CONFIDENTIAL

STUDY PROTOCOL

A window of opportunity study to assess the biological effect of enobosarm in oestrogen receptor positive, androgen receptor positive early breast cancer

Study Sponsor: University of Liverpool
1-3 Brownlow Street
Liverpool
Merseyside
L69 3BX

EudraCT number: **2016-000543-13**
ISRCTN number: **ISRCTN12213700**
Protocol version: **Final V6.0**
Date: **17/05/2021**

Study Protocol Approval

I, the undersigned, hereby approve this clinical study protocol:

Signature: _____ **Date:** _____

Professor Carlo Palmieri
Chief Investigator
Professor of Translational Oncology
Institute of Translational Oncology
Department of Molecular and Clinical Cancer Medicine
University of Liverpool

Signature: _____ **Date:** _____

Richard Jackson
Trial Statistician
Liverpool Cancer Trials Unit

Signature: _____ **Date:** _____

Neil French
Head of Clinical Operations
University of Liverpool
Sponsor

General Information

This document describes the EMERALD trial and provides information about procedures for entering patients into it. The protocol should not be used as an aide-memoir or guide for the treatment of other patients. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial, but centres entering patients for the first time are advised to contact the coordinating centre (Liverpool Clinical Trials Centre (LCTC)) to confirm they have the most up to date version. Clinical problems relating to this trial should be referred to the relevant Chief Investigator via LCTC.

Statement of Compliance

This study is designed to comply with the guideline developed by the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP) and will be conducted in compliance with the protocol, LCTC Standard Operating Procedures and EU Directive 2001/20/EC, transposed into UK law as the UK Statutory Instrument 2004 No 1031: Medicines for Human Use (Clinical Trials) Regulations 2004

UK Registration

This study will undergo HRA review and Approval before opening to recruitment. The HRA approval will bring together the assessment of governance and legal compliance in addition to an independent Research Ethics Committee (REC) review provided the through the UK research ethics service. Each centre will confirm they have the capability and capacity to deliver the study locally prior to being open to recruitment.

Furthermore, the study will hold a Clinical Trials Authorisation issued by the Medicines and Healthcare Products Regulatory Agency (MHRA).

Contact Details: Institutions

Sponsor:	**Trial Management and Monitoring:**	**Clinical Laboratory:**
The University of Liverpool Liverpool Joint Research Office 2nd Floor Block D Waterhouse Building 3 Brownlow Street Liverpool L69 3GL Tel: 0151 794 8373 Email: sponsor@liverpool.ac.uk	Liverpool Clinical Trials Centre University of Liverpool 1st Floor, Block C Waterhouse Building 3 Brownlow Street Liverpool L69 3GL Tel: 0151 794 8935 Fax: 0151 794 8250	Liverpool GCP Laboratory Facility 3rd Floor UCD Building Molecular and Clinical Cancer Medicine University of Liverpool L69 3GA Tel: 0151 706 4948
Translational Research Laboratory	**Study Drug Developer**	**Study Drug Distributor**
Leeds Institute of Cancer and Pathology (LICAP) Section of Pathology and Tumour Biology Wellcome Trust Brenner Building 4th Floor, Room 4.13 St James's University Hospital Beckett Street Leeds LS9 7TF	GTx, Inc. 175 Toyota Plaza 7th Floor Memphis Tennessee 38103 USA Tel: 001 901 271 8622 Fax: 001 901 844 8075	PCI Pharma Services Treforest Unit D8.3 Treforest Industrial Estate Main Avenue Pontypridd Cardiff CF37 5UR Tel: 01443 846111
Study Drug Manufacturer		
Catalent Pharma Solutions, LLC 2725 Scherer Drive St. Petersburg Florida 33716 USA Tel: 001 727 803 2100 Fax: 001 727 803 1639		

Contact Details: Oversight Committees

Contact	Document Title
Trial Management Group (TMG) Trial Steering Committee (TSC) Independent Data and Safety Monitoring Committee (IDSMC)	For oversight committee details please refer to the respective charters held in the Trial Master File.

Contact Details: Principal Investigators

Contact	Document Title
Principal Investigators	A list of principal investigators and sites is available on the LCTC Trial Management System

Contact Details: Individuals

Individual Authorised to Sign the Protocol and Protocol Amendments on behalf of the Sponsor:	Chief Investigator (CI):	Medical Expert who will Advise on Protocol Related Clinical Queries (If CI unavailable):
Charlotte Rawcliffe Liverpool Clinical Trials Centre Block C Waterhouse Building 1-3 Brownlow Street University of Liverpool L69 3GL Tel: 0151 794 8167 Fax: 0151 794 8250 E-mail: c.rawcliffe@liverpool.ac.uk	Professor Carlo Palmieri Professor of Translational Oncology and Consultant in Medical Oncology Department of Molecular and Clinical Cancer Medicine Institute of Translational Medicine Faculty of Health and Life Sciences University of Liverpool Sherrington Building Ashton Street Liverpool L69 3GE Tel: 0151 706 3616 Fax: 0151 706 5826 E-mail: c.palmieri@liv.ac.uk	Please refer to the Pharmacovigilance Plan available in the Trial Master File for this information.
Medical Expert who will Evaluate SAE Reports (If CI unavailable):	**Trial Coordinator**	**Trial Statistician:**
Please refer to the Pharmacovigilance Plan available in the Trial Master File for this information.	Helen Scott LCTC Block C Waterhouse Building 1-3 Brownlow Street University of Liverpool Tel: 0151 795 5289 Fax: 0151 794 8250 Email: emeraldtrial@liverpool.ac.uk	Richard Jackson LCTC Block C Waterhouse Building 1-3 Brownlow Street University of Liverpool
Data Manager	**Public and Patient Involvement**	**Lab Technician**
Data Manager Liverpool Clinical Trials Centre Block C Waterhouse Building 1-3 Brownlow Street University of Liverpool Tel: 0151 794 8209 Fax: 0151 794 8250 Email: emeraldtrial@liverpool.ac.uk	Dr Adrienne Morgan Independent Cancer Patient's Voice adrienne@icpv.org.uk	Liverpool GCP Laboratory Facility Technician 1st Floor William Henry Duncan Building University of Liverpool 6 West Derby Street Liverpool L7 8TX Tel: 0151 706 4184

Table of Contents

Glossary of terms and abbreviations

AE	Adverse Event
AI	Aromatase Inhibitors
ALP	Alkaline Phosphate
ALT	Alanine Transaminase
AnR	Androgen Receptor
AR	Adverse Reaction
AST	Aspartate Transaminase
BC	Breast Cancer
BCC	Breast Cancer Campaign
BSA	Body Surface Area
CI	Chief Investigator
CRF	Case Report Form
CRUK	Cancer Research UK
CSG	Clinical Study Group
ct	Count
CTA	Clinical Trials Authorisation
CTIMP	Clinical Trial of an Investigational Medicinal Product
CTU	Clinical Trials Unit
CV	Curriculum Vitae
DHT	Dihydrotesterone
DM	Data Manager
eCRF	Electronic Case Report Form
ER	Oestrogen Receptor
ET	Endocrine Therapy
FBC	Full Blood Count
FFPE	Formalin Fixed Paraffin Embedded Tissue
FSH	Follicle Stimulating Hormone
GCDFP	Gross Cystic Disease Fluid Proteins
GNRH	Gonadotrophin Releasing Hormone
GP	General Practitioner
HER2	Human Epidermal Growth Factor
HRT	Hormone Replacement Therapy
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH GCP	International Conference on Harmonisation Good Clinical Practice
ICPV	Independent Cancer Patient Voice
IDSMC	Independent Data and Safety and Monitoring Committee
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
LBM	Lean Body Mass
LCTC	Liverpool Clinical Trials Centre
LFT	Liver Function Test
LH	Luteinizing Hormone
MCRN CTU	Medicines for Children Clinical Trials Unit
MHRA	Medicines and Healthcare Products Regulatory Agency
MPA	Medroxyprogesterone Acetate
MRI	Magnetic Resonance Imaging
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events

NCRI	National Cancer Research Institute
NSCLC	Non-Small Cell Lung Cancer
PI	Principal Investigator
PIS	Patient Information Sheet
PK	Pharmacokinetics
POB	Probability of Benefit
PR	Progesterone Receptor
PSA	Prostate Specific Antigen
R&D	Research & Development
RAG	Red, Amber, Green System
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SARM	Selective Androgen Receptor Modulators
SHBG	Sex Hormone Binding Globulin
SOP	Standard Operating Procedures
SPC	Summary of Product Characteristics
SSA	Site Specific Assessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
TC	Trial Coordinator
TSC	Trial Steering Committee
UAR	Unexpected Adverse Reaction
UKAS	United Kingdom Accreditation Service
ULN	Upper Limit of Normal
UOL	University of Liverpool
US	Ultrasound

1 PROTOCOL SUMMARY

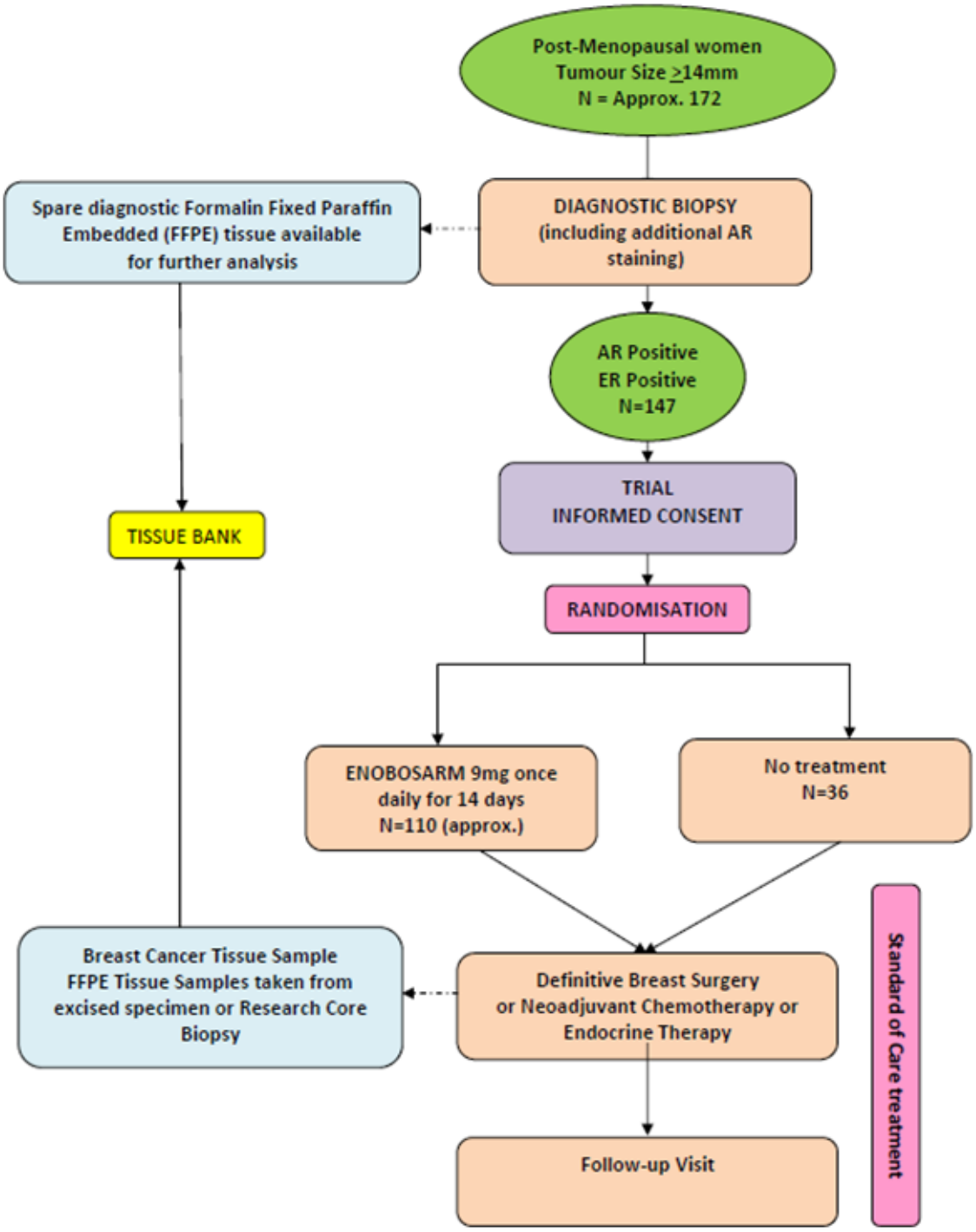
Full Title	A window of opportunity study to assess the biological effect of enobosarm (GTx-024/Ostarine®) in oestrogen receptor positive, androgen receptor positive early breast cancer
Short Title	EMERALD
Phase	Phase II
Design	Open label randomised superiority trial
Sample Size	147 patients across sites in the UK
Main Inclusion Criteria	1. Females 16 years of age or older 2. Histologically confirmed ER positive breast cancer (Allred ≥3) 3. AnR positive breast cancer (defined as ≥10% nuclear AnR staining by immunohistochemistry)[1] 4. Any HER2 status 5. Tumour measuring ≥14mm in longest diameter by ultrasound (US) examination, MRI or mammogram 6. Postmenopausal as defined by one of the following criteria: a. Women ≥55 years of age with an intact uterus and amenorrhoea ≥12 months at the time of diagnosis (or documented or current FSH and oestradiol levels within the postmenopausal range (as per local institutional/laboratory standard)) b. Prior bilateral oophorectomy c. Documented or current FSH and oestradiol levels within the postmenopausal range (as per local institutional/laboratory standard) in women aged <55 years or in women who have had a hysterectomy with intact ovaries 7. Eastern Cooperative Oncology Group (ECOG) performance status 0, 1 or 2 8. Adequate renal function defined by a serum creatinine ≤1.5 x ULN. Adequate liver function defined by total bilirubin ≤ 1.5 ULN (patients with Gilbert's Syndrome exempted), either ALT or AST ≤2.5 ULN and ALP ≤2.5 ULN 9. Acceptable risk of bleeding (e.g. bleeding diathesis, warfarin) as assessed by the PI (if the PI is unsure the CI will make the final decision) 10. Written informed consent 11. Able to comply with treatment and follow up

[1] Immunohistochemistry (IHC) for AnR: This will initially be performed by contributing centres, all of who are UKAS (UK Accreditation Service) accredited laboratories and take part in the national external quality assessment service (NEQAS) IHC assessment schemes. ALL AnR IHC will subsequently be reviewed centrally by the trial pathologists. AnR testing will be performed at the time of diagnosis, with only those patients with ER positive AND AnR positive tumours being approached for study entry in the clinic.

