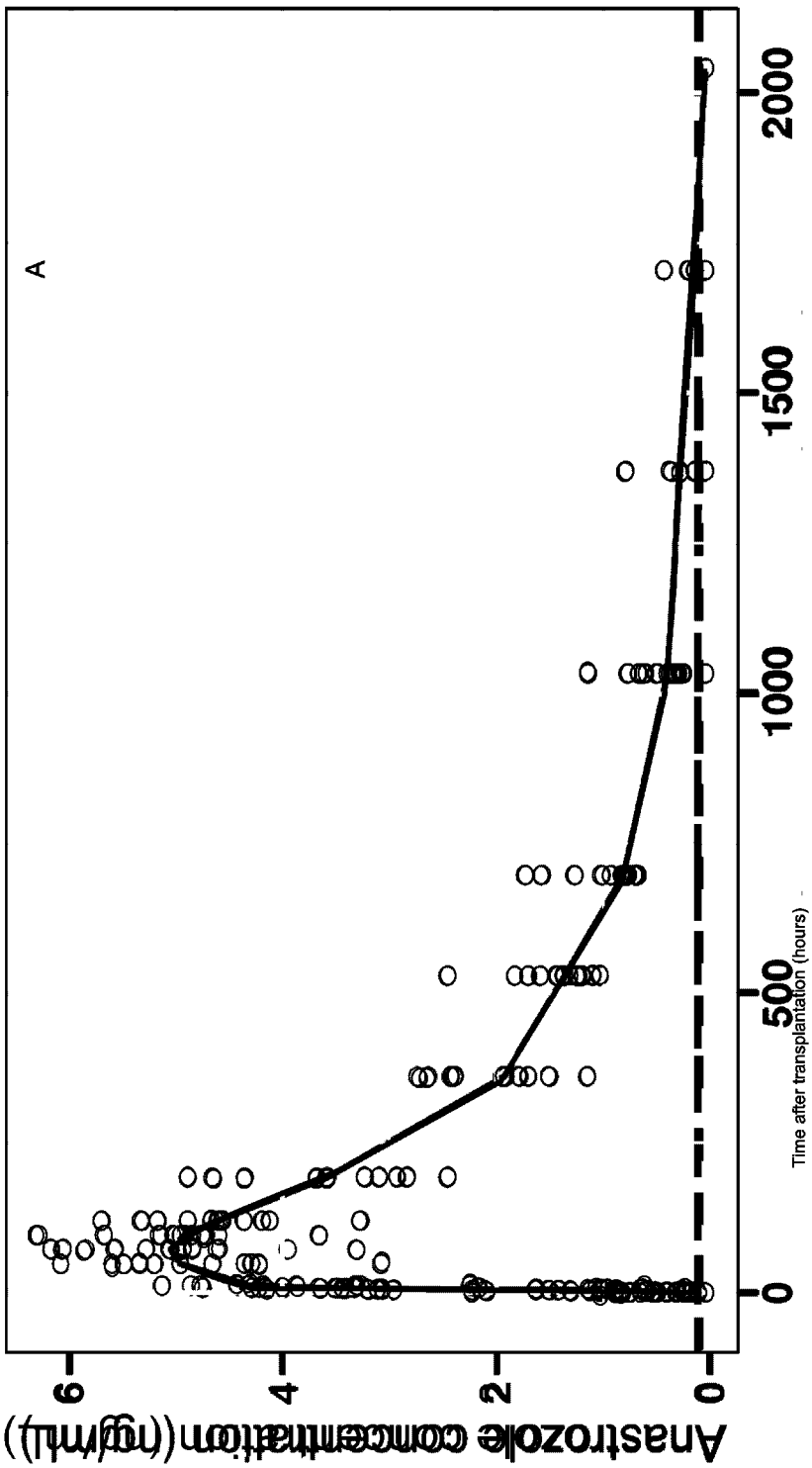

A
Anastrozole concentration (ng/mL)
0
2
4
6
0
500
1000
1500
2000
Time after transplantation (hours)

Figure 8A

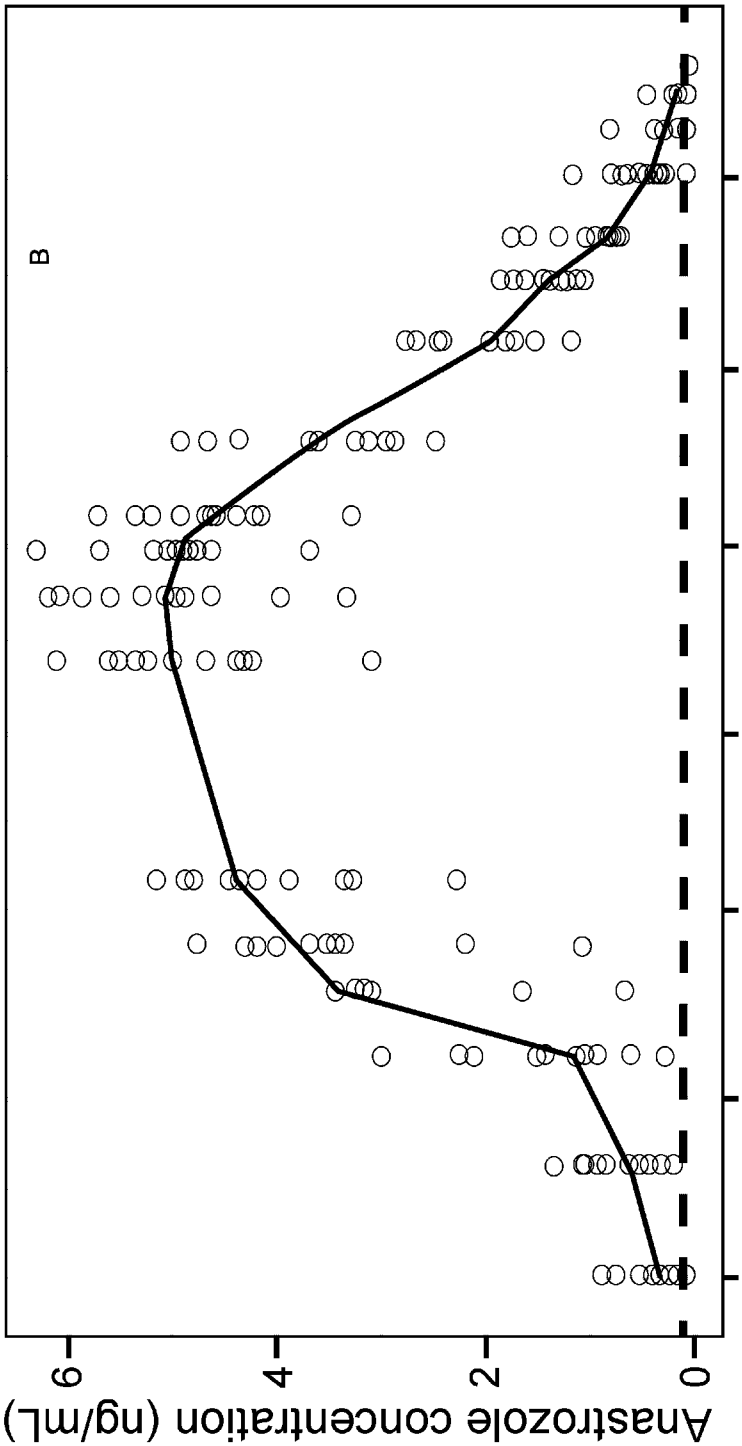

B
Anastrozole concentration (ng/mL)
0
2
4
6
1
3
10
30
100
1300
1000
Time after transplantation (hours)

Figure 8B

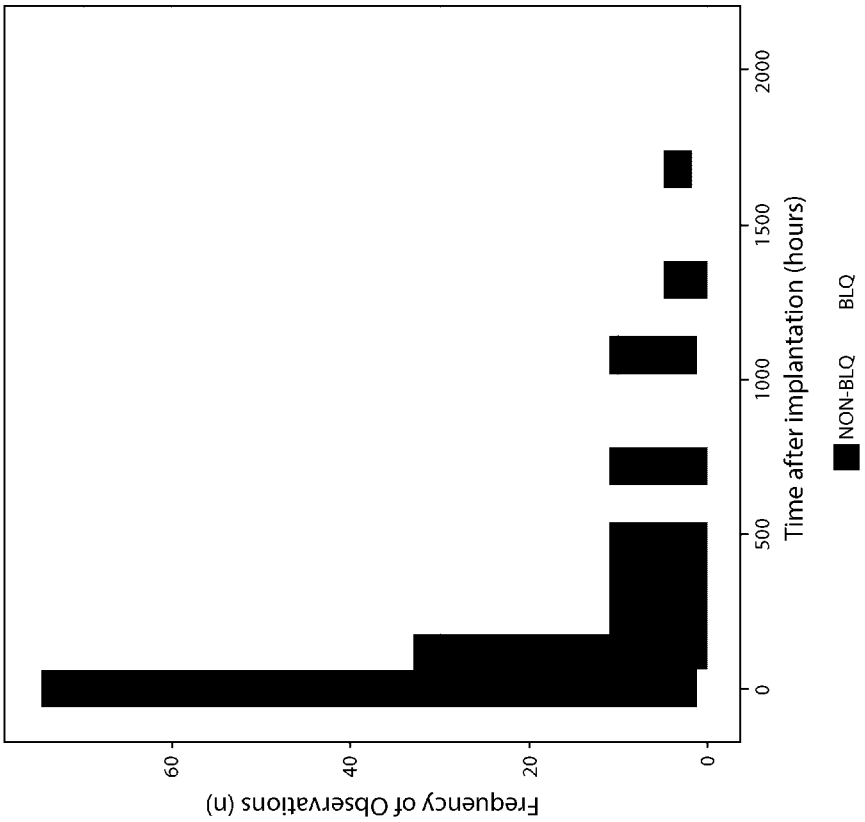

Frequency of Observations (n)
0
20
40
60
0
500
1000
1500
2000
Time after implantation (hours)
NON-BLQ
BLQ