Main Exclusion Criteria	1. Inoperable breast cancer 2. Males 3. Inflammatory tumours 4. Evidence of metastatic disease 5. Any history of invasive malignancy within 5 years of starting study treatment (other than adequately treated basal cell carcinoma or squamous cell carcinoma of the skin and cervical carcinoma in situ) 6. Prior endocrine therapy or chemotherapy for breast cancer 7. Concomitant use (defined as use within 12 weeks prior to entry) of HRT or any other oestrogen-containing medication or supplement (including vaginal oestrogens and phytoestrogens) 8. Previous use of oestrogen implants within the last 12 weeks 9. Uncontrolled abnormalities of serum potassium, sodium, calcium or magnesium levels 10. Evidence of uncontrolled active infection 11. Evidence of significant medical condition or laboratory finding which, in the opinion of the investigator, makes it undesirable for the patient to participate in the trial 12. Participation in a clinical trial of an IMP in the last 30 days
Study Duration	14 months for recruitment, 6 months for set up and 6 months for close out
Description of Agent/Intervention	Patients randomised 3:1 to 9mg of enobosarm (therapeutic arm) which will be taken orally, once daily, up to surgery/research core biopsy which should happen 2 weeks +4 days from commencing treatment
Objectives	<u>Primary Objective:</u> To determine whether enobosarm reduces Ki67 proliferation index in comparison with no treatment. <u>Secondary Objectives:</u> 1. To determine the effects of enobosarm on tumour cell apoptosis 2. To determine the effects of enobosarm on oestrogen receptor and androgen receptor regulated genes 3. To evaluate the changes in circulating steroidogenic hormones after enobosarm administration 4. To evaluate changes in prostate specific antigen (PSA) after enobosarm administration 5. To establish the safety and tolerability of enobosarm

Protocol Summary - continued

Schematic of Study Design:



EMERALD Trial Flow Chart Version 9 Date: 30/MAR/2018

2 BACKGROUND INFORMATION

2.1 Introduction

Breast Cancer and Endocrine Therapy

Breast cancer (BC) is a leading cause of morbidity and mortality in the UK with 49,936 new cases and 11,762 deaths in 2011 ('Cancer Research UK. Cancer Stats Key Facts Breast Cancer.' 2013). BC is a heterogeneous disease which may be classified by gene expression profiles (Perou et al. 2000) or immunohistochemical biomarkers (Carey et al. 2006). One key subtype (80% of BC) is defined by the expression of the nuclear transcription factor oestrogen receptor alpha (herein referred to as ER) (Harvey et al. 1999). Hence, endocrine therapies that aim to inhibit ER activity represent a cornerstone strategy in the management of ER positive BC. The majority of ER positive BC are diagnosed as early stage disease and are treated with curative intent by surgery followed by various combinations of adjuvant chemotherapy, radiotherapy, and trastuzumab (if HER2 positive and receiving chemotherapy). All women diagnosed with ER positive BC should receive endocrine therapy (ET), and the introduction and widespread use of adjuvant tamoxifen and subsequently in the postmenopausal population aromatase inhibitors (AI) have resulted in significant improvements in the overall survival of women with ER positive early BC (Early Breast Cancer Trialists' Collaborative et al. 2011; Dowsett et al. 2010). Despite these improvements, 30% of patients will suffer relapse, due to inherent or acquired resistance to ET. These women inevitably die of metastatic breast cancer (MBC). The introduction of sequential lines of different endocrine therapies has led to stepwise improvements in disease control and outcomes for women with metastatic ER positive disease (Bonneterre et al. 2001; Lonning et al. 2000; Robertson et al. 2009; Chia et al. 2008). However, resistance both *de novo* and acquired continues to limit the efficacy of ET in both early and metastatic BC (Osborne and Schiff 2011). While the introduction and use of agents which target resistance mechanisms implicated in endocrine resistance, such as trastuzumab, everolimus and palbociclib, have improved the efficacy of ET when used in combination in the metastatic setting ((Kaufman et al. 2009; Baselga et al. 2012; Finn et al. 2016) with others in clinical trial (Palmieri et al. 2014)) resistance to ET remains a major clinical problem. Therefore, continued clinical and translational research into potential novel endocrine therapies, as well as strategies to enhance the effectiveness of currently available therapies, is required if outcomes in both early and metastatic disease are to be significantly improved (Palmieri et al. 2014).

Androgens and Human Breast Cancer

Androgen hormones play a key role in breast development and homeostasis in both males and females (Yeh et al. 2003; Nieto, Rider, and Cramer 2014). The natural growth inhibitory influence of androgenic activity in normal breast development is at least partly sustained in the context of breast cancer. In nearly all pre-clinical models of ER positive disease, androgens consistently exert an antiproliferative, anti-oestrogenic effect (Poulin, Baker, and Labrie 1988; Dauvois et al. 1991; Birrell et al. 1995; Kandouz et al. 1999).This also occurs *in vivo*, whereby androgen treatment caused regression of DMBA-induced rat mammary carcinomas (Zava and McGuire 1977) or delayed their onset (Simanainen et al. 2012). In non-human primates, blockade of endogenous androgen activity enhanced oestrogen-induced proliferation of mammary epithelial cells and treatment with testosterone inhibited oestrogen mediated mammary cell proliferation. Data regarding the inhibitory effect of androgens in pre-clinical models of breast cancer are supported by the efficacy seen clinically with androgen therapies such as

dihydrotestosterone (DHT) and fluoxymesterone in the treatment of metastatic breast cancer, where disease regression was reported in up to 30% of cases (Adair and Herrmann 1946; Kennedy 1958; Goldenberg and Hayes 1961; Goldenberg 1964). More recently a retrospective study reported a clinical benefit rate of 58.5% with testosterone in women with ER positive MBC who had progressed on ER-directed therapies (Boni et al. 2014).

The androgen receptor (AnR) mediates the biological effects of androgens, and mechanistically ligand activation of AnR *in vivo* has been shown to decrease expression of ER over time (Zhou et al. 2000) and actively inhibit ER-mediated transcriptional activity (Peters et al. 2009). These mechanisms offer plausible explanations for the anti-oestrogenic, growth-inhibitory effects of androgens (Birrell et al. 1995; Hickey et al. 2012). Furthermore, clinical efficacy of a synthetic AnR agonist medroxyprogesterone acetate (MPA) was only seen in women with tamoxifen-resistant breast cancer that had a wild-type AnR with a lack of clinical benefit in women with inactivating AnR mutations (Buchanan et al. 2005).

Androgen Receptor and ER-Positive Breast Cancer

AnR is the most prevalent sex steroid receptor in primary and metastatic breast cancers occurring in up to 85% of primary and 75% of metastatic disease (Honma et al. 2012; Lea, Kvinnsland, and Thorsen 1989; Park et al. 2010). However, the frequency of AnR expression varies between BC subtypes, with ER positive cancers significantly more likely to be AnR positive as compared to ER negative cancers (Gonzalez-Angulo et al. 2009; Hu et al. 2011; Park et al. 2010; Peters et al. 2009; Yu et al. 2011; Loibl et al. 2011; Micello et al. 2010; Niemeier et al. 2009). Some studies have reported AnR expression based on luminal sub-types with luminal A cancers expressing AnR more frequently than luminal B (Yu et al. 2011; Collins et al. 2011). Furthermore, AnR expression in ER positive cancers has been associated with favourable clinico-pathological characteristics such as older age at diagnosis, lower tumour grade, lower Ki67 positivity, smaller tumours and less necrosis (Castellano et al. 2010; Hu et al. 2011; Witzel et al. 2013), and has been shown to independently predict outcome in ER positive disease. (Peters et al. 2009; Park et al. 2010; Gonzalez-Angulo et al. 2009). AnR expression was also associated with improved overall survival for women with ER positive BC in two meta-analyses (Qu et al. 2013; Vera-Badillo et al. 2014).

Pre-Surgical Windows of Opportunity Studies

Pre-surgical window-of-opportunity studies are a validated strategy to evaluate novel therapies in ER positive BC and can help to characterise the optimal target population (Dowsett et al. 2011). Such studies enable access to breast cancer tissue before, on and after treatment for pharmacodynamic and correlative translational studies. Such studies provide important information amongst others on molecular mechanism of sensitivity and resistance, aiding the identification of the optimal patient group as well as comparing the relative activity of different agents both alone and in combination.

In the first endocrine therapy preoperative window of opportunity study a short period of tamoxifen as compared to placebo was shown to cause a significant reduction in the proliferative marker Ki67 (Clarke et al. 1993). Subsequently AI treatment was shown to be associated with a greater suppression of Ki67 as compared to tamoxifen (Harper-Wynne et al. 2002; Dowsett et al. 2005; Ellis et al. 2003). The neoadjuvant IMPACT study went on to demonstrate that changes in the expression of Ki67 after 2 weeks of endocrine therapy to be closely linked with relapse free survival (Dowsett et al. 2011).

Furthermore, the data from this small and short study mirrored the results in the larger adjuvant ATAC trial, where suppression of Ki67 at 2 weeks was greater with anastrozole than with either tamoxifen or the combination of anastrozole plus tamoxifen (Baum et al. 2003). Therefore, changes in Ki67 expression in the context of window of opportunity studies can be utilised as a pharmacodynamic biomarker of effectiveness of novel agents and aid in decision making with regard to further drug development.

Aside from endocrine therapy, windows of opportunity studies have tested amongst others ('Cancer Research UK. Cancer Stats Key Facts Breast Cancer.' 2013) the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, erlotinib, which indicated activity in ER positive breast cancer outside of the EGFR overexpressing group (Perou et al. 2000; Guix et al. 2008) celecoxib, demonstrating very modest, effect on Ki67 over a 2 week period in ER positive disease, providing evidence against its acting as an aromatase suppressant (Martin et al. 2010), and (Carey et al. 2006) tyrosine kinase inhibitor, lapatinib, in HER2 negative tumours indicating antiproliferative activity in a subgroup of HER2 negative nonamplified tumours characterised by high HER3 expression (Leary et al. 2015). Pre-surgical window of opportunity studies have now become established as an accepted approach to investigate the efficacy of novel agents, and in helping decision making around subsequent drug development.

Selective androgen receptor modulators (SARMs)

Despite the therapeutic benefits of androgen therapy for BC, the strategy fell from use due to virilising side effects, concerns regarding aromatization to oestrogen, and the advent of efficacious ER-directed therapies, namely tamoxifen and later, aromatase inhibitors (Cole, Jones, and Todd 1971; Coombes et al. 1984). Furthermore, the initial use of androgen therapy predated knowledge regarding the expression and mechanistic actions of AnR in ER positive BC. However, the development of selective androgen receptor modulators (SARMs) offers a novel approach for therapeutically targeting AnR in women. SARMs have a high specificity for binding to AnR, and have the advantage that they dissociate the anabolic from androgenic effects of AnR, and therefore lack the virilising side effects seen with previously employed androgens (Mohler et al. 2009; Chen, Kim, and Dalton 2005). Given their inability to be aromatised or 5-α reduced, SARMs do not generate oestrogenic and androgenic metabolites (Coombes et al. 1984; Chen, Kim, and Dalton 2005). ***Several molecular mechanisms contribute to the observed tissue selectivity of SARMs – these include differences in the three-dimensional conformation of the AnR ligand binding domain, coactivator and corepressor recruitment, nongenomic signalling, and gene regulation induced by SARMs as compared to testosterone or other steroidal androgens*** (Bohl et al. 2005; Kazmin et al. 2006; Narayanan, Coss, et al. 2008).

Enobosarm

Enobosarm/GTx-024/Ostarine®,(S)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2 hydroxy-2-methylpropanamide (GTx Inc., Memphis, TN, USA) is an oral aryl-propinamide non-steroidal SARM, which binds and activates AnR with enantioselective affinity, potency and efficacy similar to DHT (Kim et al. 2005; Narayanan et al. 2013). Enobosarm has the advantages of having selective anabolic activity, lacking androgenic activity, and it cannot be converted to oestrogenic or androgenic metabolites (Chen, Kim, and Dalton 2005; Narayanan, Mohler, et al. 2008; Coss, Jones, and Dalton 2014). Transfection studies using oestrogen receptors (i.e. ERα and ERβ), glucocorticoid receptor, and mineralocorticoid receptor demonstrate that enobosarm has neither the ability to

stimulate agonist activity through these receptors nor the ability to inhibit the activity of their endogenous agonists (i.e. oestradiol, cortisol, or aldosterone respectively) at concentrations up to 1 µM. The underlying hypothesis regarding the selectivity of enobosarm is that while it binds to the AnR with similar affinity as testosterone, it induces conformational changes upon binding, which selectively alters the interaction of the receptor with coactivator and corepressor proteins that exist in different tissues and the ability of the receptor to regulate gene expression. The differences in the coactivators and corepressors that exist in the different tissues, coupled with the different conformation of the enobosarm/receptor complex and the steroid/receptor complex, result in a different mix of genes being turned on and off and confer more selective anabolic activities. Differences in intracellular signalling pathways (i.e. non-genomic effects) and/or interactions with steroid biosynthetic enzymes (e.g. 5α-reductase) between enobosarm and the steroids may also contribute to differences in selectivity. As a nonsteroidal SARM, enobosarm is not a substrate for aromatase or 5α-reductase and thus cannot be converted to oestradiol or dihydrotestosterone, respectively.

Enobosarm and its structurally related SARM, GTx-027 (one atom difference), have demonstrated antiproliferative activity in ER positive cell lines, which included ZR-75-1 and MCF-7 cells, and in a xenograft model of MCF-7 overexpressing AnR (Narayanan et al. 2013). Microarray analysis of MCF-7 AnR positive xenografts indicated induction of antiproliferative genes (Narayanan et al. 2013). GTx-027 as well as increasing well-known AnR-regulated genes, such as KLK3, SNAI2, and MUC1, also inhibited several ER target genes such as progesterone receptor (PR), ER, and pS2 (Narayanan et al. 2013). *In vivo* studies of intact and castrated rats have shown that enobosarm has anabolic and myoanabolic effects but lacks androgenic effects (Kim et al. 2005). Given the inability of enobosarm to undergo conversion by 5α-reductase or aromatase no oestrogenic or androgenic metabolites result (Mohler et al. 2009). Two phase II clinical trials have demonstrated the ability of enobosarm to significantly increase lean body mass and physical function without the androgenic side effects (Dobs et al. 2013; Dalton et al. 2011).

Clinical Data

Enobosarm has been evaluated in 1043 subjects and patients have been enrolled into 23 completed and ongoing clinical trials evaluating enobosarm. These studies are summarised in Table 1 below.

TABLE 1 - Summary of clinical studies with GTx-024

Protocol Number	Design/Objective	Subject/Patient Population
G100401	A Randomized Double-blind, Placebo-controlled, Phase I Study to Assess the Safety, Tolerability, Pharmacokinetics of Single Doses of GTx-024	96 healthy young males 19-45 years of age GTx-024, N=72; Placebo, N=24
G100402	A Randomized Double-blind, Placebo-controlled, Phase I Study to Assess the Safety, Tolerability, Pharmacokinetics of Multiple Doses of GTx-024	59 healthy young males 19-45 years of age 12 elderly males with truncal obesity (N=71 unique subjects) GTx-024, N=56; Placebo, N=17
G200501	A Randomized, Double Blind, Placebo Controlled, Multiple Dose Study to Assess the	60 healthy postmenopausal females 45 to 70 years of age 60 healthy elderly men over 60

	Safety, Tolerability, Pharmacokinetics, and Pharmacodynamic Efficacy of GTx-024 in Healthy Postmenopausal Women and Elderly Men	years of age GTx-024, N=96; Placebo, N=24
G100503	A "divided dose" (ten 0.3mg doses) to simulate a sustained release formulation was compared to a single 3mg dose of GTx-024 for the differences in pharmacokinetics and effect on clinical chemistries	18 healthy male volunteers ≥50 years of age 18 healthy postmenopausal female volunteers GTx-024, N=24; Placebo, N=12
003	A Randomized, Double Blind, Double Dummy, Placebo Controlled, Multiple Dose Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamic Efficacy of MK-3984 and GTx-024 in Healthy Postmenopausal Women	102 healthy postmenopausal females 45 to 75 years of age GTx-024, N=25; Placebo or other, N=77
006	Double blind, randomized, placebo controlled single and multiple dose study of GTx-024 in Japanese Women.	12 postmenopausal women of Japanese descent. GTx-024, N=9; Placebo, N=3
G200502	Phase IIb Proof-of-Concept Study in Patients with Cancer	159 patients with non-small cell lung cancer, colorectal cancer, non-Hodgkin's lymphoma, chronic lymphocytic leukemia and breast cancer GTx-024, N=107; Placebo, N=52
G100506	Phase I, Relative Bioavailability and Food Effect Study of GTx-024	27 healthy young male volunteers aged 19 to 45 years GTx-024, N=27;
G100507	Phase I Study to Assess the Pharmacokinetics and Absolute Oral Bioavailability of GTx-024 in Caucasian and African American Men and Women	48 healthy subjects (12 Caucasian males, 12 African American males, 12 Caucasian females, and 12 African American females)
G100508	Phase I study to assess the effect of mild and moderate hepatic impairment on the pharmacokinetics of GTx-024	24 subjects (8 with mild hepatic impairment, 8 with moderate hepatic impairment and 8 healthy) GTx-024, N=24; Placebo, N=0
G100509	Phase I mass balance study to determine the metabolism and excretion of radiolabeled GTx-024 in healthy volunteers	6 healthy male subjects between the ages of 21 and 65 years of age GTx-024, N=6;

G100510	A Single-Dose, Randomized, Double-Blind, Comparative, Positive and Placebo-Controlled, Four-Period Crossover Study to Define the ECG Effects of GTx-024, at Therapeutic and Supratherapeutic Doses, in Healthy Male and Female Subjects: A Thorough ECG Trial	54 healthy male and female subjects (approximately 27 females and 27 males) that were ≥ 18 and ≤ 45 years of age were enrolled in the study.
G100511	Phase I study to assess the effect of severe renal impairment on the pharmacokinetics of GTx-024	16 subjects (8 with severe renal impairment and 8 healthy). Healthy subjects are non-smokers between 19 and 75 years of age for males and PMP women age ≥30 years GTx-024, N=16; Placebo, N=0
G100512	Phase I Study to Assess the Effects of Itraconazole on the Pharmacokinetics of GTx-024	12 healthy males aged 19 – 55 years were enrolled into this study.
G100513	Phase I Study to Assess the Effects of Rifampin on the Pharmacokinetics of GTx-024	12 healthy males aged 19 – 55 years were enrolled into this study.
G100514	Phase I Study to Assess the Effects of GTx-024 on the Pharmacokinetics of Celecoxib	42 healthy males aged 19 – 55 years were enrolled into this study.
G100515	Phase I Study to Assess the Effects of Probenecid on the Pharmacokinetics of GTx-024	16 healthy males aged 19 – 55 years were enrolled into this study.
G100516	Phase I Study to Assess the Effects of GTx-024 on the Pharmacokinetics of Rosuvastatin	50 healthy males aged 19 – 55 years were enrolled into this study.
G300504	Phase III, randomized, double-blind, placebo-controlled study of the effect of GTx-024 on muscle wasting in patients with non-small cell lung cancer on first line platinum plus a taxane chemotherapy	321 men and postmenopausal women with Stage III or IV non-small cell lung cancer GTx-024 3.0mg/day, N=160; Placebo, N=161
G300505	Phase III, randomized, double-blind, placebo-controlled study of the effect of GTx-024 on muscle wasting in patients with non-small cell lung cancer on first line platinum plus a non-taxane chemotherapy	330 men and postmenopausal women with Stage III or IV non-small cell lung cancer GTx-024 3.0mg/day, N=165; Placebo, N=165

G200801	Phase II, Open Label Study to Examine Androgen Receptor Status and the Activity of GTx-024 Hormonal Therapy in Women with ER Positive Metastatic Breast Cancer Who Have Previously Responded to Hormone Therapy	22 female subjects with oestrogen receptor (ER) positive metastatic breast cancer and who have responded previously to hormone therapy. GTx-024 9.0mg/day
G200802	Phase II Open Label, Multi-Center, Multinational, Randomized, Parallel Design Study Investigating the Efficacy and Safety of GTx-024 on Metastatic or Locally Advanced ER Positive/Androgen Receptor Positive (ER+/AR+) Breast Cancer (BC) in Postmenopausal Women	36-88 post-menopausal female subjects with metastatic or locally advanced ER+/AR+ BC Randomized to GTx-024 9.0 mg or 18 mg daily for up to 24 mo
G200901	Phase II Open Label, Multi-Center, Multinational Study Investigating the Efficacy and Safety of GTx-024 on Advanced, Androgen Receptor-Positive Triple Negative Breast Cancer (AR+ TNBC)	41 female subjects with AR+ TNBC GTx-024 18 mg daily for up to 12 mo

Drug Metabolism

The *in vitro* metabolism of [14C]enobosarm was studied in human hepatocytes and shown to be via direct glucuronidation on the tertiary alcohol of enobosarm to form enobosarm glucuronide. This major metabolite does not significantly interact with receptor subtypes *in vitro* at the screening concentration of 10µM, indicating that it is an inactive metabolite.

The relative contributions of various cytochrome P450 isoforms to the oxidative metabolism of enobosarm were investigated through *in vitro* incubations with recombinant human CYP1A2, CYP2C9, CYP2C19, CYP3A4, and CYP2D6. Incubation of enobosarm with CYP3A4 and CYP2C19 produced very small amounts of singly oxidized metabolites, while incubations of enobosarm with CYP1A2, CYP2C9, and CYP2D6 resulted in no detectable metabolites. These results suggest that cytochrome P450-mediated oxidation is a relatively minor pathway for enobosarm metabolism.

Phase II metabolism of enobosarm was evaluated in human liver microsomes and S9 fraction and with recombinant human UDP-glucuronosyl transferase (UGT) isoenzymes. Incubations of GTx-024 with UGT1A1 and UGT2B7 showed an increase in the glucuronide metabolite, whereas UGTs 1A4, 1A6, 1A8, 1A10 and 2B15 did not generate the glucuronide product. These *in vitro* results suggest that UGT1A1 and UGT2B7 might be the primary UGT isoforms responsible for the Phase II biotransformation of enobosarm *in vivo*.

Inhibition of Human Liver Microsomal CYP450 Isoforms

The ability of enobosarm to inhibit the activity of individual cytochrome P450 enzymes 1A2, 2C9, 2C19, 2D6, and 3A4 was evaluated using human recombinant CYP450 isozymes. Enobosarm significantly inhibited CYP450 2C19 and 2C9 with an IC50 of approximately 0.7 and 1.6 µM, respectively, but did not appreciably inhibit the other P450 enzymes tested over the range of 0.15 to 20.0 µM. Studies using human liver microsomes indicated that enobosarm is only a moderately weak inhibitor of CYP2C19 with a Ki of 8.9 µM. Based on the cumulative *in vitro* data (IC50 and Ki) for enobosarm against CYP2C9 and 2C19, the probability of any clinical drug:drug interaction in patients administered GTx-024 at doses of up to 3mg would be considered remote, while a drug:drug interaction with a CYP2C9 substrate or inhibitor may be possible at a dose of 9mg.

Induction of CYP450 Isoforms in Human Primary Hepatocytes

An increased activity was noted for CYP3A4 when incubated with enobosarm, with an IC50 >10 µM. The activities of CYP2D6 and 1A2 were not affected by 10 µM enobosarm.

Pharmacokinetics (PK)

The pharmacokinetics of enobosarm were evaluated in two double-blind, placebo-controlled, phase I studies, a single dose (Protocol G100401) and dose ascending (Protocol G100402) study involving 155 healthy young males and 12 elderly males with truncal obesity. In protocol G100401 a single oral dose of 1mg to 100mg in 48 healthy males resulted in increases in plasma concentrations and exposures to enobosarm. PK was linear and proportional, and the half-life of enobosarm was between 21 and 24 hours. (Jones et al., Drug of the future 2013; 38:309-316). In the multiple-ascending dose study (Protocol G100402) enobosarm was administered at doses of 1.0mg, 10mg, 3.0mg and 30mg to healthy male volunteers and at doses of 3.0mg and 30mg to elderly volunteers with truncal obesity. Each dose was administered for a period of 14 days. Increases in plasma concentrations and exposures to enobosarm were observed with increasing dose at steady state. Maximum plasma concentrations after a dose of enobosarm were achieved at approximately one hour, steady state was reached prior to Day 6 and the terminal half-life ranged from 14 to 21 hours. Mean maximum plasma concentrations, time to maximum plasma concentration and exposure were similar in young and elderly subjects (Jones et al. 2013). Within this study there was a dose dependent reduction in the serum levels of sex hormone binding globulin (SHBG), with a reduction in total testosterone but no clinically significant change in free testosterone was observed (GTx 2015). No clinically apparent adverse effects involving the skin or prostate were documented (GTx 2015). The most common treatment emergent adverse effect was elevated ALT outside the normal range, there appeared to be a dose dependent rise in mean ALT following enobosarm administration with peak mean elevations noted on days 7 and 14. Mean AST values remained within the reference range for all study groups although there was a notable increase in mean AST values in the 30mg dose cohort. Other documented adverse events (AE) were headaches and constipation (GTx 2015).

A subsequent double-blind, placebo-controlled, Phase I study assessed the PK and safety of 14 days of enobosarm as a 3mg once daily dose and as a 'divided dose' (ten 0.3mg doses) to simulate a sustained release formulation in 18 healthy males and 18 healthy postmenopausal females (Protocol G100503). The overall exposure to enobosarm was similar following ten divided doses of 0.3mg as compared to a single oral dose of 3mg as measured by $AUC_{(0-24)}$, while peak concentrations as measured by C_{max} were lower. Treatment-emergent AEs reported in more than 10% of patients

included headache, myalgia, mild insomnia, nausea, mild rhinorrhea, mild abdominal distension and mild back pain. Three patients experienced ALT elevation (≤ 2.9 x ULN) on Day 7 which returned to normal levels by day 21 (GTx 2015)

In a mass balance study to determine the metabolism and excretion of radiolabelled enobosarm (Protocol G100509), healthy men received a single oral dose of 14C-enobosarm. The parent compound [14C] enobosarm and M6, the glucuronide metabolite, were the major circulating radioactive components in plasma following a single oral dose. The oxidation metabolite, M8, was a minor component in plasma. The main route of excretion was the urine, accounting for 80.8-97.2% of the administered dose, with M6 the predominant radioactive component detected in the urine, enobosarm could only be detected by mass spectrometry. The minor route of excretion was in the faeces, accounting for 3.8-16.1% of the administered dose. [14C] enobosarm was the major component observed in faeces, accounting for 3.8-14.9% of the administered dose. Final pharmacokinetic data is not yet available from this study.

In a single dose crossover relative bioavailability and food effect study (Protocol G100506). Hardshell capsule and softgel formulation of enobosarm were shown to be bioequivalent with regard to rate and extent of absorption. Food had no effect on the extent of absorption of enobosarm in softgel formulation as demonstrated by the $AUC_{(0\text{-}inf)}$ values. However, a reduction in C_{max} was observed with food compared to the fasting state.

PK studies in male and females Caucasians and African-American (Protocol G100507) have shown no significant differences in oral bioavailability following the oral administration of 3mg enobosarm. While a study in postmenopausal Japanese women (Protocol 006) demonstrated that multiple dose PKs of 3mg enobosarm in this group were similar to those previously observed in non-Japanese subjects (Study G100503).

The pharmacokinetics of enobosarm was assessed in patients with mild (Child-Pugh classification score 5 to 6) and moderate hepatic impairment (Child-Pugh classification score 7 to 9) following a single 3mg dose of enobosarm. There was no statistically significant effect of hepatic impairment on the systemic exposure of enobosarm following a single 3.0mg dose of GTx-024 in subjects with mild or moderate hepatic impairment when compared to subjects with normal hepatic function.

The pharmacokinetics of enobosarm was assessed in patients with severe renal impairment (creatinine clearance <30 mL/min) and matched control subjects with normal renal function (creatinine clearance ≥80 mL/min) following a single 3mg dose of enobosarm. The plasma protein binding of enobosarm was not altered in subjects with severe renal impairment. While final PK data is not yet available from this study, preliminary PK data for enobosarm glucuronide suggest that plasma concentrations of this metabolite were about 6-fold higher in subjects with severe renal impairment compared to subjects with normal renal function. Given that enobosarm glucuronide has no pharmacologic activity, that glucuronides are commonly eliminated by renal mechanisms, and the observed safety of enobosarm in this study, suggests that the dose of enobosarm does not need to be adjusted in patients with renal impairment. Moreover, preliminary analyses suggest that the PK of enobosarm in subjects with severe renal impairment did not differ significantly from control subjects with normal renal function.

In a drug-drug interaction study in healthy male volunteers (protocol G100512), a single 3mg oral dose of enobosarm was administered either alone or after repeated daily doses of 200mg itraconazole, (CYP3A4 inhibitor). Mean maximum (based on C_{max}) and overall (based on AUC_{0-t}, AUC_{0-24}, and AUC_{∞}) of plasma enobosarm exposures following a single dose of enobosarm were not affected by co-administration of itraconazole. Median T_{max} and mean T1/2 values of enobosarm were also similar following enobosarm alone or in combination with itraconazole. Similarly, PK of plasma enobosarm glucuronide were not affected by co-administration of itraconazole.

In a drug-drug interaction study in healthy male volunteers (protocol G100513), a single 3mg oral dose of enobosarm was administered alone or after repeated daily doses of 600mg rifampin, (CYP3A4 inducer). Co-administration of enobosarm with rifampin lowered the peak concentrations and exposure to enobosarm by all measures. The mean plasma C_{max} value for enobosarm following administration of enobosarm and rifampin was 22% lower compared to that observed following administration of enobosarm alone. Mean plasma AUC_{0-t}, AUC_{0-24}, and AUC_{∞} for enobosarm were also lower (approximately 30 to 45% lower) when enobosarm was co-administered with rifampin relative to when enobosarm was administered alone. Median T_{max} following administration of enobosarm and rifampin occurred approximately 1 hour later and mean $T_{½}$ more than 5 hours shorter when compared to when enobosarm was administered alone. Similarly, peak concentrations and exposure to enobosarm glucuronide were lower by all measures when enobosarm was co-administered with rifampin.

In a drug-drug interaction study in healthy male volunteers (Protocol G100514) a single 200mg oral dose of celecoxib (CYP2C9 substrate) was initially administered alone, followed by a single 200mg oral dose of celecoxib with repeated daily doses of enobosarm 3.0mg. Multiple doses of enobosarm did not affect the single-dose PK of oral celecoxib. A single dose of celecoxib did not affect the multiple-dose PK of enobosarm and enobosarm glucuronide. These results suggest that there is no DDI between celecoxib and enobosarm.

In a drug-drug interaction study in healthy male volunteers (protocol G100515), a single 3mg oral dose of enobosarm was administered either alone or after repeated twice daily doses of 500mg probenecid (UDP glucuronosyl transferase inhibitor). Co-administration of probenecid produced 6%, 39%, and 49% increases in enobosarm AUC_{0-24}, AUC_{0-t}, and AUC_{0-inf}, respectively, compared to treatment with enobosarm alone with a slightly lower C_{max}. Enobosarm glucuronide AUC values increased by ~38%, 97%, and 112% for AUC_{0-24}, AUC_{0-t}, and AUC_{0-inf}, respectively, after enobosarm with probenecid compared to treatment with enobosarm alone.

In a drug-drug interaction study in healthy male volunteers (protocol G100516), a single 10mg oral dose of rosuvastatin (BCRP Substrate) was administered either alone or during repeated daily doses of 3mg enobosarm. Mean maximum (based on C_{max}) and overall (based on AUC_{0-24}, AUC_{0-t}, and AUC_{∞}) plasma rosuvastatin exposures were approximately 24% – 37% higher when rosuvastatin was co-administered with enobosarm compared to when rosuvastatin was administered alone. Mean plasma rosuvastatin $T_{1/2}$ values and median plasma rosuvastatin T_{max} values were not affected on co-administration of rosuvastatin with enobosarm. Co-administration of a single dose of rosuvastatin

with multiple doses of enobosarm did not alter the PK of enobosarm or enobosarm glucuronide compared to multiple doses of enobosarm alone.

Clinical Data

Effect on lean body mass and physical function

A 12 week double-blind, placebo-controlled phase II study (protocol G200501) involving 120 healthy men (>60 years of age) and postmenopausal women investigated the effect of enobosarm on total lean body mass at five different dose levels (doses of 0.1mg, 0.3mg, 1mg and 3mg) (Dalton et al. 2011). This study demonstrated a significant dose-dependent increase in total lean body mass (P<0.001, 3mg vs. placebo) and resistance (P=0.013, 3mg vs. placebo). There was also an improvement in physical function as measured by changes in stair climb power. Changes in circulating steroidogenic hormones were also measured within the study, and in male subjects no statistically significant differences in free testosterone, DHT, oestradiol, follicle-stimulating hormone (FSH), or luteinizing hormone (LH) from baseline values as compared to placebo. However, sex hormone binding globulin (SHBG) was significantly reduced with enobosarm, 3mg versus placebo, and this decrease in SHBG was accompanied by a significant reduction in serum total testosterone with 1mg or 3mg dose of enobosarm. While in female subjects free testosterone, total testosterone, DHT, and oestradiol levels did not differ between enobosarm and placebo. There was however a significant reduction in LH and FSH with 3mg dose of enobosarm, as well as with SHBG at 1mg and 3mg dose compared to placebo. (Dalton et al. 2011). Significant reductions in both glucose and insulin resistance were documented at 3mg and 1mg/3mg respectively. Changes in plasma lipids were also measured, compared to placebo there was a non-significant reduction in serum triglycerides at 1mg and 3mg-dose and a statistically significant reduction in total cholesterol was observed at 0.3mg, 1mg, and 3mg dose groups. While there was no significant effect on low-density lipoprotein (LDL) observed among treatment groups, there was a significant dose-dependent reduction in high-density lipoprotein (HDL). Enobosarm was well tolerated with the most common adverse events reported being headache and back pain. No serious adverse events (SAE) were reported during the study. With regard to haematological and biochemical changes on enobosarm, small, statistically significant increases in haemoglobin and hematocrit were observed with enobosarm 3mg compared to placebo, as well as transient increases in transaminases. Elevation in transaminases to above the upper limit of normal were documented in eight subjects, with resolution in seven of eight subjects while on drug. In one subject enobosarm was discontinued due to an elevation in ALT to 4.2 times the upper limit of normal, this subsequently normalised after discontinuation of drug (Dalton et al. 2011)**.**

A subsequent randomised, double-blind, placebo controlled phase II trial recruited patients diagnosed with NSCLC, colorectal cancer, non-Hodgkin lymphoma, chronic lymphocytic leukaemia, or breast cancer who had had at least a 2% weight loss in the 6 months before entry into the study (protocol G200502). Patients were randomised to receive once-daily oral enobosarm 1mg, 3mg, or placebo for up to 113 days (Dobs et al. 2013). The primary endpoint was change in total lean body mass from baseline. Secondary endpoints included effects on total bodyweight, physical function, bone turnover markers, total body fat mass, hair growth, prostate-specific antigen, haemoglobin and quality of life. A significant increase in total lean body mass at 1mg (median 1.5 kg, range –2.1 to 12.6, p=0.0012) and 3mg doses. 1.0 kg, –4.8 to 11.5, p=0·046) (Dobs et al. 2013). Both stair climb time and power were significantly better in the two treatment arms compared to placebo. The drug was tolerated at the 1mg and 3mg dose levels, most AEs were grade 1 and 2, the most common in the placebo group were

fatigue and nausea; in the enobosarm 1mg group were nausea, anaemia and constipation, and in the enobosarm 3mg group were fatigue, diarrhoea and cough. Three patients in the enobosarm 3mg treatment group had transient (returning to normal while still on drug) ALT increases that were two to three times the upper limit of normal; one patient each in the enobosarm 3mg and placebo groups had a transient ALT increase to more than three times the upper limit of normal. There were no discontinuations because of increases in ALT.