Figure 9

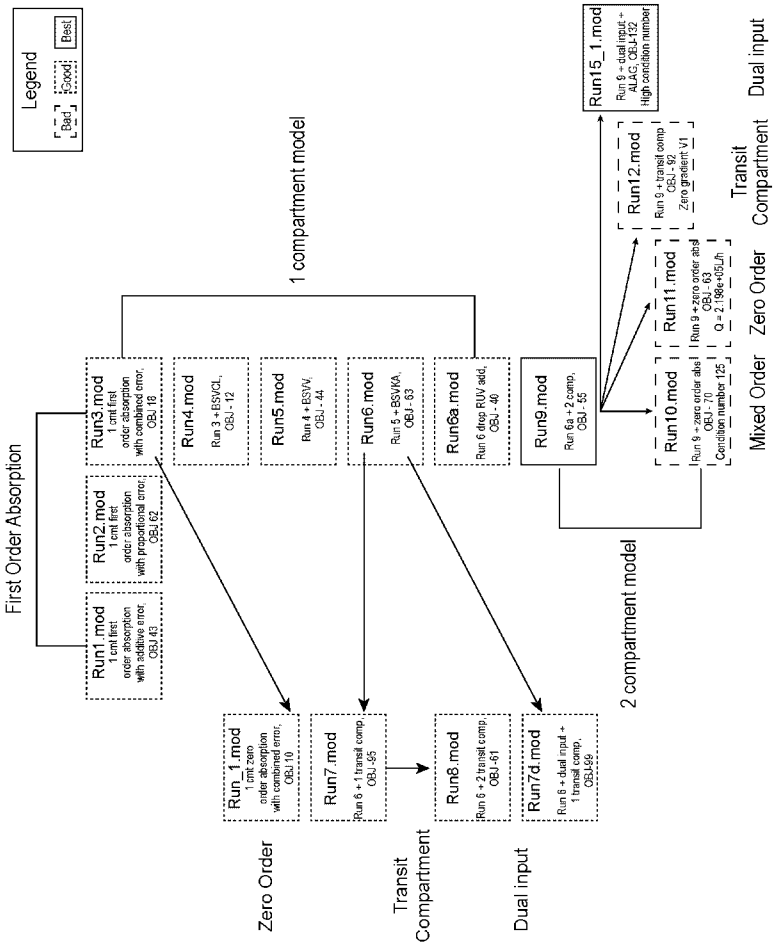

Legend
Bad
Good
Best
First Order Absorption
1 compartment model
2 compartment model
Run1.mod
1 cmt first order absorption with additive error
OBJ 43
Run2.mod
1 cmt first order absorption with proportional error
OBJ 62
Run3.mod
1 cmt first order absorption with combined error
OBJ 18
Run4.mod
Run 3 + BSV(CL, OBJ - 12
Run5.mod
Run 4 + BSV(V, OBJ - 44
Run6.mod
Run 5 + BSV(KA, OBJ - 53
Run6a.mod
Run 6 drop RUV add, OBJ - 40
Run9.mod
Run 6a + 2 comp, OBJ - 55
Run10.mod
Run 9 + zero order abs
OBJ - 70
Condition number 125
Run11.mod
Run 9 + zero order abs
OBJ - 63
Q = 2.196e+05/h
Run12.mod
Run 9 + transit comp
OBJ - 92
Zero gradient v1
Run15_1.mod
Run 6 + dual input + ALAG, OBJ-122
High condition number
Run_1.mod
1 cmt zero order absorption with combined error,
OBJ 10
Run7.mod
Run 6 + 1 transit comp, OBJ-95
Run8.mod
Run 6 + 2 transit comp, OBJ-61
Run7d.mod
Run 6 + dual input + 1 transit comp, OBJ-99
Zero Order
Transit Compartment
Dual input
Mixed Order
Zero Order
Transit Compartment
Dual input

Figure 10

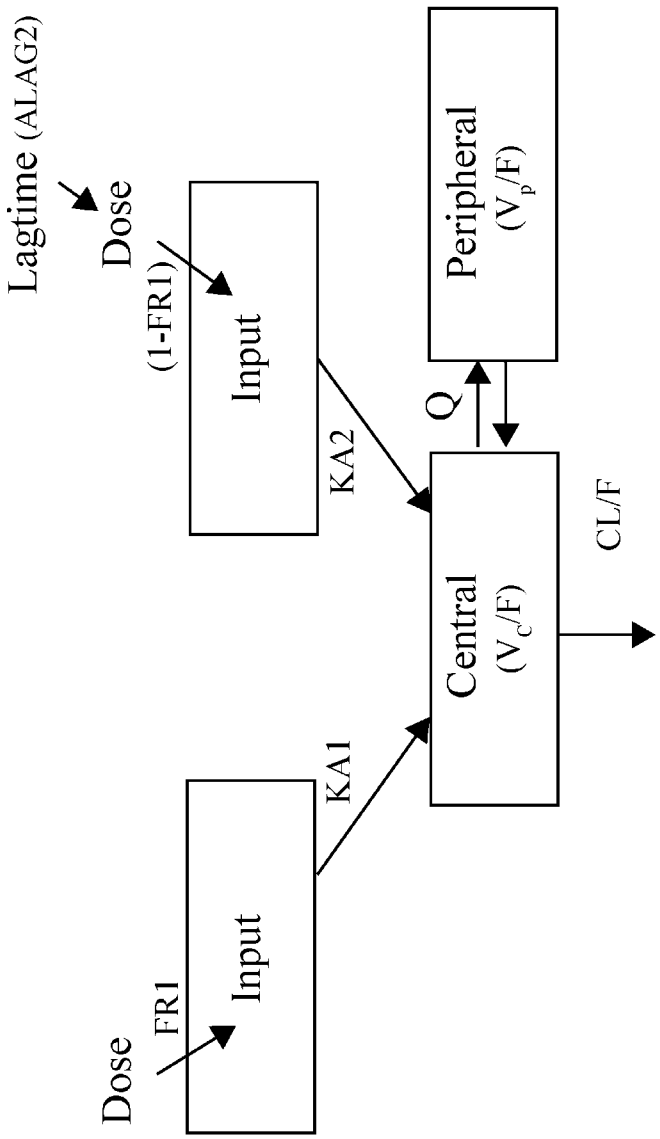

Lagtime (ALAG2)
Dose
(1-FR1)
Input
KA2
Peripheral
(V_p/F)
Q
Central
(V_c/F)
CL/F
KA1
Dose
FR1
Input