POWER Studies

Given the observed benefits of enobosarm on total lean body mass (LBM) and physical function in cancer patients, two phase III studies investigating the effect of enobosarm in patients with non-small cell lung cancer (NSCLC) were initiated, these were entitled 'Prevention and treatment Of muscle Wasting in patiEnts with Cancer1 [(POWER1) (NCT01355484) and POWER 2(NCT01355497)] (NCT01355484). Both studies recruited patients at the time of initiation of first-line chemotherapy for stage III/IV NSCLC, patients being randomised to receive placebo or enobosarm 3mg for 147 days beginning at the time that they initiated chemotherapy [POWER1 (platinum and paclitaxel or docetaxel) and POWER2 (platinum and gemcitabine, pemetrexed, or vinorelbine)), 321 and 320 patients were recruited respectively. The trials had identical co-primary endpoints of total LBM response and physical function response for enobosarm vs. placebo after 3 months of treatment, with response (clinical benefit) defined as no loss or a gain of total LBM and at least a 10% increase in stair climb power, respectively, at day 84. Secondary endpoints for the trials include survival for safety, durability of benefit in LBM and stair climb power at 147 days, tolerability to chemotherapy, and quality of life (Dalton et al. 2013). In both POWER1 and POWER2 enobosarm resulted in significant increases in LBM compared to placebo at day 147. The incidence of adverse events was similar between enobosarm and placebo in both trials (Crawford et al. 2014). However, statistically significant effects on physical function (stair climb power) were observed only in patients receiving taxane-based chemotherapy and enobosarm (POWER1) as compared to placebo (Dalton et al. 2013).

Breast Cancer

While enobosarm at a dose of 3mg was chosen for its anabolic activity in muscle in the completed POWER studies, a dose of 9mg once daily of enobosarm was selected for use as endocrine therapy in this study in order to achieve a higher exposure that is both safe and more likely to be efficacious in women with advanced BC. While data on reductions in sex hormone binding globulin (SHBG), one of the most sensitive serum biomarkers for AnR signalling, from protocol G100402 supports the use of a dose beyond 3mg. Within G100402 SHBG was reduced by 15.1%, 15.6%, 18.2%, and 18.4% in young, healthy volunteers who received oral enobosarm 1mg, 3mg, 10mg, and 30mg daily for 14 days respectively, these data suggest that doses above 3mg maximally stimulate AnR activity.

Given this a dose of 9mg was taken forward in a proof of concept open label phase II study in post-menopausal women with ER positive MBC (Protocol G200801;NCT01616758). This study assessed the safety and efficacy of enobosarm at a dose of 9mg daily in postmenopausal women with ER positive metastatic breast cancer who had previously been treated with endocrine therapy. The primary endpoint was clinical benefit rate (complete response + partial response + stable disease). Patients to be considered eligible for the study had to have been receiving adjuvant therapy for ≥ 3 years or their most recent endocrine therapy for metastatic disease for ≥ 6 months prior to progression. Twenty two subjects were enrolled into this study (Overmoyer et al. 2014). Of the AnR positive patients 35% derived clinical benefit, with the current six month Kaplan-Meier estimate of progression free survival

being 40.1% (95% CI: 18.1% -62.1%). Correlative work within this study suggests that serum PSA maybe a surrogate marker for AnR activity (Overmoyer et al. 2014). The drug at 9mg appeared safe with 95% of adverse events recorded being grade 1/2, and included pain, fatigue, nausea, hot flashes/night sweats, arthralgia and anxiety (50). The ALT rises documented with enobosarm in this study and others may be related to ALT regulation by androgens in non-hepatic tissue (Coss et al. 2012). Additional information on the PK and drug metabolism of enobosarm as well as clinical studies and safety data is provided in the enobosarm Investigator's Brochure (GTx 2015).

2.2 Rationale

Hypothesis

Enobosarm provides an opportunity to reinstate a therapeutic strategy previously shown to have clinical benefit in BC using a novel contemporary agent that lacks the negative features of the previous androgenic compounds. In the current study in early breast cancer we hypothesize that in ER positive early BC that selective activation of AnR will lead to a reduction in tumour cell proliferation. We hypothesise that in early, untreated ER positive, AnR positive BC, that targeting AnR with the SARM, enobosarm, for 2 weeks prior to surgery/research core biopsy will result in clinical activity. This will be demonstrated by a significant reduction in the proliferation marker Ki67 as measured by immunohistochemistry.

Scientific Rationale for targeting AnR in ER positive cancers

- Endocrine therapy is a key treatment in the management of ER positive breast cancer
- The efficacy of endocrine theory is limited by both de novo and acquired resistance and this remains a major clinical challenge in ER positive breast cancer.
- Approximately 70-80% of ER positive breast cancers also express the AnR.
- Historically, androgens were an effective agent in the management of breast cancer. Their side effect profile and the advent of other endocrine therapies resulted in them falling from use
- Preclinical and clinical data supports a role for AnR in antagonising the proliferative effects of ER in ER positive breast cancer

Rationale for enobosarm

- Enobosarm is a selective androgen receptor modulator (SARM)
- It has demonstrated non-clinical tumour activity in ER positive breast cancer models
- Phase II studies have demonstrated clinical activity by enobosarm in ER positive metastatic breast cancer.
- It has demonstrated an acceptable toxicity profile in clinical studies and does not have the side effects associated with androgens.

Rationale for the use of treatment during the short-preoperative window

- Short-term preoperative studies are validated strategy to gain insight and evidence with regard to the biological activity of novel agents in breast cancer.
- Offers the potential to help streamline and prioritize the development of novel agents.
- Short-term preoperative/pre-treatment studies do not delay breast surgery/chemotherapy/endocrine therapy given the time between the diagnosis breast cancer and definitive breast surgery/chemotherapy/endocrine therapy is generally 2-4 weeks.
- Enables access to the breast cancers before and after treatment allowing pharmacodynamic and correlative studies to be carried out which can help define the optimal patient group as well as identify mechanisms of sensitivity and resistance.

Rationale for Ki67 endpoint

- Enobosarm has been shown to inhibit tumour cell proliferation.
- Ki67 expression can be easily measured by IHC (using the MIB-1 antibody) and is a validated biomarker of tumour cell proliferation.
- A set of international criteria have been set out to ensure its optimal use in clinical trials.
- Prospective clinical trials have demonstrated that 2-week preoperative therapy with an AI or tamoxifen markedly reduces breast cancer cell proliferation as measured by Ki67
- A highly significant relationship between 2-week Ki67 expression and relapse free survival has been confirmed on multivariate analysis

2.3 Trial Objectives

2.3.1 Primary objective

- To determine whether enobosarm reduces Ki67 proliferation index in comparison with no treatment.

2.3.2 Secondary objective:

- To determine the effects of enobosarm on tumour-cell apoptosis
- To determine the effects of enobosarm on the expression of ER and AnR-regulated genes
- To evaluate changes in circulating steroidogenic hormones after enobosarm administration
- To evaluate changes in PSA after enobosarm administration
- To determine the safety and tolerability of enobosarm in this population

2.3.3 Exploratory Objectives

To explore whether subgroups based on Ki67 response to enobosarm can be identified in molecular profiles performed in pre-treatment samples.

To characterize the molecular effects of enobosarm and to evaluate potential biomarkers that may help predict response to enobosarm.

To assess the association of estrogenreceptor, progesterone receptor and HER-2 receptor by AR status (either negative or positive) in all patients who were stained for AR as part of the screening procedure for the study.

2.4 Potential Risks and Benefits

2.4.1 Potential Risks

GTx-024 has generally been well-tolerated in clinical trials conducted to date. Certain AEs associated with GTx-024 may occur. The most commonly reported side effects (≥0.5%) for GTx-024 in the previous 23 clinical studies include headache, nausea, alanine aminotransferase increased, diarrhoea, dizziness, back pain, constipation, vomiting, pain in extremity, hyperhidrosis, pruritus, somnolence, dyspnoea, fatigue, abdominal pain, hot flush, muscle spasms, myalgia, dizziness postural, insomnia and rash

The main potential risks with this study are:

- Enobosarm could theoretically stimulate proliferation in some tumours. This would be an important finding and once the subtype was formally identified would represent an exclusion from future enobosarm/SARM studies.
- The patient may experience side effects as a result of enobosarm.
- Patients as a result of providing translational blood samples may experience additional pain, bruising, infection or inflammation at the sample site, and/or feel faint during the blood collection procedure.

All those involved in the care of patients entered into the study will be experienced in clinical trials and managing toxicities of novel agents within the context of such studies.

Three Serious Adverse Events of hypercalcemia have been reported in the ongoing Phase II trial NCT02463032, two of which were felt to be possibly related to GTx-024 by the investigator. In all cases, the patients had ER positive breast cancer with bone metastases, and were being treated with the 18mg dose. No adverse events related to hypercalcemia were reported in previous trials with GTx-024. However, as the EMERALD trial will be using the 9mg dose over 14 (+4) days, with only patients with early breast cancer and no metastases, no serious adverse events relating to hypercalcemia are anticipated.

Furthermore, patients may be discontinued from treatment if there are any severe or life threatening toxicities. Patients will be informed of all of these facts in the Patient Information Sheet. In addition, all trials endorsed by the LCTC undergo a risk assessment review with the aim to put appropriate measures in place, such as monitoring and safety, to reduce any risks. The trial protocol and patient information sheet will also be reviewed by a Research Ethics Committee (REC) and the study will hold a clinical trial authorisation form issued by the MHRA.

2.4.2 Known Potential Benefits

No direct benefit is expected from participation in this study. However, it is hoped that this study will provide evidence that will enable the development of future studies with this agent and lead to the introduction of novel treatments for the management ER positive, AnR positive early breast cancer.

3 SELECTION OF CENTRES/CLINICIANS

Each participating centre (and PI) has been identified and selected for their expertise in breast cancer. A lead in each speciality at each site has been identified. Each centre will complete an EMERALD feasibility questionnaire which will identify any centre specific logistics in setting up or running the study and will ensure the centre is able to work to the current approved protocol.

3.1 Centre/Clinician Inclusion Criteria

a. Confirmation of local capacity and capability to conduct the study via the HRA
b. Be listed on the application given approval by MHRA
c. Be listed on the application given approval by REC
d. Completed and signed Research Site Agreement including material transfer clauses
e. Completion and return of Signature and Delegation Log to LCTC
f. Suitable MDT meeting structure to identify potential patients
g. Curriculum Vitae (CV) and a certificate of International Conference for Harmonisation of GCP (ICH-GCP) training- Principal Investigator (PI)
h. CV including a record of ICH GCP training – all other personnel on the delegation log
i. Clinical Study Protocol Receipt Form
j. IB Receipt Form
k. Patient Information Sheet, Informed Consent Form and GP Letter on local hospital trust headed paper
l. Attendance at site initiation training by PI, research nurse(s) and pharmacist, delegated individuals must also attend MACRO training for remote data entry
m. Local laboratory accreditation/quality check
n. Local laboratory reference ranges
o. Ability to recruit required number of patients
p. Sites should have the ability to perform MRI, ultrasound and mammogram scans on patients.
q. A centrifuge capable of generating 850g RCF
r. A freezer able to store samples at -80°C.
s. Ability to process blood samples
t. Able to provide FFPE tissue samples, sites will need a histology tissue processor, histology tissue cassettes and embedding moulds.
u. Sites will require an immunohistochemistry stainer, and the capacity to perform AR and ER receptor analysis on all patients where a breast cancer biopsy is taken.

3.2 Centre/Clinician Exclusion Criteria

Those centres that do not fulfil the above inclusion criteria will not be permitted to participate in the trial.

4 TRIAL DESIGN

4.1 Overall Design

This is a multicentre secondary/tertiary care setting, two-arm parallel group, open label, pre surgical "window of opportunity" randomised superiority trial, recruiting to a ratio of 3:1 (treatment: standard of care).

4.2 Primary Endpoint

Change in the proliferation marker Ki67 (% positive tumour cells) from baseline to after 2 weeks of treatment in the two arms.

4.3 Secondary Endpoint(s)

- Changes in cleaved caspase 3 from baseline to after 2 weeks of treatment
- Changes in the expression of PSA, Gross Cystic Disease Fluid Proteins (GCDFP)-24 &-15; PgR, GREB1, BCL2, TFF1/PS2, SEC14L2, FKBP5 and prolactin-induced protein, PIP and the 142-gene AR signature from baseline to after 2 weeks of treatment
- Changes in serum levels of circulating steroidogenic hormones such as oestradiol, oestrone, oestrone sulfate, androstenedione, follicle stimulating hormone, luteinizing hormone, DHT, progesterone, sex hormone binding globulin, and total/free testosterone in blood samples taken prior to and after 2 weeks of study treatment
- Changes in serum levels of PSA prior to and after 2 weeks of study treatment
- Safety and tolerability in terms of:
    - Incidence of SAEs
    - Incidence of all AEs of all grades
    - Incidence of grade 3 and 4 AEs as classified by National Cancer Institute Common Terminology Criteria for Adverse Events(NCI-CTCAE) v4.03
    - Incidence of withdrawal from trial treatment due to toxicity
    - Delays to scheduled surgery/neoadjuvant chemotherapy or endocrine therapy

4.4 End of Trial

The end of the trial is defined to be the date of the last follow up visit of the last patient recruited (21 - 28 days after recruitment (+4 days depending on surgery/neoadjuvant chemotherapy/endocrine therapy date)) and when all data fields have been completed. However, the Trial Steering Committee (TSC) may recommend that the trial be stopped prematurely. Such premature termination or suspension of the trial will be notified to the MHRA and REC as required. Ongoing patients must be contacted to notify them of the end of the study.

5 STUDY POPULATION

5.1 Target Population

The target population for this trial is women with histologically confirmed ER positive breast cancer (Allred ≥3) who are also AnR positive (defined at ≥10% nuclear AnR staining by immunohistochemistry). Patients must not have received prior endocrine therapy or chemotherapy for breast cancer.