Figure 11

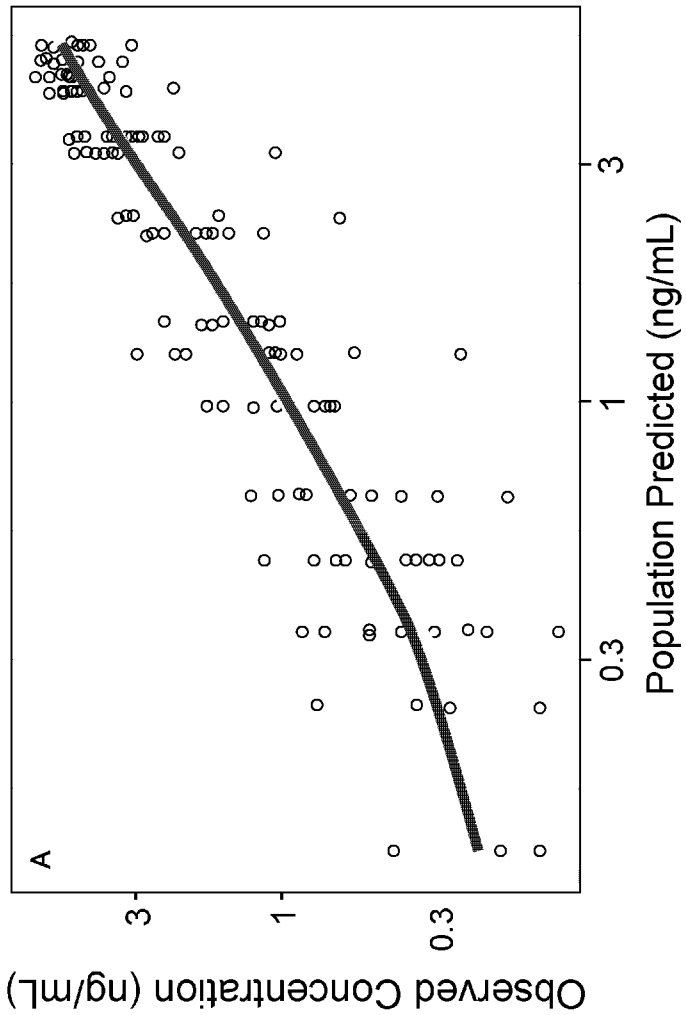

A
Observed Concentration (ng/mL)
Population Predicted (ng/mL)
0.3
1
3
0.3
1
3

Figure 12A

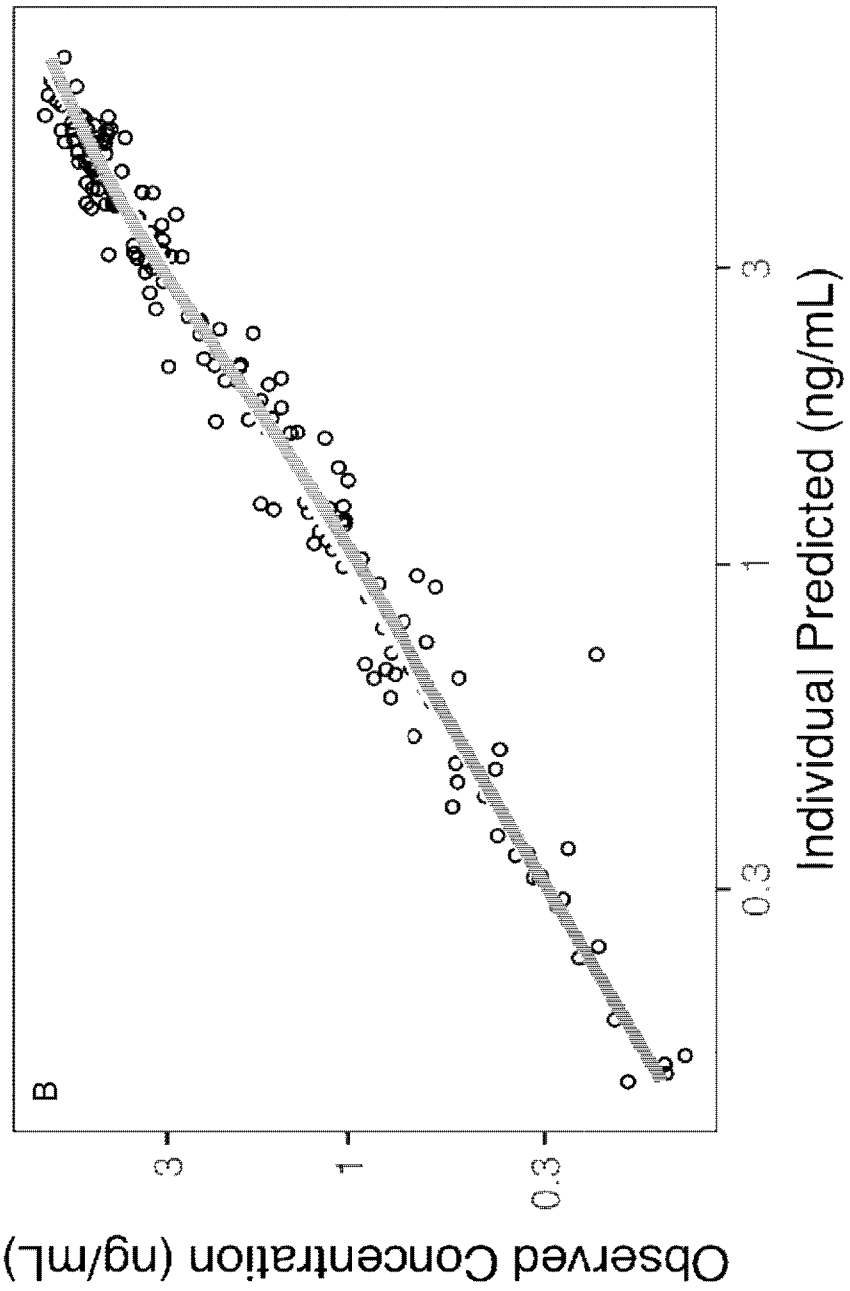

B
Observed Concentration (ng/mL)
3
1
0.3
Individual Predicted (ng/mL)
0.3
1
3

Figure 12B

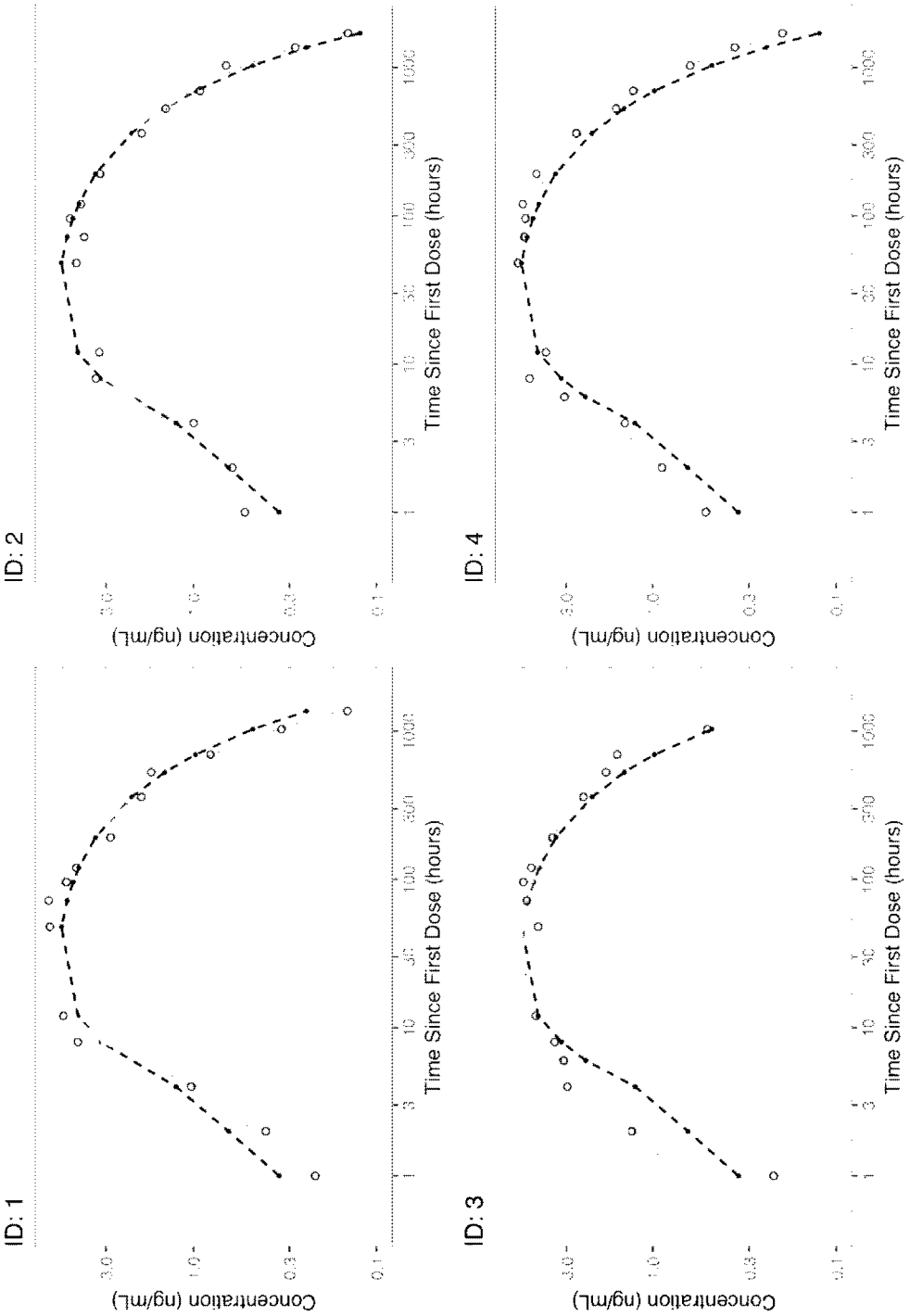


Figure 13 (1 of 3)

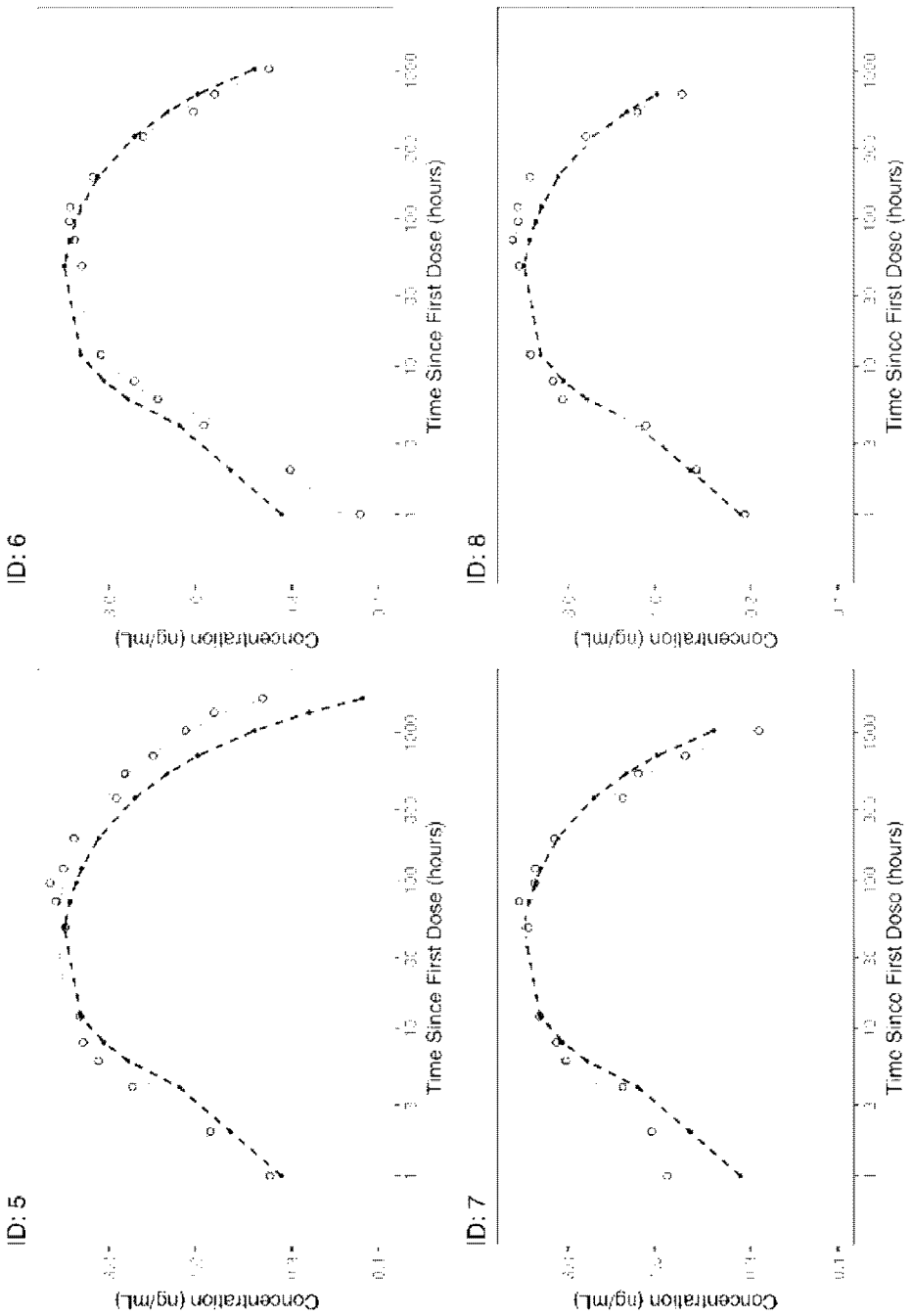


Figure 13 (2 of 3)

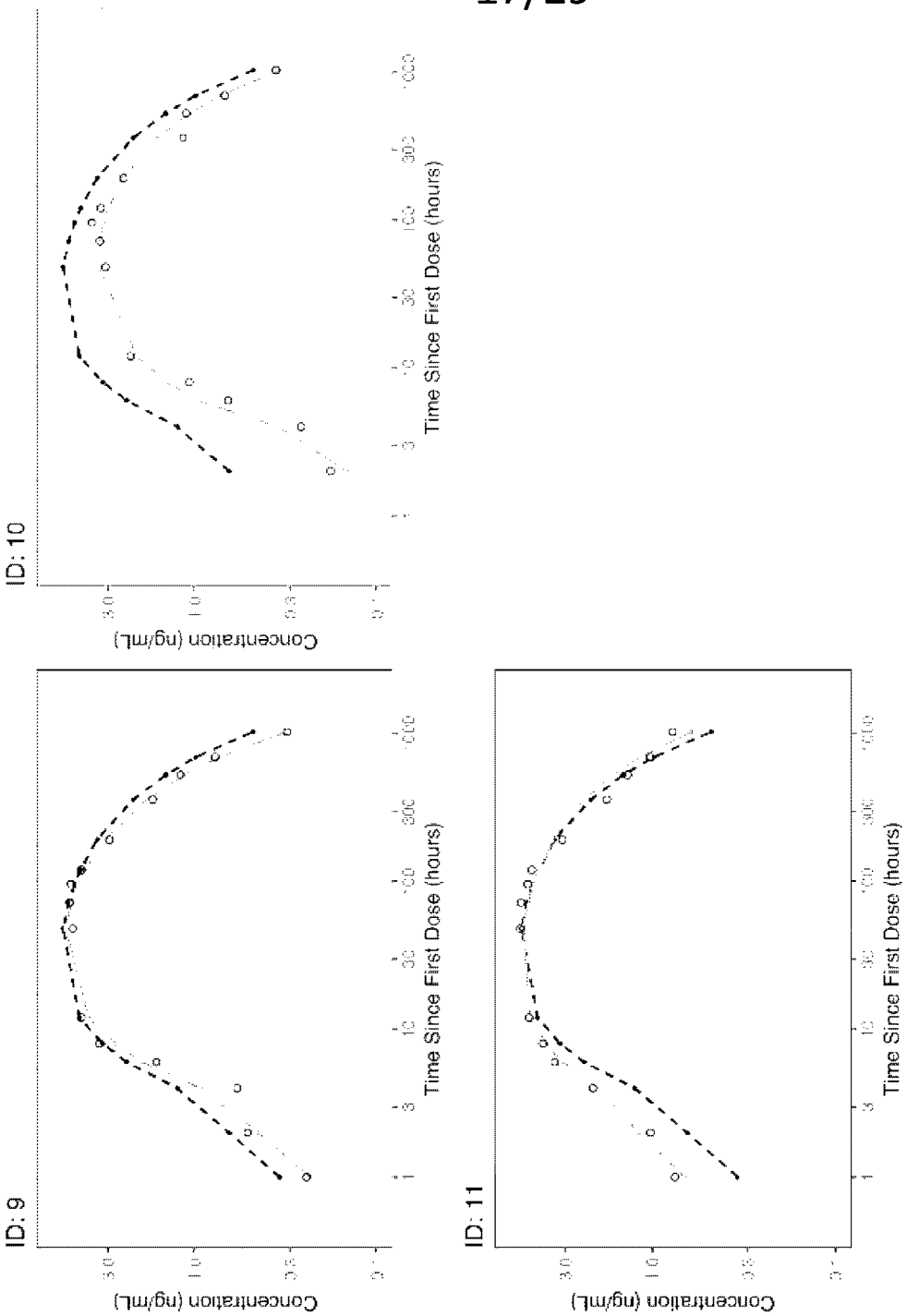


Figure 13 (3 of 3)

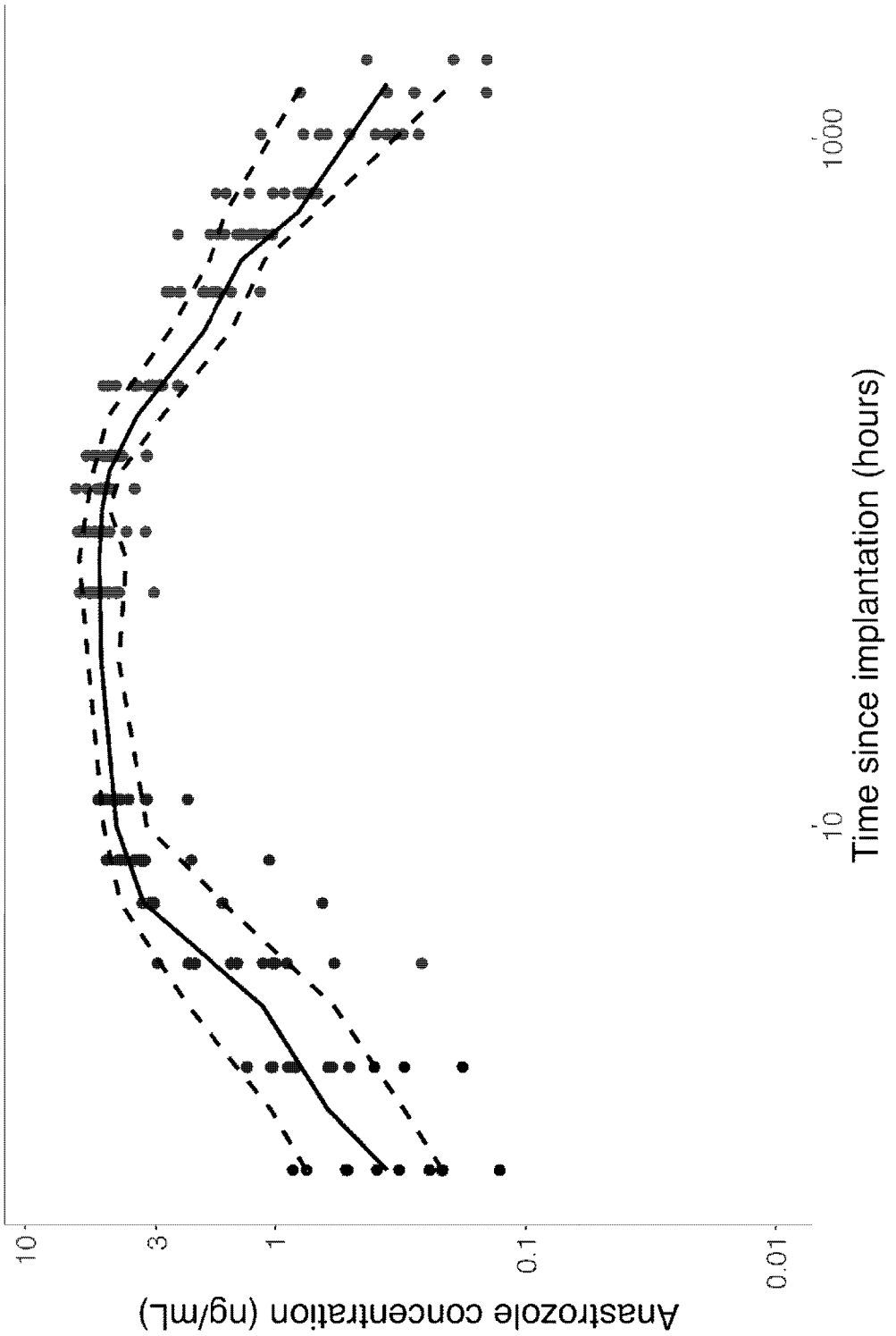

Anastrozole concentration (ng/mL)
10
3
1
0.1
0.01
10
1000
Time since implantation (hours)