5.2 Inclusion Criteria

1. Females 16 years of age or older
2. Histologically confirmed ER positive breast cancer (Allred ≥3)
3. AnR positive breast cancer (defined as ≥10% nuclear AnR staining by immunohistochemistry)[2]
4. Any HER2 status
5. Tumour measuring ≥14mm in longest diameter by ultrasound (US) examination, MRI or mammogram
6. Postmenopausal as defined by one of the following criteria:
    - Women ≥55 years of age with an intact uterus and amenorrhoea ≥12 months at the time of diagnosis (or documented or current FSH and oestradiol levels within the postmenopausal range (as per local institutional/laboratory standard))
    - Prior bilateral oophorectomy
    - Documented or current FSH and oestradiol levels within the postmenopausal range (as per local institutional/laboratory standard) in women aged <55 years or in women who have had a hysterectomy with intact ovaries
7. Eastern Cooperative Oncology Group (ECOG) performance status 0, 1 or 2
8. Adequate renal function defined by a serum creatinine ≤1.5 x ULN. Adequate liver function defined by total bilirubin ≤ 1.5 ULN (patients with Gilbert's Syndrome exempted), either ALT or AST ≤2.5 ULN and ALP ≤2.5 ULN
9. Acceptable risk of bleeding (e.g. bleeding diathesis, warfarin) as assessed by the PI (if the PI is unsure the CI will make the final decision)
10. Written informed consent
11. Able to comply with treatment and follow up

5.3 Exclusion Criteria

1. Inoperable breast cancer

[2]Immunohistochemistry (IHC) for AnR: This will initially be performed by contributing centres, all of who are UKAS (UK Accreditation Service) accredited laboratories and take part in the national external quality assessment service (NEQAS) IHC assessment schemes. ALL AnR IHC will subsequently be reviewed centrally by the trial pathologists. AnR testing will be performed at the time of diagnosis, with only those patients with ER positive AND AnR positive tumours being approached for study entry in the clinic.

2. Males
3. Inflammatory tumours
4. Evidence of metastatic disease
5. Any history of invasive malignancy within 5 years of starting study treatment (other than adequately treated basal cell carcinoma or squamous cell carcinoma of the skin and cervical carcinoma in situ)
6. Prior endocrine therapy or chemotherapy for breast cancer
7. Concomitant use (defined as use within 12 weeks prior to entry) of HRT or any other oestrogen-containing medication or supplement (including vaginal oestrogens and phytoestrogens)
8. Previous use of oestrogen implants at ANY time
9. Uncontrolled abnormalities of serum potassium, sodium, calcium or magnesium levels
10. Evidence of uncontrolled active infection
11. Evidence of significant medical condition or laboratory finding which, in the opinion of the PI, makes it undesirable for the patient to participate in the trial
12. Participation in a clinical trial of an IMP in the last 30 days

5.4 Patient Transfer and Withdrawal

In consenting to the trial, patients are consented to trial treatment, follow-up and data collection. If voluntary withdrawal occurs, the patient should be asked to allow continuation of scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any adverse event resolve or the patient's condition becomes stable. Patients who do discontinue from the trial will be contacted for safety assessments for 30 days after the last dose of enobosarm. The investigator must take every reasonable effort to keep patients on study for the duration of the trial. Provided consent is not withdrawn, patients will be traced through their GP and national records to assist in the collection of long term follow-up information and the closure of all SAEs.

5.4.1 Patient Transfers

For patients moving from the area, every effort should be made for the patient to be followed-up at another participating trial centre and for this trial centre to take over responsibility for the patient or for follow-up via GP.

A copy of the patient workbooks/clinical data should be provided to the new site. The patient will have to sign a new consent form at the new site, and until this occurs, the patient remains the responsibility of the original centre. The LCTC should be notified in writing of patient transfers.

5.4.2 Withdrawal from Trial Intervention

Patients may be withdrawn from treatment for any of the following reasons:

a. Withdrawal of consent
b. Unacceptable toxicity.
c. Intercurrent illness preventing further treatment.

d. Any change in the patient's condition that justifies the discontinuation of treatment in the clinician's opinion.
e. Serious patient violation of the study protocol, e.g. failure to attend

If a patient wishes to withdraw from trial treatment, centres should nevertheless explain the importance of remaining on trial follow-up, or failing this, of allowing routine follow-up data to be used for trial purposes. Generally, follow-up will continue unless the patient explicitly also withdraws consent for follow-up (see section 5.4.3).

5.4.3 Withdrawal from Trial Completely

Patients are free to withdraw consent at any time without providing a reason. Patients who withdraw from the trial for other reasons have previously consented to follow-up in the trial. Data up to this time can be included in the trial if anonymised. They may need to reaffirm that they consent to follow-up through usual NHS mechanisms. If the patient explicitly states their wish not to contribute further data to the study, a withdrawal CRF should be completed.

5.5 Co-enrolment Guidelines

Patients may not participate in another clinical trial of Investigational Medicinal Product (IMP) with any investigational drug within 30 days prior to registration until the Follow-Up Visit has been completed on Day 21 - 28.

Where recruitment into another trial is considered to be appropriate and without having any detrimental effect on the EMERALD Trial this must first be discussed with the LCTC who will contact the CI Professor Carlo Palmieri.

6 SCREENING AND RANDOMISATION

6.1 Screening

Screening will be performed upon a patient's possible eligibility for the study and must be documented on the LCTC Web Portal "Screening and Enrolment Log". Screening details should be entered into the Portal and this will automatically generate a screening number and a confirmation email with these details will be sent to site staff. The screening log can be printed at any time from the Portal to allow for storage in the Investigator Site File.

Step-by-step guides will be issued to research site staff and the process will also be demonstrated during site initiations.

Start of screening is defined as the patient being first discussed for eligibility in the local MDT meeting this should be followed by the signing of the Informed Consent Form. Patient hospitals notes should be screened by the research team prior to the patient being approached to ensure no obvious exclusion/inclusion criterion are not met. As this is a window of opportunity study and to enable timely identification, enrolment and treatment of patients prior to surgery/neoadjuvant chemotherapy or endocrine therapy, we will perform AnR staining at the time of diagnosis on the diagnostic core biopsy, at the same time that the BC is assessed by IHC for ER, PR and HER2. This will enable identification of those patients with tumours that are ER positive and AnR positive and these patients will be approached to participate in the trial. Consequent to this test, informed consent must be obtained before further study-specific screening evaluations are performed and must be documented in the patient's medical records. For patients whose samples had AnR staining performed, but who were not consented or entered onto the study, a member of their direct care team will remove all identifiable information from their diagnostic biopsy FFPE block and standard care surgery FFPE block. The blocks will be sent to the University of Liverpool GCP Laboratory (the block numbers will remain on the FFPE blocks and they will be sent with a sample information sheet containing the patient's screening number and the block number). The pathology report for the diagnostic block and standard care surgery block will also be anonymised and uploaded to the LCTC portal.

A patient ID log MUST be kept at each trial centre detailing patient's full name, date of birth, hospital number and NHS number in accordance with local standard operating procedures (SOP). This log will not be sent outside of the trial centre, but may be monitored onsite by authorised personnel.

A screening and randomisation log of all potential patients who have provided written informed consent will be kept at each trial centre, including individuals who later decide not to participate in or who are found to be unsuitable for the study following the screening procedures. This will capture the patient screening number, date of screening, patient initials trial number, and date of randomisation (if applicable) or reason why patient was not eligible to participate in the trial.

Assessments made as part of routine care can only be used in screening if performed within the specified trial windows. The following screening assessments must be performed within 14 days of randomising the patient:

- Signed Informed Consent Form

- Assessment of eligibility criteria
- Review of medical history and past drug history
- Physical examination including vital signs (BP, pulse, temperature, height and weight)
- ECOG Performance Status
- Haematology and clinical biochemistry (including full blood count (FBC), liver function test (LFT), urea and electrolytes, calcium, clotting and glucose)

The following screening assessments must be performed within 28 days of randomising the patient:

- Breast ultrasound, mammogram or MRI
- Tumour size measurement
- Tissue analysis for AnR expression (on diagnostic tissue at the time of ER, PR and HER2 diagnostic testing)

6.2 Randomisation

Patients who have given written informed consent and satisfy the inclusion criteria will be randomised to the study by trained staff at the LCTC. The PI or other trial centre staff with delegated responsibility (as per the signature and delegation log) should call the LCTC: 0151 795 5289 to inform LCTC staff of a suitable patient. The appropriate fields of the online screening log should also be completed.

To ensure essential entry criteria are fulfilled, randomisation can only occur following the completion and forwarding of the trial registration documents by the investigators:

- Completed Randomisation Forms signed by the PI or a co-investigator
- A copy of the signed Consent Form
- Anonymised pathology report of AnR staining

The documents should be faxed to the LCTC between Monday - Friday from 09:00 to 17:00, fax number: 0151 794 8250.

To randomise a patient for the EMERALD study please fax the required documents to 0151 794 8250
(Note that the LCTC is open from 0900 – 1700, Monday – Friday, excluding public holidays)

If a site does not have access to a fax machine then the informed consent form should be uploaded to the LCTC Portal and the Trial Coordinator informed by telephone. This process is detailed in the work instruction Transferring Files via the LCTCPortal.

The LCTC will query any issues with the documents listed above and will provide written confirmation of randomisation into the study to the trial centre research team and pharmacy department by email. The patient will be issued with a trial specific number and this number should be used on study documentation throughout.

7 ASSESSMENTS AND PROCEDURES

7.1 Laboratory Screening Procedure

7.1.1 AnR Staining

AnR staining should be conducted on all biopsy samples of early breast cancer in females over 45 years of age at the same time as ER, PR and HER2 testing of the diagnostic sample.

Ethical approval has been granted to perform additional diagnostic testing on all patient samples who meet the following criteria:

- Female
- Over 45 years of age
- Invasive breast cancer

Each staining result should be recorded on the LCTC Portal pre-screening log along with:

- patient gender (female)
- date of birth
- ER, PR and HER2 status
- Grade and breast cancer histological subtype

Results for all AnR staining should be included alongside the ER, PR and HER2 results on any pathology reports for all patients, and for should be available for the next MDT.

Positive result
If a result comes back ER and AnR positive then please inform the research nurse working on the trial or the principal investigator as soon as possible.

Negative result
If the ER or AnR result is negative this patient should not be approached to join the study. Their result should be recorded on the pre-screening log and no further additional testing should be carried out on this sample for the purposes of this research.

Please follow SSEME_D036 EMERALD Pathology Manual for more information on the IHC staining process.
Antibodies will be provided for the AnR staining.

7.2 Schedule of Trial Procedures – Table 2

Time Points	**Screening**	**Screening**	**Baseline (Visit 1)**	**Mid-Treatment Phone Call**	**Tissue Collection (Visit 2)**	**End of study/ Follow up (Visit 3)**	**Premature Discontinuation**
Day Assessments	**-28 days**	**-14 days**	**Day 0**	**Day 7**	**Day 14 (+4 days)**	**Day 21 - 28 (+4 days)**	
Informed Consent		**X**					
Eligibility Checklist		**X**					
Demography		**X**					
Medical History		**X**					
Breast ultrasound, mammogram and/or MRI	**X**						
Tumour size measurement (radiological)	**X**						
Physical examination including vital signs		**X**					
Symptom led physical examination					**X**	**X**	**X**
Height			**X**				
Weight			**X**		**X**	**X**	**X**
ECG		**X[1]**			**X[1]**		
ECOG Performance Status		**X**	**X**		**X**	**X**	**X**
Haematology and clinical biochemistry[2]		**X**	**X**		**X**	**X[3]**	**X**
Tissue analysis for AnR expression (on diagnostic tissue)[4]	**X**				**X[5]**		
Excised tumour tissue biopsy or research core biopsy					**X[6]**		
Dispense study drug			**X**				
Study drug compliance				**X**	**X**		
Adverse events			**X**	**X**	**X**	**X**	**X**
Concomitant medication			**X**	**X**	**X**	**X**	**X**
Collection of blood – trial sample			**X**		**X[7]**		**X**

1 As part of standard care only. **2** Include full blood count, liver function tests (including ALT, AST and γGT), urea and electrolytes, calcium, clotting and glucose. **3** Only required if results at the Tissue Collection Visit were outside of reference ranges. **4** Immunohistochemistry (IHC) for AnR: This will initially be performed by contributing centres, all of who are UKAS (UK Accreditation Service) accredited laboratories and take part in the national external quality assessment service (NEQAS) IHC assessment schemes. ALL AnR IHC will subsequently be reviewed centrally by the trial pathologists. AnR testing will be performed at the time of diagnosis, with only those patients with ER positive AND AnR positive tumours being approached for study entry in the clinic. **5** Central lab only. **6** For patients having surgery two core biopsies will be taken from excised tumour tissue at the time of surgery; for neoadjuvant chemotherapy/endocrine therapy patients two core biopsies will be taken via a research core biopsy after at least 14 days of treatment and prior to commencing neoadjuvant chemotherapy/endocrine therapy. **7** Samples to be collected within 24 hours of last dose of IMP.

7.2.1 Trial Schedule

Screening
Trial specific screening tests may be up to 14 days prior to randomisation and Baseline Visit. If a test falls outside of these dates then it should be repeated (excluding core biopsy and IHC results, scans and tumour measurement).

Baseline Visit 1 (Day 0)
Baseline must be at least 14 days but not more than 18 days before planned surgery/neoadjuvant chemotherapy or endocrine therapy commencement.[1]

Mid-treatment Phone Call (Day 7)
A phone call should be made to the patient ±2 days of Day 7 depending on weekends to check for adverse events, changes in concomitant medications and drug compliance. Every effort should be made to reach the patient for this phone call (at least 3 attempts on different days).

If deemed necessary the patient should be brought in for blood investigations and/or a symptom led physical examination.

Tissue Collection Visit 2 (Day 14 (+4 days))
The Tissue Collection Visit may be the pre-surgical or surgery appointment or a visit prior to commencing neoadjuvant chemotherapy or endocrine therapy where a research core biopsy will be scheduled. This should be no more than 24 hours before surgery/commencing neoadjuvant chemotherapy or endocrine therapy, and the patient must have had at least 14 doses of enobosarm.[1] Enobosarm should not be stopped more than 24 hours prior to surgery.[2]

Follow-up Visit 3 (Day 21 - 28 (+4 days))

Surgery Patients
Follow-up should ideally be 14 days (+4 days) from the date of surgery, but can be 7 days from the date of surgery in line with scheduled follow-up (between Day 21 and Day 28). If blood results were within normal reference ranges at the Tissue Collection Visit then blood investigations are not required at this visit.

Neoadjuvant Chemotherapy and Endocrine Therapy Patients
If a patient does not have a scheduled follow-up visit and blood results were within normal reference ranges at the Tissue Collection Visit, then follow-up can be conducted via phone call between Day 21 and Day 28 (+4 days)(ideally 14 days after the final dose of enobosarm). Blood investigations are not required if the follow-up is conducted via phone call.

However, if a patient reports an adverse event that requires a symptom led physical exam then the patient MUST be asked to attend the hospital for this and investigations conducted, likewise if blood results were abnormal at the Tissue Collection Visit (Day 14 (+4 days)) then the patient must attend the visit in person and blood investigations conducted. Abnormal blood results and adverse events should be followed up until resolution.
After the visit or phone call, if all adverse events/abnormal results are resolved, the patient has completed involvement on the trial.

[1] Patients who meet all eligibility criteria but have surgery/neoadjuvant chemotherapy/endocrine therapy scheduled for 12 or 13 days after randomisation may be included in the trial at the discretion of the Chief Investigator.

[2] If a patient's surgery/neoadjuvant chemotherapy/endocrine therapy is delayed for any reason treatment may be continued until surgery/neoadjuvant chemotherapy/endocrine therapy at the discretion of the Chief Investigator.

7.3 Trial procedures

7.3.1 Physical Exam and Vital Signs

To be conducted at the screening visit.

- A clinically directed physical examination to document that the patient is eligible for the trial and to identify any pre-existing conditions
- Measurement of weight
- Temperature
- Blood Pressure
- Pulse

Height should be measured at baseline.

7.3.2 Symptom Led Physical Exam

After the initial full physical exam any further physical exam should be symptom led or carried out at the discretion of the treating physician based on any clinical concerns or signs.

7.3.3 ECG

If a 12 lead ECG is being carried out at screening/baseline or at Visit 2 as part of standard care the results should be recorded on the CRF.

7.3.4 ECOG Status

To be conducted at all visits by a clinician.

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair
5	Dead

Oken, MM., Creech, R.H., Tormey, D.C., Horton, J. Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am J Oncol 5:649-655, 1982.

7.3.5 Haematology and Biochemistry

To be conducted at all visits. Haematology and biochemistry investigations should include FBC, LFT (including ALT, AST and ɣGT), urea and electrolytes, calcium, clotting and glucose.

All tests should be performed in accordance with local practice and reviewed by a PI or a delegated medically qualified person.

FBC, LFT and urea and electrolytes results should be checked at each visit (and prior to each dispensation). Toxicities are graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), v4.03. Grade 3 hepatotoxicity must be discussed with the CI to determine whether to withdraw the patient or interrupt study treatment, all other grade 3 AEs should be considered for withdrawal/interruption from the study by the local PI. See Section 7.4.