Figure 14

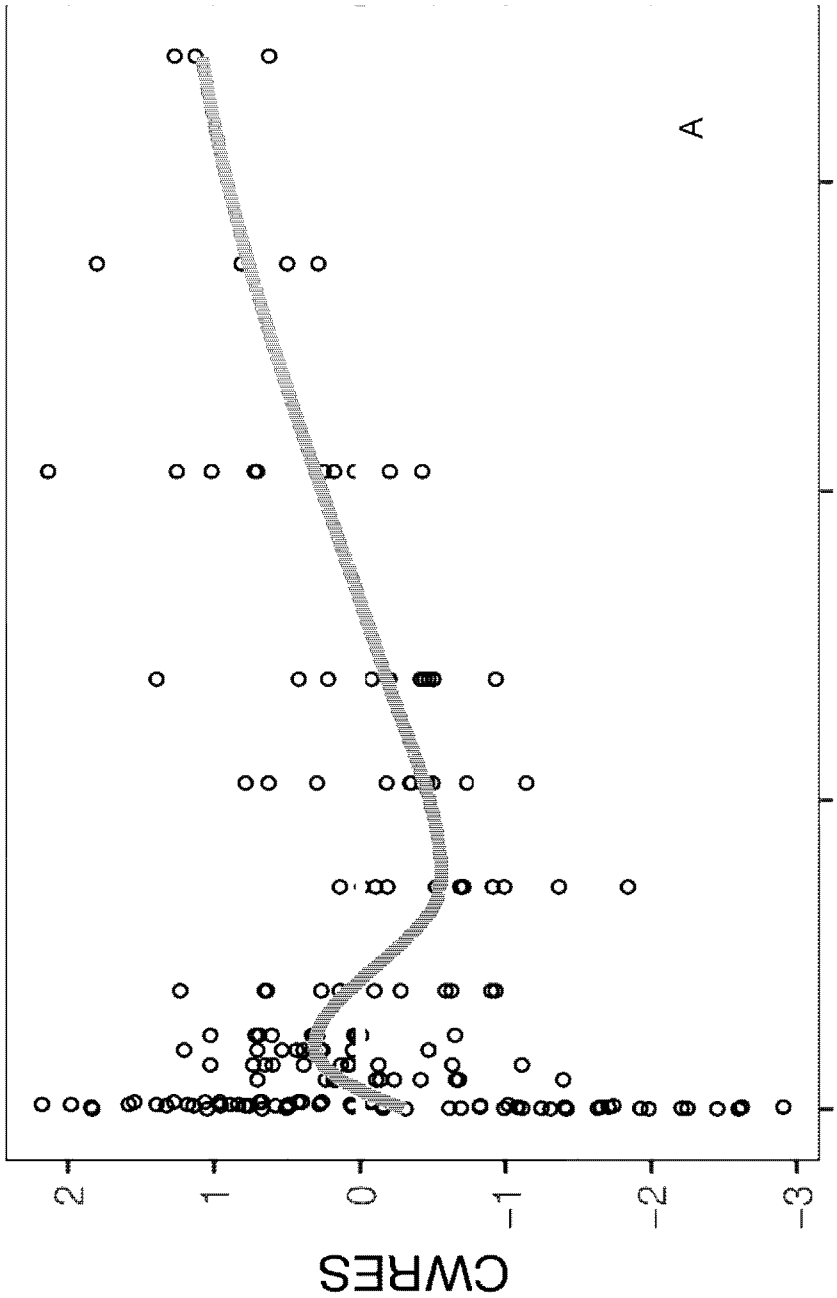


Figure 15A

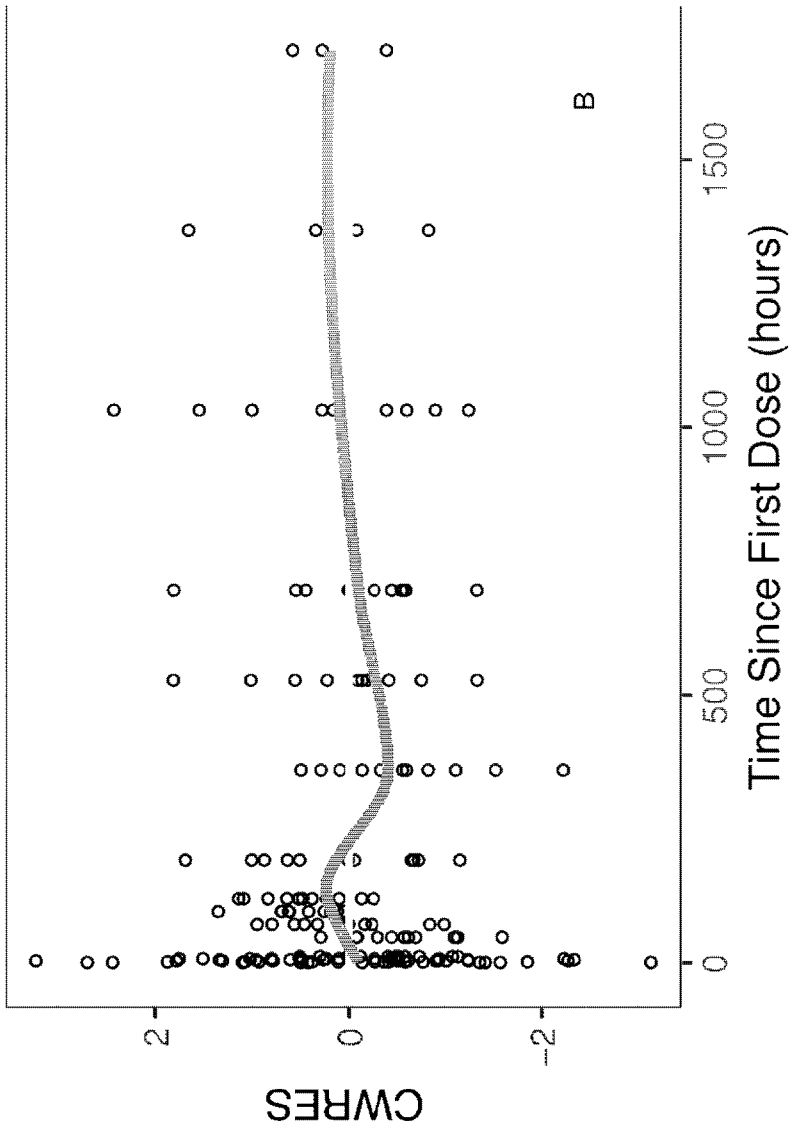


Figure 15B

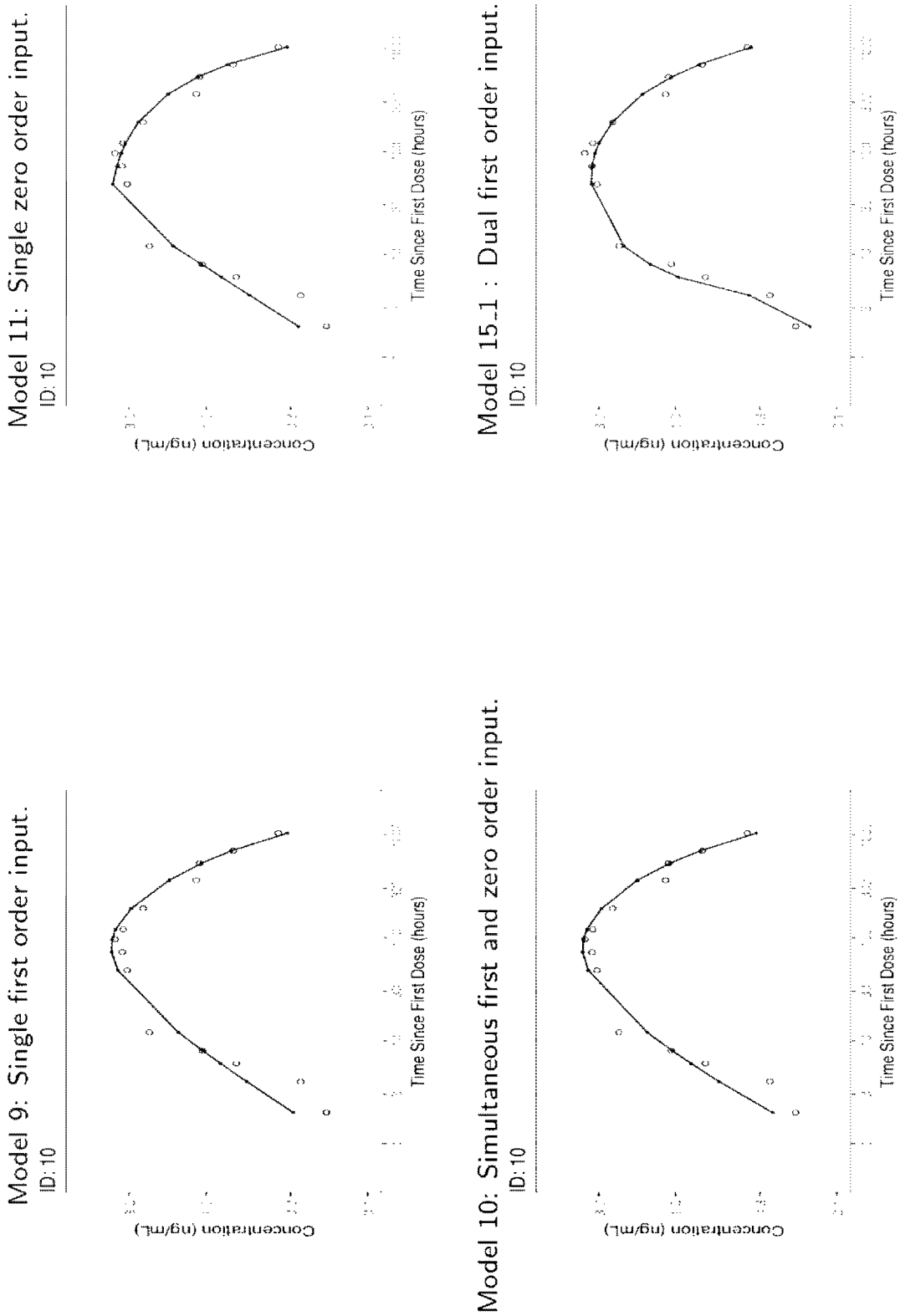

Model 11: Single zero order input.
ID: 10
Concentration (ng/mL)
Time Since First Dose (hours)
Model 15_1 : Dual first order input.
ID: 10
Concentration (ng/mL)
Time Since First Dose (hours)
Model 9: Single first order input.
ID: 10
Concentration (ng/mL)
Time Since First Dose (hours)
Model 10: Simultaneous first and zero order input.
ID: 10
Concentration (ng/mL)
Time Since First Dose (hours)

Figure 16

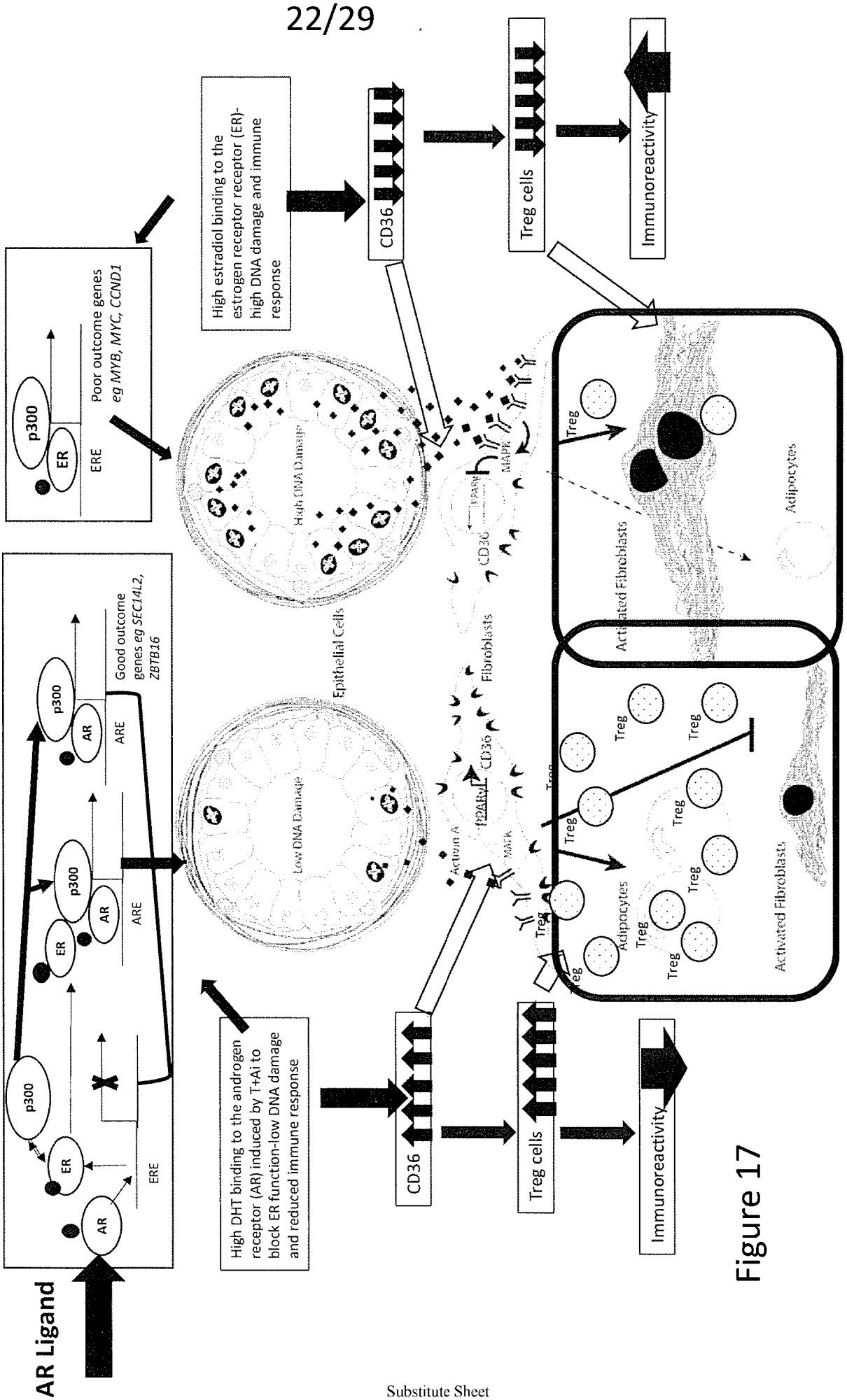

AR Ligand
AR
ERE
ER
p300
AR
ARE
Good outcome genes eg SEC14L2, ZBTB16
High DHT binding to the androgen receptor (AR) induced by T+Ai to block ER function-low DNA damage and reduced immune response
CD36
Treg cells
Immunoreactivity
Low DNA Damage
Epithelial Cells
High DNA Damage
High estradiol binding to the estrogen receptor receptor (ER)-high DNA damage and immune response
Poor outcome genes eg MYB, MYC, CCND1
Fibroblasts
Activated Fibroblasts
Adipocytes
Treg
Immunoreactivity
Treg cells
CD36

Figure 17

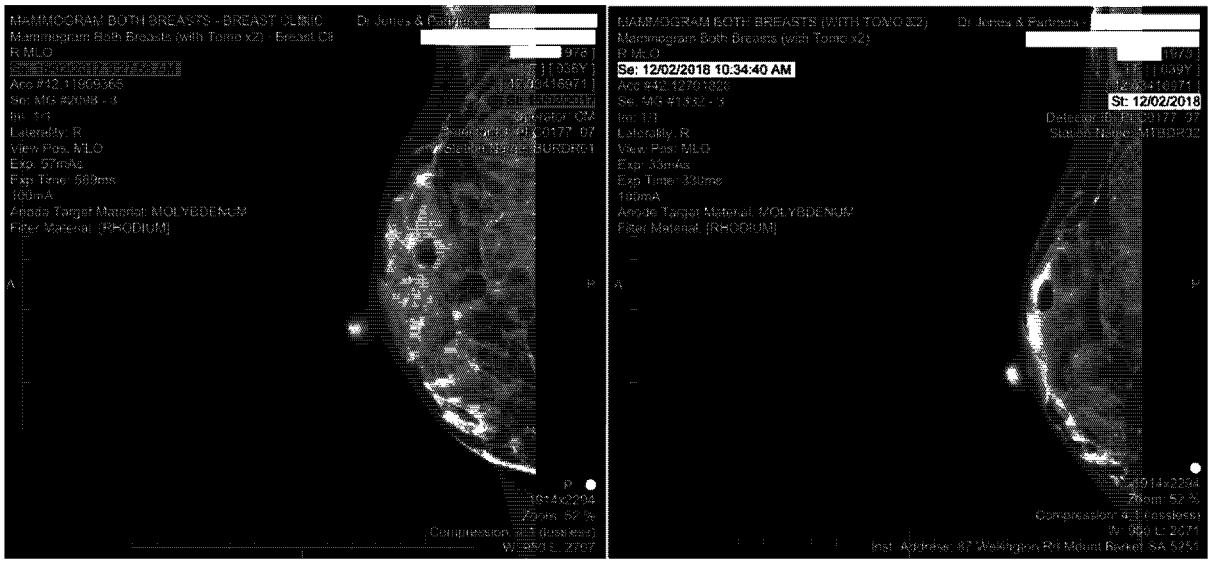
Figure 18

Pre-Treatment			Post-Treatment (24 Hours)		
Patient 1	Patient 2	Patient 3	Patient 1	Patient 2	Patient 3

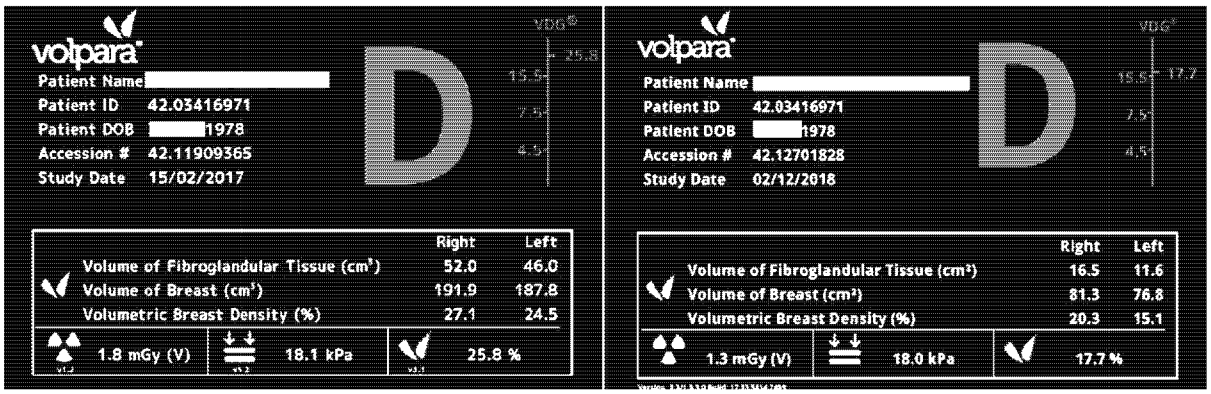


Figure 19

A



B



C

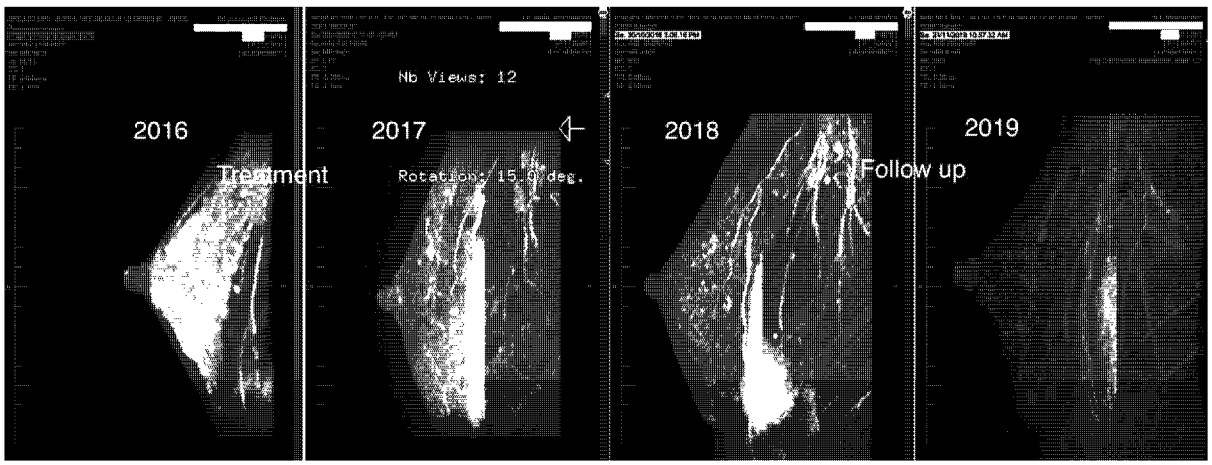
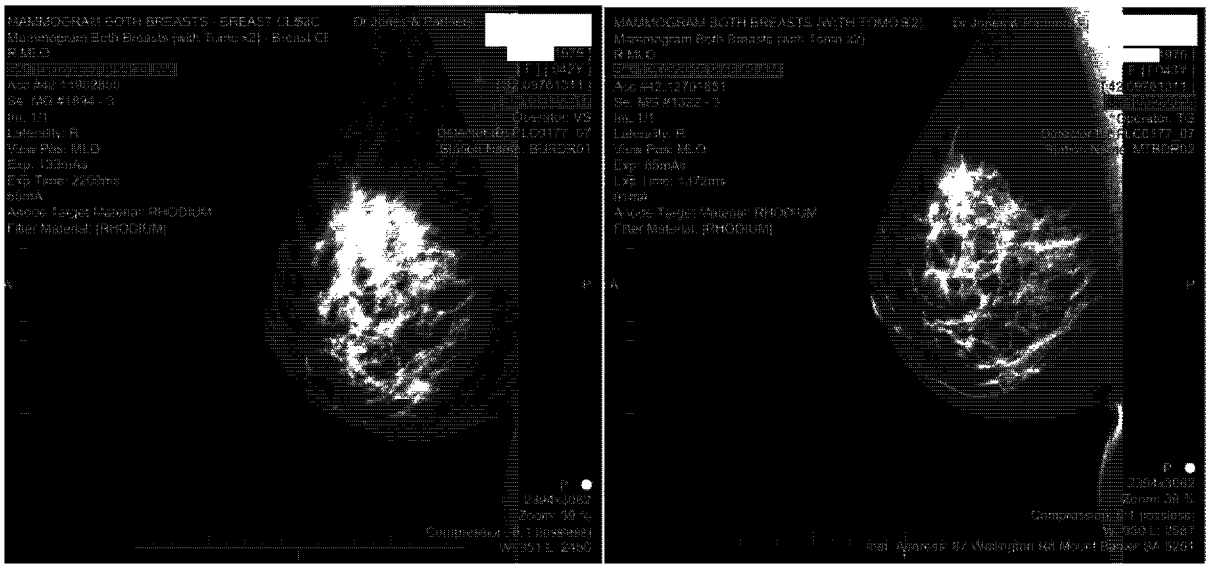