7.3.6 Surgery/Research Core Biopsy

Surgery

The breast surgery will be pre-arranged before the patient begins the enobosarm treatment as part of the patient's standard of care. This visit should be within 24 hours of taking the last dose of enobosarm, this may be the day before surgery or the day of surgery depending on the time the last dose was taken and the time of surgery. Samples taken at surgery should ideally be taken via core biopsy (2 cores) once the tumour has been removed BUT BEFORE the tissue has been placed in formalin.

Research Core Biopsy

Patients who have pre-arranged neoadjuvant chemotherapy or endocrine therapy in place of surgery or before surgery will be required to have an additional core biopsy using a core-cut or tru-cut device under imaging guidance prior to commencement of neoadjuvant chemotherapy/endocrine therapy. All efforts should be made to coincide this biopsy with any planned interventions such as insertion of marker clips (for example to mark tumour site in case of complete pathological response to neoadjuvant chemotherapy). Two cores should be taken (one for an FFPE block and one for the AllProtect sample (see Section 8)). If only one core is available due to the size of the tumour or the patient does not wish to have multiple cores (due to pain at the time of the procedure), the core MUST be used for the FFPE block.

The following samples will be collected for all patients at Visit 2:

- Blood samples
- Core taken and placed in formalin to create an FFPE block (from excised tumour or via research core biopsy)
- Core taken and placed in AllProtect solution (from excised breast tumour tissue or via research core biopsy)

7.3.7 Breast Ultrasound/Mammogram/MRI

A breast ultrasound, mammogram and/or MRI must have been conducted or be conducted within 28 days of screening and randomisation to the trial. This will be used to ensure the tumour size is in line with eligibility criteria (≥14mm).

7.3.8 Tissue Analysis for AnR Expression (on diagnostic tissue)

AnR Expression testing will be performed on the core biopsy at the same time as the ER, PR and HER2 diagnostic tests (standard of care). The AnR test will be performed at site following the EMERALD Study Specific Procedures Manual.

7.4 Procedures for Assessing Safety

Patients will be assessed at screening using the inclusion and exclusion criteria that have been designed to ensure safety for those who join the study, and to rule out anyone for whom it may be unsafe. At screening, a patient may be judged by the PI or a psychiatrist to be ineligible based on medical mental health history as listed in the exclusion criteria.

Each patient will attend the hospital on Day 14 (+4) for the Surgery Visit (Visit 2) and the Day 21 -28 Follow-Up Visit (Visit 3) where the PI, or delegated medically qualified person, will formally assess and record any AEs as described in Section 11. A phone call to check for any AEs, changes in concomitant medications and drug compliance will be made on Day 7, and if for any reason it is deemed necessary the patient will be brought in for clinical investigations and a symptom led physical exam. AEs will be described using the NCI-CTCAE Version 4.03.

Toxicities are graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), v4.03. Patients who experience a Grade 3 or higher toxicity that is attributed to enobosarm (except LFTs) and cannot be ameliorated by the use of adequate medical intervention should interrupt treatment with the study drug. Hypercalcemia should also be considered an AE that should necessitate discontinuation of enobosarm. Treatment interruption and re-initiation should be discussed with the CI. Blood and tumour samples should continue to be collected as scheduled.

All patients discontinued from dosing should be followed up until abnormal values return to normal.

ALL DISCONTINUATIONS SHOULD BE DISCUSSED WITH THE CHIEF INVESTIGATOR PRIOR TO DISCONTINUATION.

7.5 Procedures for Assessing Efficacy

Response will be measured by changes in Ki67 from baseline to the Day 14 (+4) Pre-surgical/Surgery visit. Testing of core biopsy samples from screening/baseline and surgery will be carried out externally and the technician performing the test will be blinded

Response will be measured by changes in Ki67 as measured by immunohistochemistry between the baseline sample and the sample taken at the time of surgery (Day 14 +4 days). Samples will be assessed and scored centrally in a blinded fashion at the laboratories of Leeds Institute of Cancer and Pathology (LICAP), Section of Pathology and Tumour Biology, University of Leeds once all samples have been collected.

7.6 Other Assessments

7.6.1 Patient Drug Diary

Patients who are randomised to receive enobosarm will be asked to complete a drug diary once they are commenced on treatment. Patients should fill this in every day and document any reason why they have not taken the enobosarm on that day. The drug diary will be reviewed at the hospital by the research team at Visit 2 (Day 14+4).

7.6.2 Concomitant Medication

Data on concomitant medication will be collected at ALL visits and entered onto MACRO. At the baseline visit any concomitant medication taken within the last 3 months which has been subsequently stopped should also be recorded. Concomitant medication should also be recorded at the time of SAE reporting. Use of concomitant medication may be checked in the source notes during any routine site monitoring.

7.6.3 Adverse Event Reporting

See Section 11.2 and 11.8 for adverse event reporting.

7.6.4 Translational Samples

Blood samples (plasma and serum) for translational research must be taken at Visit 1 (Baseline), and Visit 2 (Day 14 +4).

Formalin fixed paraffin embedded (FFPE) tissue of breast will be collected at the pre-planned breast surgery.

Formalin fixed paraffin embedded excess tissue remaining from the diagnostic core biopsy will be collected from ALL patients in the EMERALD study. This will be requested immediately upon randomisation and sent to the Liverpool GCLP Facility at the same time as the surgical sample or as soon as possible thereafter.

Details of the procedure for taking and processing translational samples will be provided in the EMERALD Laboratory Manual.

7.6.4.1 Non-consented patients

For patients whose samples had AR staining performed, but who were not consented or entered onto the study, a member of their direct care team will remove all identifiable information from their diagnostic biopsy FFPE block and standard care surgery FFPE block. The blocks will be sent to the University of Liverpool GCP Laboratory (the block numbers will remain on the FFPE blocks and they will be sent with a sample information sheet containing the patient's screening number and the block number). The pathology report for the diagnostic block and standard care surgery block will also be anonymised and uploaded to the LCTC portal.

7.7 Loss to Follow-up

A patient is considered to be "lost to follow up" if a reasonable number of steps has been taken to unsuccessfully contact the patient including several telephone calls, written attempts or via the patient's GP. Attempts to contact a patient should be documented in the source documents.

If a trial patient is lost to follow up, contact will initially be attempted through the PI at each trial site. If the PI at the site is not the patient's usual clinician responsible for their speciality care, follow-up will also be attempted through this clinician. Where all of these attempts are unsuccessful, the patient's GP will be asked to provide follow-up information to the recruiting centre. Loss to follow up is expected to be very low, therefore there are no plans to replace patients lost to follow up.

7.8 Trial Closure

Trial enrolment may be stopped at a trial site when the total requested number of patients for the trial has been obtained. PIs will be informed by the LCTC when patient recruitment is to cease. A letter to patients to inform them of the study results will be provided to sites to distribute by post.

Otherwise, the end of the trial is defined to be the date on which data for all participants is frozen and data entry privileges are withdrawn from the trial database after the last follow up visit of the last patient recruited (21 - 28 days after recruitment (+4 days depending on surgery)). However, the Trial Steering Committee (TSC) may recommend that the trial be stopped prematurely. Such premature termination or suspension of the trial will be notified to the MHRA and REC as required. Ongoing patients must be contacted to notify them of the end of the study.

8 TRANSLATIONAL RESEARCH

The collection of biological material is a key part of this study and will enable translational research. All EMERALD samples should be collected, processed, stored and shipped as detailed in the EMERALD Laboratory Manual provided to sites. Sampling kits will be provided and postage costs will be covered by the trial.

8.1 Blood (Plasma & Serum) Collection

In addition to the patient's routine haematology blood samples, a further 20ml (approximately) of blood will be taken, at specified time points throughout the study.

These samples will be processed at each trial site, stored at -80°C and then transferred in batches to the Liverpool GCLP Facility, University of Liverpool. Samples will then be stored under appropriate conditions for up to 10 years from study start and will be used in future translational studies subject to ethical approval.

Blood samples for translational research must be taken at the Baseline (Visit 1) and Tissue Collection Visit (Visit 2). If a patient is deemed too unwell to receive treatment, every effort should still be made to take the samples.

A separate translational plan will be completed detailing analysis of samples. A laboratory manual will be provided to site on collection procedures and transferring samples to Liverpool before the trial begins at each site.

8.2 Formalin Fixed Paraffin Embedded Tissue of Breast

FFPE tissue of breast will be taken at the pre-planned Tissue Collection Visit (Day 14(+4)) as part of surgery (ideally a core biopsy of the resected tumour tissue) or as a research core biopsy. The cores should be put in formalin and a trial specific FFPE block created for the primary endpoint analysis.

FFPE tissue remaining from the diagnostic core biopsy will be requested immediately upon randomisation and sent to the Liverpool GCLP Facility at the same time as the surgical sample or as soon as possible thereafter.

FFPE blocks created as part of standard care from the resected tumour tissue will also be requested for the trial.

For patients whose samples had AR staining performed, but who were not consented or entered onto the study, a member of their direct care team will remove all identifiable information from their diagnostic biopsy FFPE block and standard care surgery FFPE block. The blocks will be sent to the University of Liverpool GCP Laboratory (the block numbers will remain on the FFPE blocks and they will be sent with a sample information sheet containing the patient's screening number and the block number). The pathology report for the diagnostic block and standard care surgery block will also be anonymised and uploaded to the LCTC portal.

8.3 AllProtect® Sample

A fresh sample of tumour tissue (not placed in formalin) will be collected at the pre-planned Tissue Collection Visit (Day 14 (+4)) as part of surgery (ideally a core biopsy of the resected tumour tissue) or as a research core biopsy. This sample should be taken by the surgeon following surgery or by the pathologist when they receive the resected tumour tissue and should be stored in an AllProtect® tube and frozen at -80°C.
If it is not possible, due to local procedures, to take the AllProtect Sample then an agreement can be made and filenoted with the EMERALD Trial Team to not collect this sample.

8.4 Summary of Collection of Biological Material for Translational Research (Table 3)

Sample to be collected	Visit 1 (Baseline)	Visit 2 (Pre-Surgical/ Surgery)	Visit 3 (End of Study/Follow Up)
Time Point	Day 0	Day 14 +4 days	Day 21- 28
Plasma & serum samples	X	X[1]	
FFPE tissue of breast		X	
AllProtect® sample		X	
FFPE tissue remaining from diagnostic core biopsy	Samples are requested following randomisation and sent to the Liverpool GCLP Facility after the patient's pre-planned surgery visit.		

[1]Samples to be taken from patients within 24 hours of last dose of IMP

9 TRIAL TREATMENT

9.1 Introduction

Patients will be randomised 3:1 to 9mg of enobosarm (therapeutic arm) to a control arm receiving "treatment as usual" with no placebo. Enobosarm will be taken orally, once daily, up to surgery/neoadjuvant chemotherapy/endocrine therapy which should take place 14 days +4 days from commencing treatment. Enobosarm will be prescribed in hospital and is self-administered at home by the patients. Patients will be dispensed two bottles containing 35 enobosarm softgels (enough in each for a 7 day course +4 days/wastage) at Visit 1 (Baseline). Compliance to treatment will be checked through a patient drug diary on Day 14 (Visit 2) hospital visit. Remaining unused enobosarm will be collected and accounted for at Visit 2.

9.2 Enobosarm

9.2.1 Formulation, Packaging, Labelling, Storage and Stability

GTx-024 3.0mg softgels will be supplied as opaque, white to off-white, size 5, oval softgel capsules with "GTx" imprinted in black ink on the outer shell containing 3.0mg of GTx-024. The liquid softgel fill is composed of GTx-024 dissolved in polyethylene glycol 400.

GTx-024 3.0 mg softgels will be packaged in high density polyethylene (HDPE) bottles with induction seal and child-resistant closure. Each bottle will contain thirty-five (35) GTx-024 3.0 mg softgel capsules. Two (2) 35-ct bottles will be packed into a single kit carton, with each kit carton containing sufficient drug supply to cover the 14 days +4 days dosing period with overage. Patients will be prescribed two bottles at baseline, patients will be requested to return both bottles at Visit 2 on Day 14(+4).

Each bottle and each kit carton will be labeled with dosing and storage instructions.

Enobosarm (GTx-024) should be stored at room temperature, 15°C to 25°C (59°F to 77°F), with excursions permitted to 30°C, and protected from moisture. Stability will be confirmed for at least the length of the clinical trial or new material will be manufactured and supplied. The expiry date of the study drug will be on each bottle.

9.2.2 Dosage and Administration of Study Treatment

One daily dose of 9mg enobosarm (3x3.0mg softgels) will be taken orally with water and with food where possible at approximately the same time each day. Patients will be asked to commence the enobosarm at a time convenient to them on Day 1 and to attempt to keep this time consistent.

9.2.3 Dose Modifications

No dose modifications are allowed as part of study given the short duration.

9.2.4 Accountability Procedures for Study Treatment

The PI is responsible for the correct storage of study medication according to GTx, Inc. recommendations outlined in the Investigator's Brochure. The study medication made available for this clinical trial must be used in accordance with the protocol and dispensed only under the supervision of the Investigator and documented sub-Investigators. The Investigator must maintain complete and accurate records, showing the receipt and disposition of all supplies of the study medication delivered by the GTx, Inc., authorized representative. These records must include a master record which lists the date of receipt of all study medication shipments, batch numbers, expiration date, and quantities received. In addition, a dispensing record which includes all quantities dispensed, identification of the person to whom study medication was dispensed, the date of each dispensing, and the identification of the dispenser will also be maintained. The master dispensing records are separate from records kept for individual trial patients.

It is the Investigator's responsibility to ensure that study medication used by trial patients plus unused study medication equal the total amount received from the GTx, Inc. authorized representative. Damaged and/or contaminated packets must also be accounted for in the dispensing records. All discrepancies must be explained in writing. The study personnel responsible for study medication administration to the patient will record the date and time the initial treatment is given to the patient. In addition, the Drug Accountability electronic case report form (eCRF) will document any treatment interruptions or discontinuations.

9.2.5 Assessment of Compliance with Study Treatment

Patients should be instructed to return all unused study medication and containers at Visit 2 prior to surgery/commencing neoadjuvant chemotherapy or endocrine chemotherapy so that drug accountability can be performed. If the patient forgets to bring the bottle with them, they will be able to bring the bottle back at the next visit (Visit 3 for follow-up). The research nurse is required to count all returned study capsules and record this on the eCRF, a reason must be provided for any missing or damaged study drug. The dispensing pharmacy should also count returned study capsules and record this on the drug accountability log.

All study medication returned by patients must be accounted for and verified by the EMERALD Trial Coordinator. After verification of study medication, a drug destruction form should be completed and sent to the EMERALD Trial Coordinator who will then authorise the destruction of returned, damaged and unused study medication according to local pharmacy SOPs.

9.3 Concomitant Medications/Treatments

Forbidden medications and treatments during the study include:

- Major surgery within 28 days before randomization
- Testosterone, methyltestosterone, oxandrolone (Oxandrin®), oxymetholone, danazol, fluoxymesterone (Halotestin®), testosterone-like agents (such as dehydroepiandrosterone, androstenedione, and other androgenic compounds,

including herbals), or antiandrogens. Previous therapy with testosterone and testosterone-like agents is acceptable with a 28-day washout (if previous testosterone therapy was long-term depot within the past 6 months, the site should contact the Medical Monitor). Treatment with any of the following hormone replacement therapies, unless discontinued at least 28 days prior to randomization

- Oestrogens
- Megesterol acetate
- Treatment with any investigational agent within <4 half-lives for each individual investigational product OR within 28 days prior to randomization
- All other anticancer treatments (including, but not limited to, all SERMs, AIs, and fulvestrant)
- Caution should be exercised when administering potent CYP3A4 inducers, UDP inhibitors, and substrates of BCRP with GTx-024. Drug-drug interaction studies conducted with GTx-024 3mg have demonstrated that concomitant use of CYP3A4 inducers may decrease the rate and extent of exposure of GTx-024 and its glucuronide metabolite. UDP inhibitors may increase GTx-024 and glucuronide exposure. Concomitant administration of GTx-024 with BCRP substrates may lead to increased exposure of the BCRP substrate.

9.4 Missed Dose and Vomiting Post Dose

If a dose is missed, enobosarm should be taken immediately and continue on the following day with the once daily dose as recommended. The dose should not be doubled within the same day to make up for a missed dose. Missed doses should be recorded in the patient drug diary along with the time and date of the next dose.

If a patient vomits within 6 hours of dose, they should take another complete dose (3 x 3.0mg enobosarm Softgels).

9.5 Overdose

Inadvertent overdosing, such as administration of a higher dose than dictated in the protocol, should be followed by rigorous monitoring for potential adverse reactions. Patients experiencing toxicities upon misdosing or overdosing must be treated at the discretion of the treating physician with adequate supportive care and followed until recovery.

All cases of misuse of enobosarm, including overdose, should be reported to the EMERALD Trial Coordinator.

10 STATISTICAL CONSIDERATIONS

10.1 Method of Randomisation

The sequence of allocations will be centrally generated by the LCTC study statistician using the Stata package *ralloc* employing permuted block randomisation with variable block length, and 3:1 (enobosarm : control) allocation to the two treatment arms.

10.2 Outcome Measures

10.2.1 Primary outcome measures

Change in the proliferation marker Ki67 (% positive tumour cells) determined by tissue immunohistochemistry at baseline and 2 weeks.

10.2.2 Secondary outcome measures

- Amount of cleaved caspase 3 determined by tissue immunohistochemistry at baseline and two weeks
- Expression of PSA, Gross Cystic Disease Fluid Proteins (GCDFP)-24 &-15; PgR, GREB1 by tissue immunohistochemistry at baseline and two weeks
- Amount in serum levels of circulating steroidogenic hormones oestradiol, oestrone, oestrone sulfate, androstenedione, follicle stimulating hormone, luteinizing hormone, DHT, progesterone, sex hormone binding globulin (SHBG) and total testosterone in blood determined by blood assay at baseline and two weeks; free testosterone to be derived from SHBG and total testosterone
- Amount in serum levels of PSA and steroidogenic hormones determined by blood assay at baseline and two weeks

10.2.3 Safety Endpoints

- Occurrence of grade 3+ toxicity as classified by NCI-CTCAE v 4.03
- Occurrence of serious adverse events
- Withdrawal from trial treatment due to toxicity
- Experience of delay to scheduled surgery/neoadjuvant chemotherapy or endocrine therapy

10.3 Sample Size

In the absence of a clearly defined or a clinically relevant difference for Ki67, the primary efficacy parameter will be the probability that the response in one group will be “better” than the response in the other group. This may be referred to as the Probability of Benefit or PoB, and is directly estimated by the (scaled) Mann-Whitney statistic; in the present context a “good” response is a greater reduction in the Ki67 index in the treated arm, and a useful reduction in Ki67 is taken to be a PoB of 0.67 (odds of 2:1 favouring the intervention, and equivalent to a standardised effect size of 0.62).

To demonstrate a PoB (in terms of a reduced Ki67 index) of 0.67, with 5% two-sided significance and 90% power we require 147 patients (with allocation 3:1 enobosarm: "treatment as usual" control). This can be achieved from a minimum of 7 sites assuming an average of 2 pts/site/month and 2 sites opened/month (actually yielding 150 patients), with duration of accrual of 14 months.

The recruitment rate/site estimate is based on the recruitment to the POETIC study (Smith et al. 2011) from the selected centres. Based on previous studies loss to follow-up is expected to be minimal (<1%) as this is a highly motivated group of participants, follow-up is brief and assessment non-invasive.

10.4 Interim Monitoring and Analyses

There is no stopping rule for efficacy or futility because there is no formal clinical definition of success and the intervention is not intended as a formal practical treatment. Stopping early on safety grounds is possible however, and a single ISDMC (Independent Safety and Data Monitoring Committee) meeting to review safety will be convened when half of the target number of patients has been evaluated for outcome. The analysis conducted by the Trial Statistician will be unblinded. The results of any efficacy analysis will be confined to the ISDMC and Trial Statistician, and the results of the review will be regarded as guidance only.

10.5 Analysis Plan

Statistical analyses will be performed in Stata v13 or higher. The trigger for the statistical analysis is the end of the trial.

10.5.1 Patient Groups for Analysis

Full Analysis set: In order to follow the Intention to Treat (ITT) principle this will consist of all randomised patients with assessment of the primary outcome excepting for: a) patients withdrawing consent between randomisation and starting therapy b) patients withdrawn from the study after randomisation because of irregularities with the consent process and c) patients whose information determining ineligibility existed before randomisation but was not read until after randomisation. Mis-randomised patients will be analysed as randomised.

Per protocol (PP) set: This will consist of those patients in the Full Analysis set without any major protocol deviations.

Safety set: All patients.

Major deviations from protocol that lead to exclusion of a patient from the per-protocol set will be those that either prevent evaluation of the primary endpoint or which imply insufficient exposure to reasonably expect a treatment effect. While it is possible to identify some deviations that will be a priori catastrophic (e.g. missing primary endpoint or missing all the treatment doses) it is difficult to determine in advance the impact of many others: for instance

would missing ½ of the dose for each day of treatment have the same impact as missing the dose completely on ½ of the treatment days? And would the impact differ depending on whether the missed days were early or late in the treatment period? A table of foreseen major deviations together with provisional assessments of impact is provided below (Table 3), but the final decision will be made in a review towards the end of the study before data lock. Patients who are excluded because of missing baseline or response data may still be included in the ITT population if the missing values can be imputed (see section 10.5.5). Minor violations by definition do not matter in this sense and will be ignored.

Efficacy analyses will be performed on the full analysis set and the per protocol set. Safety summaries will be performed on the safety set.

Table 3 Foreseen Major Deviations

Foreseeable deviation	**Action**
Missing primary endpoint	Exclude from per protocol analysis
All treatment doses missed	Exclude from per protocol analysis
Samples outside allowable window	Review and assign overall severity (0-1) weight*
Assessments outside allowable window	Review and assign overall severity (0-1) weight*
Some treatment days missed	Review and assign overall severity (0-1) weight*
Inadequate doses taken	Review and assign overall severity (0-1) weight*
Multiple deviations	Review and assign overall severity (0-1) weight*
*Patients may be excluded from per protocol analysis if weight is >0.5, included otherwise	

10.5.2 Assessment of Study Quality & Exposure to Treatment/Compliance

Study quality will be summarised by arm in terms of withdrawals/losses to follow-up, frequency of deviations and numbers of patients with 0,1,2… deviations, and extent of missing critical data.

Exposure to treatment by arm will be assessed by mean dose/patient and mean planned dose.

10.5.3 Study centre effects

No adjustment will be made for this.

10.5.4 Identification and handling of outliers

For continuous variables potential outliers will be defined as follows (Tukey 1977) after testing for symmetry and if non-symmetric, transforming to approximate symmetry using a “ladder of powers” approach using the Stata command ladder:

“Mild” outliers: UQ+1.5×IQR to UQ+3×IQR or LQ-1.5×IQ to LQ-3×IQR
Severe outliers: values more extreme than the above

(Note: UQ=Upper Quartile, LQ=Lower Quartile, IQR=Inter Quartile Range)

If after transformation no outliers are apparent then no action will be taken even if values appear as outliers on the original scale, apart from use of the transformation if normality is required for a particular statistical procedure, or to remove the leverage effect of the outlying values.

Otherwise outliers in quantitative data will be queried but no action taken if the result is not amended.

10.5.5 Missing data

No adjustment for missing data will be made for secondary endpoints. If more than 10% of patients have missing baseline or final Ki67 values, then multiple imputation will be attempted for these variables, performing at least 10 separate sets of multiple imputations by treatment arm using chained equations, as implemented in Stata v13 or later. Examples of variables to be included in the imputation process will be baseline and final values of other response variables, and covariates predictive of survival such as age and performance status.

The multiply-imputed data sets can then be recombined for formal analysis, either using the *mim* command prefix or if necessary a bespoke routine to estimate parameter values and combine them using Rubin's rules.

10.5.6 Description of baseline subject characteristics

Categorical baseline variables will be summarised as frequencies and percentages, while continuous variables will be summarised as mean and standard deviation. Variables to be summarise will include baseline values of endpoints together with laboratory values (biochemistry, haematology), demographic and clinical variables.

Patient accrual will be summarised graphically and disposition (ineligibility, losses to follow-up etc.) in a standard CONSORT diagram.

10.5.7 Levels of significance, width of confidence intervals

For analysis of the primary outcome statistical significance will be declared if a two-sided P-value of <0.05 is obtained in favour of enobosarm. All other significance levels will also be 5% two-sided and summary statistics will be presented with 95% confidence intervals. No adjustment will be made for multiple comparisons.

10.5.8 Analysis of primary and secondary outcomes

The primary analysis will be a Mann-Whitney test comparing the change in Ki67 index from baseline between the two arms. The null hypothesis is that enobosarm is no more effective than standard care alone in terms of reduction in Ki67 score from baseline; that is, the Probability of Benefit is not statistically different from 0.5, while the alternate hypothesis is that addition of enobosarm is superior to standard care alone with a Probability of Benefit of

0.67 or more. Results will be presented in terms of the mean, median and SD of the baseline and final Ki67 scores by arm together with the Mann-Whitney statistic and p-value.

Sensitivity analyses
A sensitivity analysis will be performed with an analysis stratified on baseline Ki67. Similar analyses will be performed on secondary efficacy endpoints.

Tests of assumptions
Like the t-test, the Mann-Whitney test may fail to detect a shift in location if there is also a change in variance, hence as a test of shift in location it assumes equal variance. If the primary analysis or the stratified analysis fails to demonstrate statistical significance then a Beta Regression Model will be fitted that simultaneously estimates changes in mean and location, including terms for baseline and treatment for both mean and scale parameters. If the Beta regression model gives a p value<0.05 for the treatment effect on the mean then the result will still be declared statistically significant.

For secondary endpoints comparison of change from baseline will be performed by a suitable regression-type model including terms to adjust for baseline and account for differences in variance.

Subgroup analyses
No subgroup analyses are planned.

10.5.9 Analysis of safety & tolerability

The number and percentage of patients withdrawing from trial treatment due to toxicity or experiencing delay to scheduled surgery/neoadjuvant chemotherapy/endocrine therapy will be tabulated, as will the number and percentage of patients reporting a Serious Adverse Event (SAE) and Grade 3 or higher toxicity (summarised by treatment, overall and by NCI-CTCAE short term). Comparisons between treatment arms will be by an exact test.

Further details of the statistical analyses (dummy tables and graphs) will be included in a formal Statistical Analysis Plan, to be signed off before patient recruitment begins.

11 PHARMACOVIGILANCE

11.1 Terms and Definitions

The following definitions have been adapted from European Directive 2001/20/EC and ICH GCP E6

Adverse Event (AE)
Any untoward medical occurrence [i.e. any unfavourable or unintended sign (including abnormal laboratory results), symptom or disease} in a research patient to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.

Adverse Reaction (AR)
Any untoward and unintended response in a patient to an investigational medicinal product which is related to any dose administered to that patient.

Unexpected Adverse Reaction (UAR)
An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the IB relating to the trial in question.

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)
Any adverse event or adverse reaction is classified as serious if it:

a) results in death
b) is life-threatening* (patient at immediate risk of death)
c) requires in-patient hospitalisation or prolongation of existing hospitalisation**
d) results in persistent or significant disability or incapacity, or
e) consists of a congenital anomaly or birth defect

Suspected Unexpected Serious Adverse Reaction (SUSAR)
A serious adverse reaction that is 'unexpected' (the nature and severity of which IS NOT consistent with the IB (see expected classifications)) is termed as a suspected unexpected serious adverse reaction.

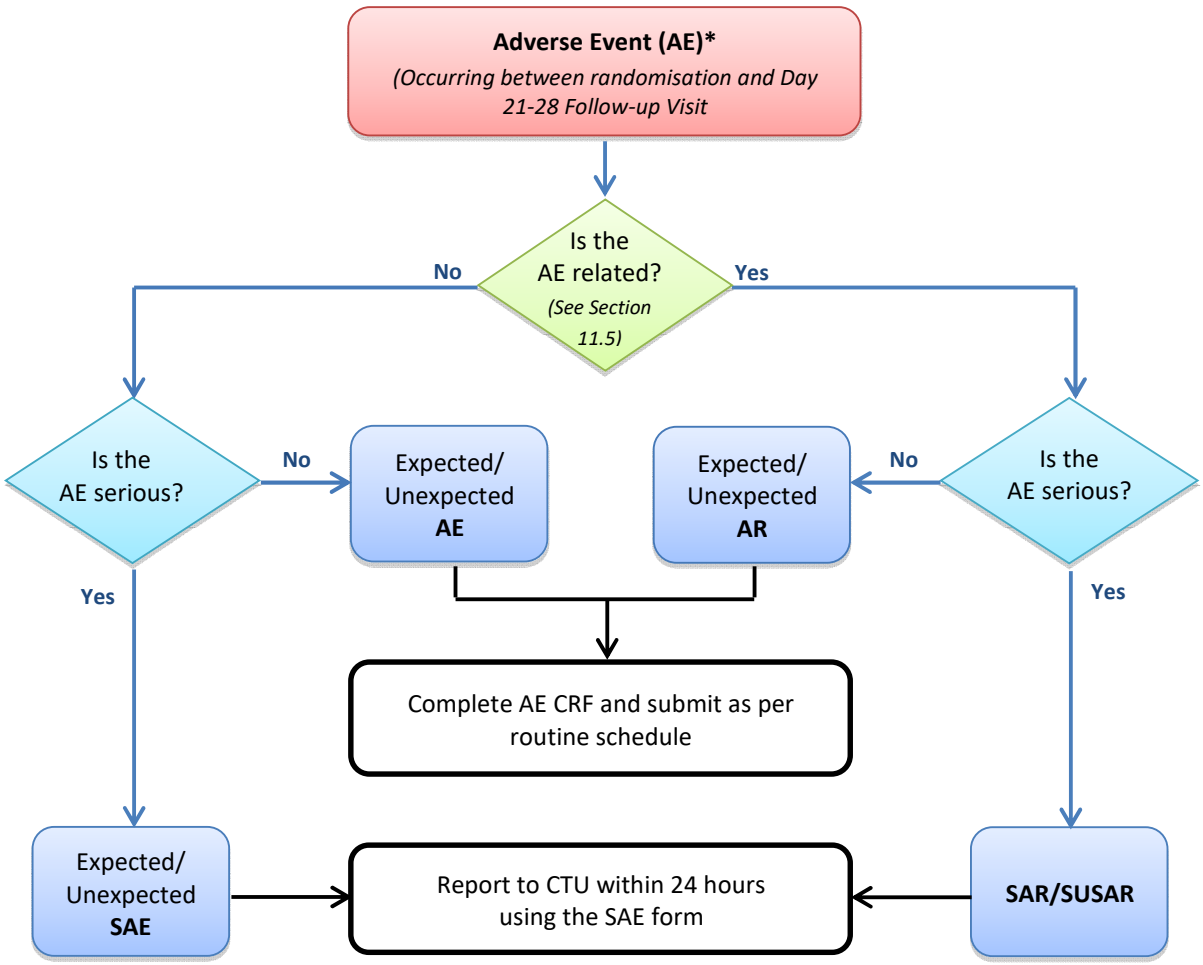
*'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
**Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, including elective procedures that have not worsened, do not constitute an SAE.

11.2 Time Period for Safety Reporting

Safety reporting of Serious Adverse Events or Reactions will be reported during the clinical trial from the period of randomisation until Day 21 - 28 Follow-up Visit.

Investigators are expected to report any SAE that 'they become aware of' and feel is related to trial treatment after the reporting time period stated in the protocol.

11.2.1 Flowchart for Reporting Requirements of Adverse Events



**If an adverse event occurs outside of this time window and the local investigator feels that the event is related to the trial treatment administered, the above process should still be followed.*

11.3 Notes on Adverse Event Inclusions and Exclusions

11.3.1 Include

- An exacerbation of a pre-existing illness
- An increase in frequency or intensity of a pre-existing episodic event/condition
- A condition (even though it may have been present prior to the start of the trial) detected after trial drug administration
- Continuous persistent disease or symptoms present at baseline that worsens following the administration of the study/trial treatment
- Laboratory anomalies that require clinical intervention or further investigation (unless they are associated with an already reported clinical event)
- Abnormalities in physiological testing or physical examination that require further investigation or clinical intervention
- Injury or accidents
- Changes in libido

11.3.2 Do Not Include

- Medical or surgical procedures - the condition which leads to the procedure is the adverse event
- Pre-existing disease or conditions present before treatment that do not worsen
- Situations where an untoward medical occurrence has occurred e.g. cosmetic elective surgery
- Overdose of medication without signs or symptoms
- The disease being treated or associated symptoms/signs unless more severe than expected for the patient's condition
- Neoadjuvant chemotherapy or endocrine therapy related adverse events (see Section 11.3.4)

11.3.3 Notes on Adverse Events Relating to Change in Libido

Patients should be asked at each visit if they have had a change in libido since baseline. Changes in libido were recorded in mouse model trials of enobosarm but there is no data to support this in humans. Changes in libido should be recorded according to the NCI-CTCAE Version 4.03, however the grading system for this event may not apply to all patients so instead a grading of 1 – mild, 2 – moderate, 3 – high should be used based on the patients answers.