Figure 20

A



B

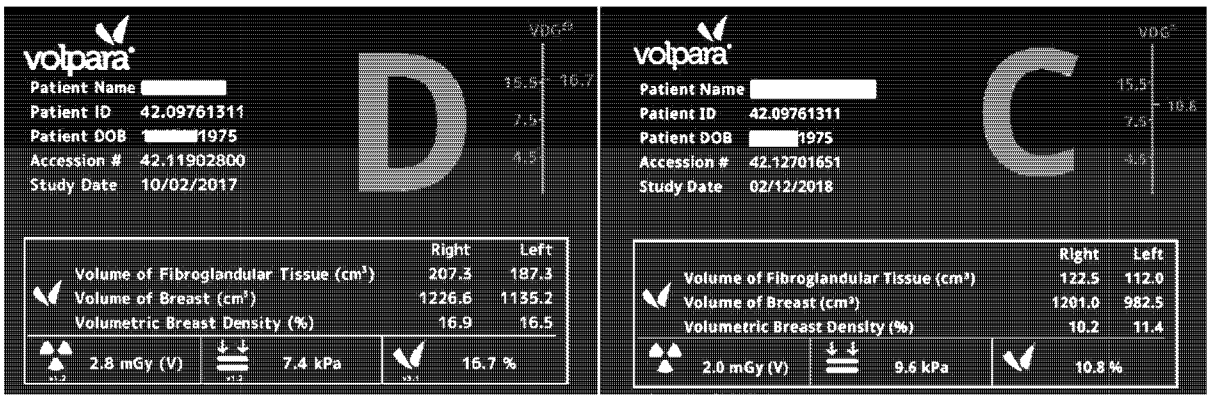
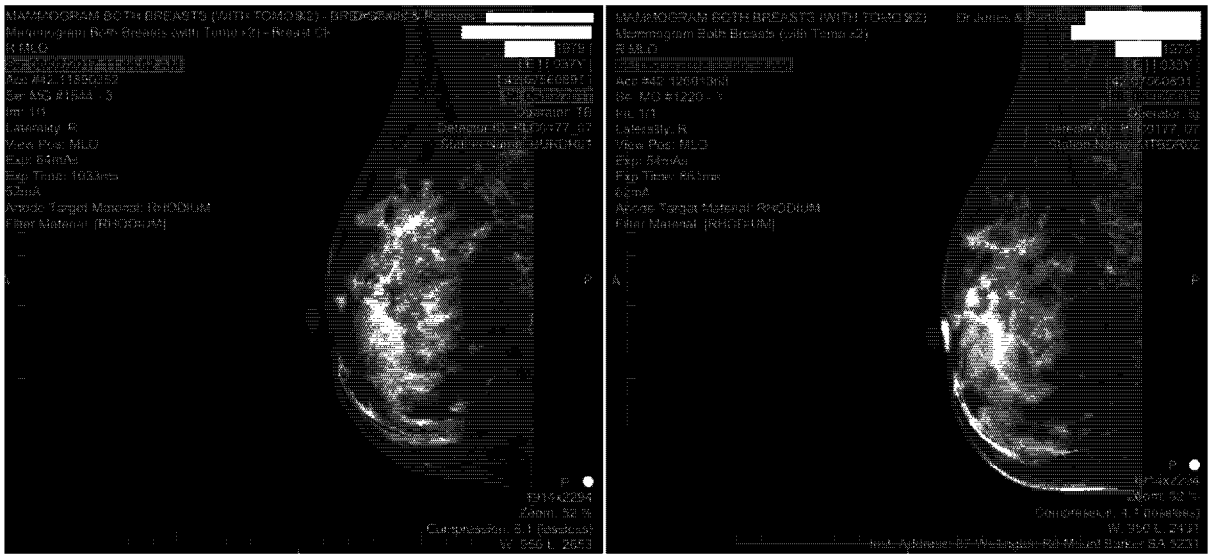

volpara
Patient Name
Patient ID 42.09761311
Patient DOB 1975
Accession # 42.11902800
Study Date 10/02/2017
D
Right Left
Volume of Fibroglandular Tissue (cm³) 207.3 187.3
Volume of Breast (cm³) 1226.6 1135.2
Volumetric Breast Density (%) 16.9 16.5
2.8 mGy (V)
7.4 kPa
16.7 %
volpara
Patient Name
Patient ID 42.09761311
Patient DOB 1975
Accession # 42.12701651
Study Date 02/12/2018
C
Right Left
Volume of Fibroglandular Tissue (cm³) 122.5 112.0
Volume of Breast (cm³) 1201.0 982.5
Volumetric Breast Density (%) 10.2 11.4
2.0 mGy (V)
9.6 kPa
10.8 %

Figure 21

A



B

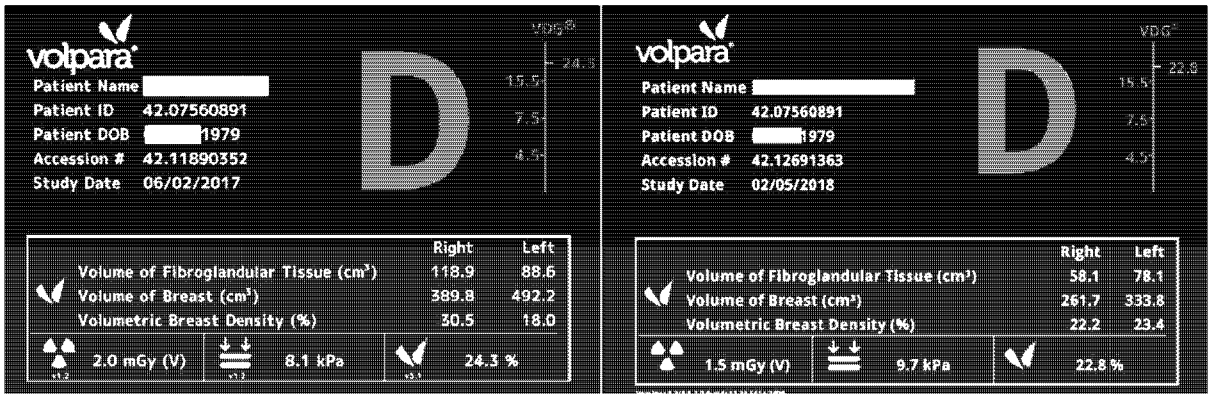
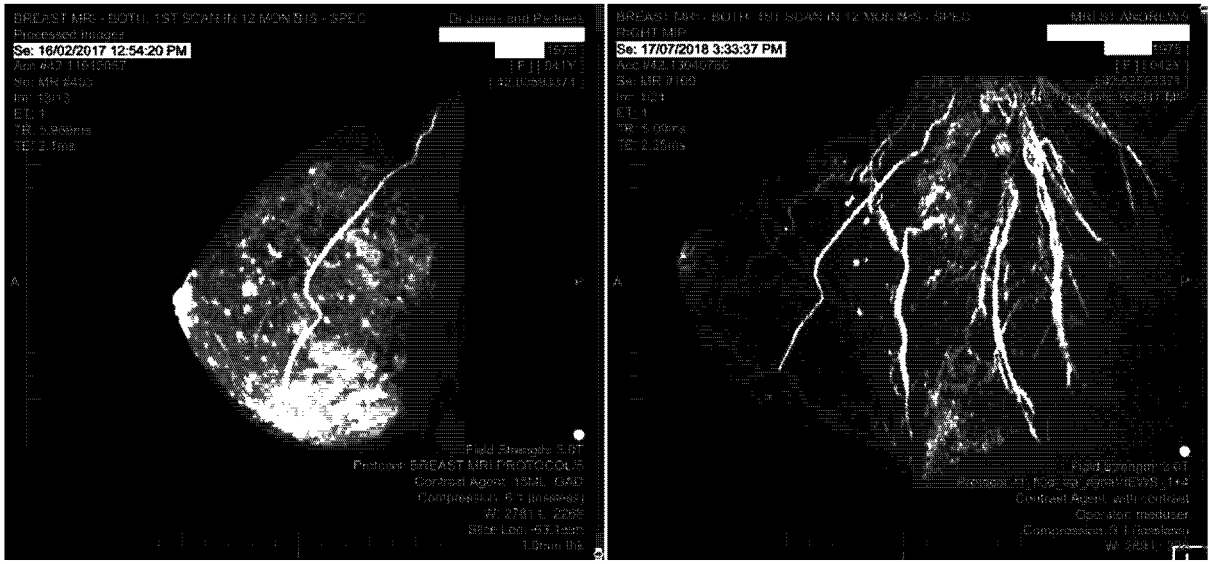

volpara
Patient Name
Patient ID 42.07560891
Patient DOB 1979
Accession # 42.11890352
Study Date 06/02/2017
VDG
D
24.5
15.5
7.5
4.5
Right Left
Volume of Fibroglandular Tissue (cm³) 118.9 88.6
Volume of Breast (cm³) 389.8 492.2
Volumetric Breast Density (%) 30.5 18.0
2.0 mGy (V)
8.1 kPa
24.3 %
volpara
Patient Name
Patient ID 42.07560891
Patient DOB 1979
Accession # 42.12691363
Study Date 02/05/2018
VDG
D
22.8
15.5
7.5
4.5
Right Left
Volume of Fibroglandular Tissue (cm³) 58.1 78.1
Volume of Breast (cm³) 261.7 333.8
Volumetric Breast Density (%) 22.2 23.4
1.5 mGy (V)
9.7 kPa
22.8 %

Figure 22

A



B



C

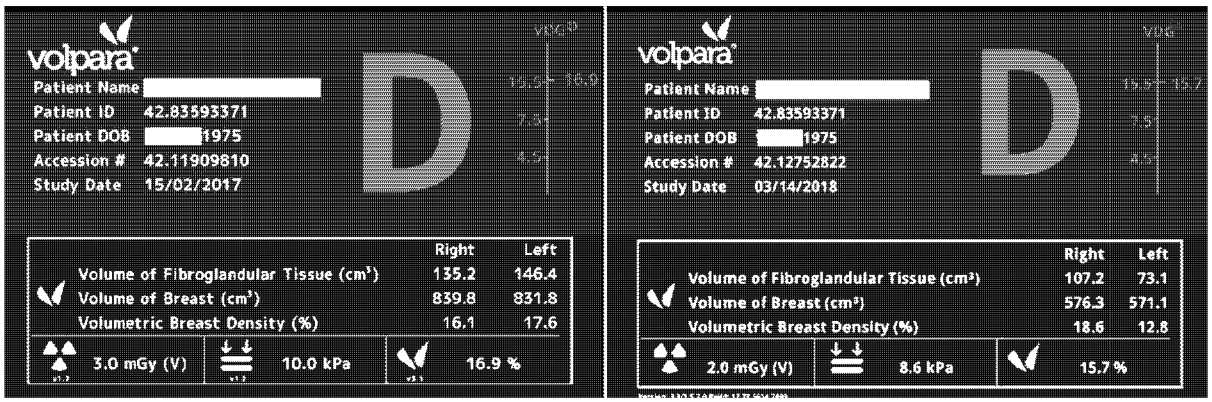


Figure 23

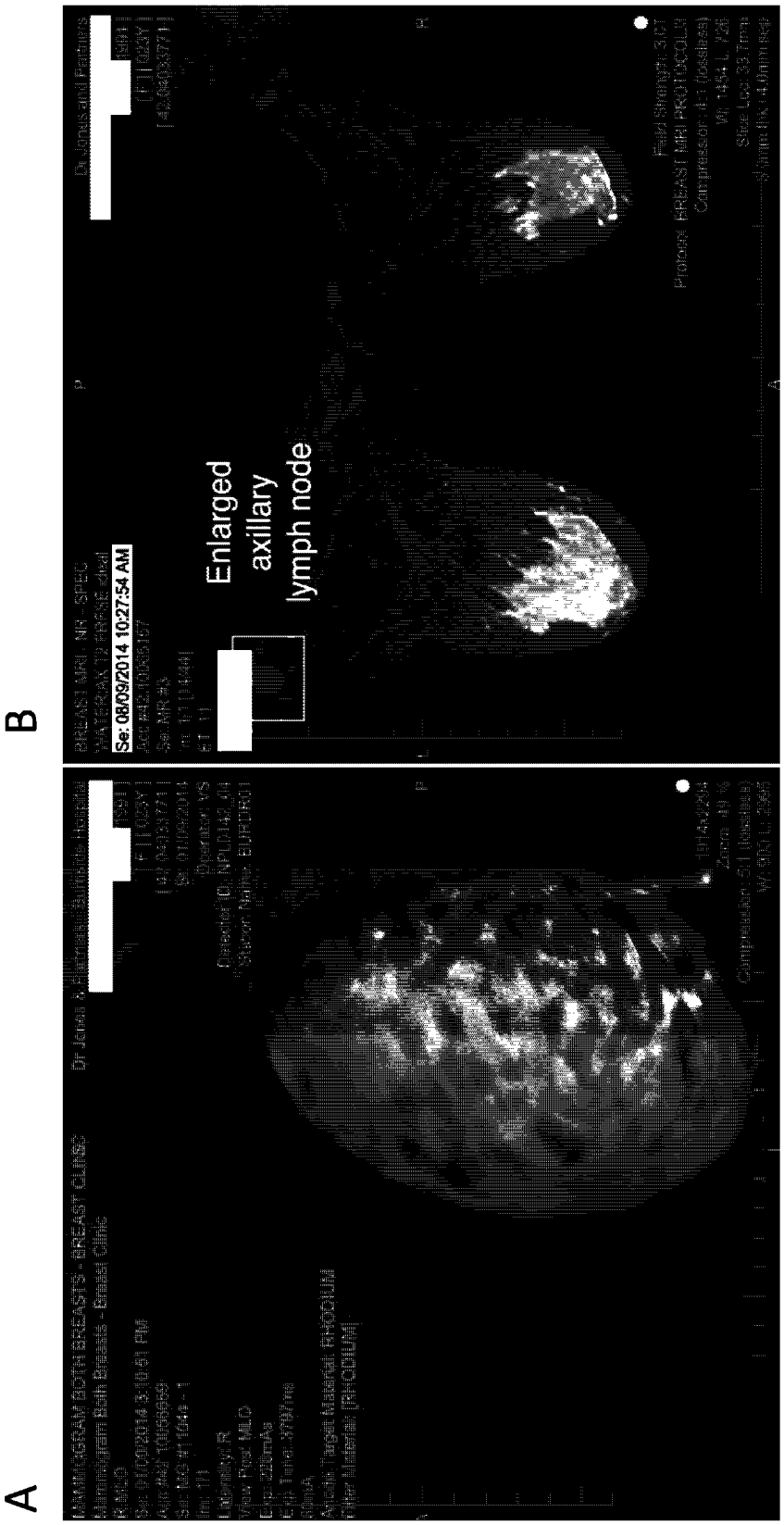
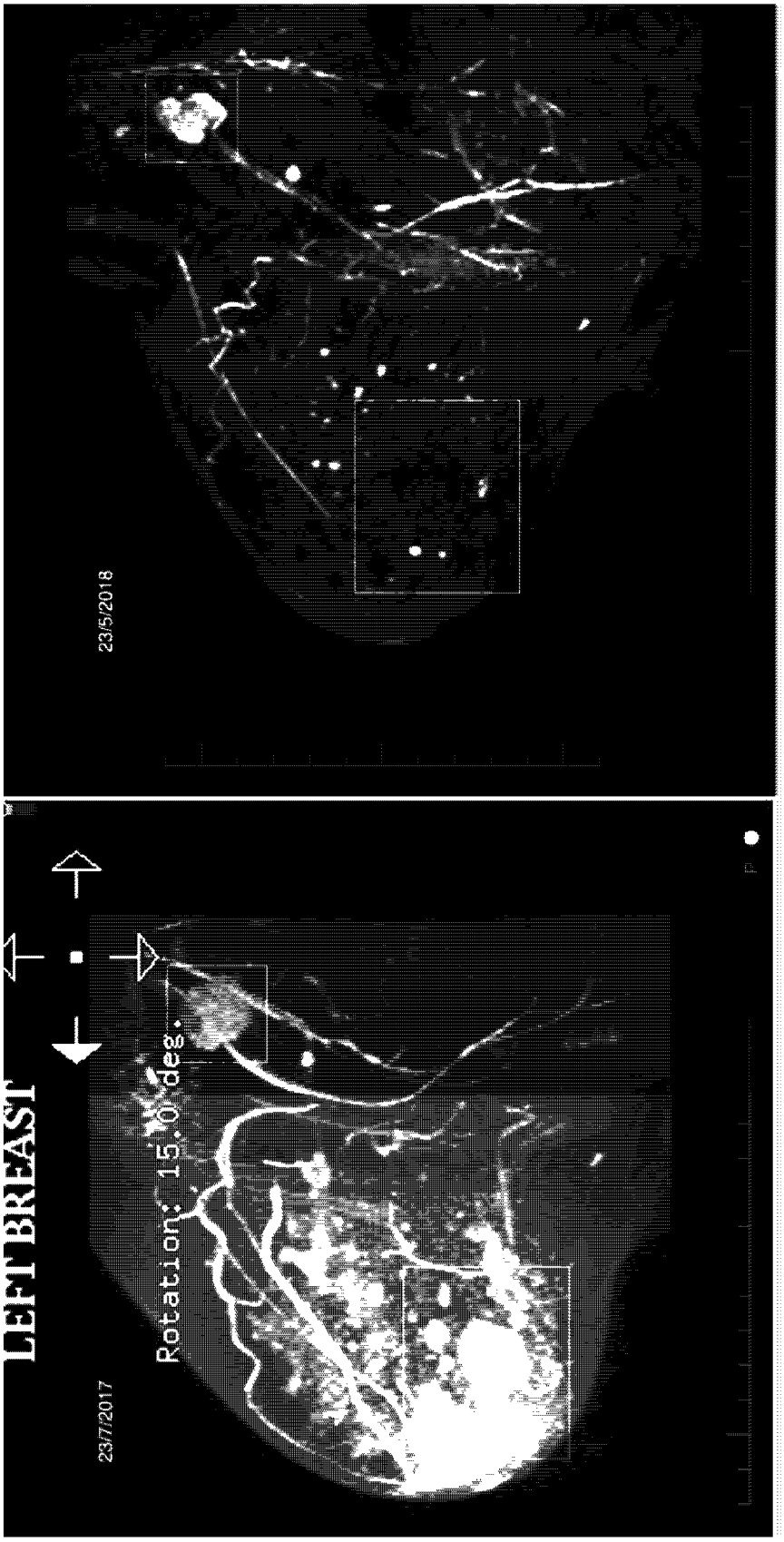


Figure 24



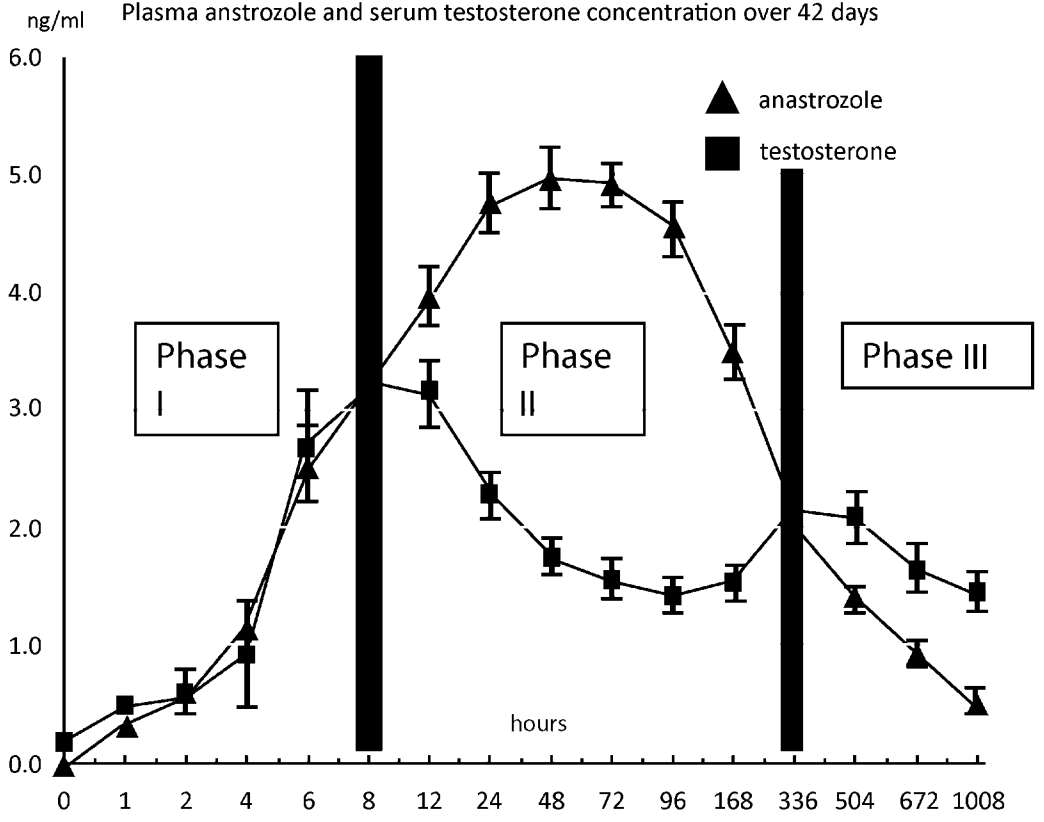

ng/ml
Plasma anstrozole and serum testosterone concentration over 42 days
6.0
5.0
4.0
3.0
2.0
1.0
0.0
anastrozole
testosterone
Phase I
Phase II
Phase III
hours
0
1
2
4
6
8
12
24
48
72
96
168
336
504
672
1008

Figure 2