11.3.4 Notes on Adverse Events Relating to Neoadjuvant Chemotherapy or Endocrine Therapy

Any adverse events or serious adverse events relating to neoadjuvant chemotherapy or endocrine therapy should NOT be reported to the LCTC, unless there is suspicion that these may also be related to enobosarm or the research core biopsy. If the AE is not possibly, probably or highly probably related to the chemotherapy or endocrine therapy drug then the adverse event should be reported to the LCTC. If the relationship to chemotherapy, endocrine therapy, enobosarm or the research core biopsy is unclear the AE/SAE should be reported.

11.4 Notes Severity/Grading of Adverse Events

Regardless of the classification of an AE as serious or not, its severity must be assessed according to medical criteria alone.

The assignment of the severity/grading should be made by the investigator responsible for the care of the patient using the NCI-CTCAE Version 4.03.

If the AE/SAE is not covered by the NCI-CTCAE then the investigator should use the definitions below.

Mild: does not interfere with routine activities
Moderate: interferes with routine activities
Severe: impossible to perform routine activities
Life threatening
Death

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity (see above) whereas seriousness is defined using the criteria in section 11.1, hence, a severe AE need not necessarily be a Serious Adverse Event.

11.5 Relationship to Trial Treatment

The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in Table 4.
If any doubt about the causality exists the local investigator should inform the study coordination centre who will notify the Chief Investigators. In the case of discrepant views on causality between the investigator and others, the MHRA will be informed of both points of view.

Table 4: Definitions of Causality

Relationship	Description
None	There is no evidence of any causal relationship. N.B. An alternative cause for the AE should be given
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).
Possibly	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
Probably	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Highly Probable	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

11.6 Expectedness

An AE whose causal relationship to the study drug is assessed by the investigator as "possible", "probable", or "highly probable" is an Adverse Drug Reaction.
All events judged by the investigator to be possibly, probably, or highly probably related to the IMP, graded as serious and **unexpected** should be reported as a SUSAR. In accordance with the Reference Safely Information (See Section 11.7) there are no expected serious adverse reactions, therefore all events that are deemed related and serious must be reported as SUSARs.

11.7 Reference Safety Information

The Reference Safety Information (RSI) for the IMP used in this trial is as follows:

Enobosarm (GTx – 024) – Investigator Brochure – Section 8.1, Appendix A

GTx-024 is the pharmaceutical company (GTx Inc.) reference name for enobosarm, the brand name is Ostarine.

The current version RSI documents applicable to the trial for the current reporting period are available on the LCTC portal (www.lctu.org.uk).

11.8 Follow-up After Adverse Events

All adverse events should be followed until satisfactory resolution or until the investigator responsible for the care of the patient deems the event to be chronic or the patient to be stable.
When reporting SAEs and SUSARs the investigator responsible for the care of the patient should apply the following criteria to provide information relating to event outcomes: resolved; resolved with sequelae (specifying with additional narrative); not resolved/ongoing; ongoing at final follow-up; fatal or unknown.

11.9 Reporting Procedures

All adverse events should be reported from the point of consent until 21 -28 days post treatment, depending on the patient's scheduled follow-up visit. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the LCTC in the first instance.

11.9.1 Non serious ARs/AEs

All such events, whether expected or not, should be recorded on the AE Form and sent to the LCTC via post, email or fax within 30 days of completion of the follow-up visit.

11.9.2 Serious ARs/AEs/SUSARs

SARs, SAEs and SUSARs should be reported within 24 hours of the local site becoming aware of the event via email or fax using the SAE Form, either method should be accompanied by a

phone call. The SAE Form asks for the nature of event, date of onset, severity, corrective therapies given/whether treatment was stopped, outcome and causality. The responsible investigator should sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

The LCTC will notify the MHRA and REC of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study. Local investigators should report any SUSARs and/or SAEs as required by their R&D Office.

11.10 Responsibilities – Investigator

The investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study product.

All SAEs must be reported immediately by the investigator to the LCTC on an SAE form (available on the LCTC Portal) unless the SAE is specified in the protocol or IB as not requiring immediate reporting. All other adverse events should be reported on the Adverse Events Form.

Minimum information required for reporting:

- Study identifier
- Study centre
- Patient number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

i. The SAE form should be completed by the responsible investigator i.e. the consultant named on the 'signature list and delegation of responsibilities log' who is responsible for the patient's care. The investigator should assess the SAE for the likelihood that it is a response to an investigational medicine. In the absence of the responsible investigator the form should be completed and signed by a designated member of the site trial team and faxed to the LCTC immediately. The responsible investigator should check the SAE form, make changes as appropriate, sign and then re-fax to the LCTC as soon as possible. The initial report shall be followed by detailed, written reports.

ii. Send the SAE form by fax (within 24 hours or next working day) to the LCTC

Fax Number: 0151 794 8250

If no fax machine is available then the SAE form should be uploaded to the LCTC Portal and the Trial Coordinator informed by telephone. This process is detailed in the work instruction Transferring Files via the LCTC Portal.

iii. The responsible investigator must **notify** their R&D department of the event (as per standard local procedure).
iv. In the case of an SAE the patient must be followed-up until clinical recovery is complete and laboratory results have returned to normal, or until the event has stabilised. Follow-up may continue after completion of protocol treatment if necessary.
v. Follow-up information is noted on another SAE form by ticking the box marked 'follow-up' and faxing to the LCTC as information becomes available. Extra, annotated information and/or copies of test results may be provided separately.
vi. The patient **must** be identified by trial number, date of birth and initials only. The patient's name **should not** be used on any correspondence.

11.11 Responsibilities – CRUK LCTC

The LCTC is undertaking duties delegated by the trial sponsor, University of Liverpool, and is responsible for the reporting of SUSARs and other SARs to the regulatory authorities (MHRA, competent authorities of other European member states in which the trial is taking place and, if required, the REC) as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the LCTC is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the LCTC first becoming aware of the reaction.
- A list of all SARs (expected and unexpected) must be reported annually.

It is recommended that the following safety issues should also be reported in an expedited fashion:

- An increase in the rate of occurrence or a qualitative change of an expected serious adverse reaction, which is judged to be clinically important
- Post-study SUSARs that occur after the patient has completed a clinical trial and are notified by the investigator to the sponsor
- New events related to the conduct of the trial or the development of the IMPs and likely to affect the safety of the patients, such as:
    a. A serious adverse event which could be associated with the trial procedures and which could modify the conduct of the trial
    b. A significant hazard to the patient population, such as lack of efficacy of an IMP used for the treatment of a life-threatening disease
    c. A major safety finding from a newly completed animal study (such as carcinogenicity)
    d. Any anticipated end or temporary halt of a trial for safety reasons and conducted with the same IMP in another country by the same sponsor
- Recommendations of the Data Monitoring Committee, if any, where relevant for the safety of the patients

Staff at the LCTC will liaise with the designated Clinical Co-ordinator who will evaluate all SAEs received for seriousness, expectedness and causality. Investigator reports of suspected SARs will be reviewed immediately and those that are SUSARs identified and reported to regulatory authorities and REC. The causality assessment given by the Local Investigator at the hospital cannot be overruled and in the case of disagreement, both opinions will be provided with the report.

The LCTC will also send an annual safety report containing a list of all SARs to regulatory authority and REC.

All SAEs and SARs will be reported to GTx, Inc. via CMED upon resolution. SUSARs and all follow-up information will be reported to GTx, Inc. and CMED concurrently to reports made to the competent authority as above (within 7 days for SUSARs which are fatal or life-threatening and within 15 days for SUSARs that are not fatal or life-threatening). Any additional information will be provided within 7 days of receipt at the LCTC. Furthermore a full list of AEs will be provided annually. Reports to CMED will be automatically generated via the MACRO Pharmacovigilance database.

Patient safety incidents that take place in the course of research should be reported to the National Patient Safety Agency (NPSA) by each participating NHS Trust in accordance with local reporting procedures.

12 ETHICAL CONSIDERATIONS

12.1 Ethical Considerations

The trial will be conducted to conform to the principles of the Declaration of Helsinki as adopted by the 18th World Medical Assembly, 1964, and amendments.

The study will be conducted in accordance with the EU Directive 2001/20/EC, the Medicines for Human Use (Clinical Trials) Regulations 2004 and the principles of Good Clinical Practice.

Patients will be asked to consent that data are recorded, collected, stored and processed and may be transferred to other countries, in accordance with any national legislation implementing the EU Data Protection Directive (95/46/EC).

This study may be terminated at the request of the CI, TSG, REC, IDSMC or the MHRA if, during the course of the study, concerns about the safety of further dosing emerge.

The CI will update the REC of any new information related to the study drug when appropriate and this will also be disseminated to the Principal Investigators at each trial centre.

- Enobosarm could theoretically stimulate proliferation in some tumours. This would be an important finding and once the subtype was formally identified would represent an exclusion from future enobosarm/SARM studies
- Patients may experience side effects as a result of enobosarm
- Patients, as a result of providing translational blood samples, may experience additional pain, bruising, infection or inflammation at the sample site, and/or feel faint during the blood collection procedure.
- Patients will have to make additional clinic visits whilst on the study treatment and if they require a research core biopsy (if this does not coincide with insertion of a marker clip)

12.2 Patients who will require a research core biopsy in order to join the study will be asked for specific consent on the informed consent form. The procedure and the additional risks will be described in the patient information sheet and it will be made clear the procedure is optional. Ethical Approval

The trial protocol has received the favourable opinion of the Haydock Research Ethics Committee which, in line with HRA Approval, has also been given on behalf of all research sites. A copy of all site approval documents and a copy of the PIS and ICF on local headed paper should be forwarded to LCTC before patients are entered. The REC Number for EMERALD is 16/NW/0807.

12.3 Informed Consent Process

Informed consent is a process initiated prior to an individual agreeing to participate in a trial and continues throughout the individual's participation. Informed consent is required for all patients participating in LCTC coordinated trials. In obtaining and documenting informed

consent, the investigator should comply with applicable regulatory requirements and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki.

Discussion of objectives, risks and inconveniences of the trial and the conditions under which it is to be conducted are to be provided to patients by staff with appropriate experience. An appropriate Patient Information Sheet and Consent Form, describing in detail the trial interventions/products, trial procedures and risks will be approved by a REC and the patient will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the patient and answer any questions that may arise. A contact point where further information about the trial may be obtained will be provided.

After being given adequate time to consider the information, the patient will be asked to sign the informed consent document. A copy of the informed consent document will be given to the patient for their records, a copy will be sent to the LCTC at the time of randomisation and a copy will be placed in the medical records, with the original retained in the Investigator Site File.

The patient may withdraw from the trial at any time by revoking the informed consent. The rights and welfare of the patients will be protected by emphasising to them that the quality of medical care will not be adversely affected if they decline to participate in this study.

As part of the trial an additional test will be carried out on all screened patients for AnR status. Ethical approval has been given NOT to seek informed consent from patients to carry out this test in order to:

- save crucial time in identifying the correct cohort for the study
- avoid approaching and consenting ineligible patients for the study
- reduce patient burden by avoiding an additional visit/consent process
- save research nurse/PI time

The following has been considered as part of this approval:

- the test does not require any additional procedures involving the patient, only the biopsy that has already been taken
- the testing will not delay or effect standard clinical care
- results of the staining will be made available to clinicians for all patients tested
- if the test is negative or the patient does not consent to the study no further tests will be performed on this sample
- if the patient does not consent to the study no further additional tests will be performed
- the test is similar to the ER, PR and HER2 testing already done, it uses a different antibody

- patient identifiable data will not leave the NHS site conducting the research until informed consent to join the study has been gained
- the trial has funding to pay for the additional AR testing
- the staining is not a genetic test
- where possible information about this test will be provided on the clinical letter to the patient before the test is done

12.4 Study Discontinuation

In the situation in which the study is prematurely discontinued (e.g. due to safety) the reason should be written in the end of study form for each patient and all patients must be informed. If a patient has been withdrawn completely from the trial whilst the study is ongoing, an end of study form should be completed.

13 REGULATORY APPROVAL

This trial has been registered with the MHRA and has been granted a Clinical Trial Authorisation (CTA). The CTA reference is 04196/0043/001-0001.

14 TRIAL MONITORING

Central and on site monitoring will be conducted to ensure protection of patients participating in the trial, and that trial procedures including study treatment administration and sample and data collection processes are of high quality and meet sponsor and regulatory requirements as appropriate. A risk assessment will be carried out to determine the level of monitoring required, and a substantial monitoring plan will be developed to document who will conduct the central and on site monitoring; and the frequency and detail of monitoring required to be conducted. A monitoring plan has been created for the study and is available in the Trial Master File, this will be the principal document with regard to monitoring.

14.1 Risk Assessment

In accordance with the LCTC SOPs a risk assessment has been completed in partnership with:

- Representatives of the Trial Sponsors
- Chief Investigator
- Trial Coordinator
- Trial Statistician
- LCTC Operational Director

In conducting this risk assessment, the contributors considered potential patient, organisational and study hazards, the likelihood of their occurrence and resulting impact should they occur.

The outcome of the risk assessment is categorised into three groups:

CTIMP Type A = Comparable to the risk of standard medical care.

CTIMP Type B = Somewhat higher than the risk of standard medical care.

CTIMP Type C = Markedly higher than the risk of standard medical care.

The risk assessment resulted in this trial being categorised as a Type C CTIMP.

14.2 Source Documents

- Source data is all information, original records of clinical findings, observations or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patient files, and records kept at the pharmacy and laboratory departments involved in the clinical trial.

- Each participating site should maintain appropriate medical and research records for this trial, in compliance with ICH E6 GCP, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of patients.
- Source data will include the patient completed drug diary which should be entered into the database and stored at site until requested by the TC or until archiving. Any data collected directly from the patient at visits for the purposes of study data collection should be noted concurrently in the patient notes as source data, e.g. height, weight, ECOG Performance Status, temperature.

14.3 Data Capture Methods

Trial data will be captured using remote data entry at research sites. This does not include AE and SAE data, which should be sent using the AE and SAE Forms. You may complete paper copies of the workbooks as an aid to data collection, however data should be entered via eCRF on MACRO. The LCTC do not require copies of the workbooks, however a paper copy of the ICF is still required. If MACRO is not in service and the deadline for data entry completion is close you may send a paper copy of the workbook to the LCTC for data entry, however this must first be discussed with the EMERALD Data Manager by telephone.

14.3.1 Electronic Case Report Forms

The study electronic case report form (eCRF) is the primary data collection instrument for the study. All data requested on the eCRF must be recorded. All missing data must be explained. Data should be entered in a timely fashion, with all data being entered within 2 weeks of the patient's final follow-up.

A guide for entering data on MACRO will be available in the LCTC PORTAL Investigator Site File section and training will be given to delegated staff at the Site Initiation Visit.

14.4 Monitoring at LCTC

Data stored at LCTC will be checked for missing or unusual values (range checks) and checked for consistency within patients over time. If any such problems are identified, a PDF list of queries will be emailed to the local site for checking and confirmation or correction as appropriate. The PDF list should be completed and the original copy returned to the LCTC or alternatively data can be amended on MACRO and in the data query (data clarification request on MACRO) should be updated, the LCTC should be informed that the query has been updated by site staff. LCTC will send reminders for any overdue and missing data.

There are a number of monitoring features in place at the LCTC to ensure reliability and validity of the trial data.

14.5 Clinical Site Monitoring

14.5.1 Direct access to data

In order to perform their role effectively, monitors and persons involved in quality assurance and inspection will need direct access to primary patient data, e.g. patient records, laboratory reports, appointment books, etc. As this affects the patient's confidentiality, this fact is included on the Patient Information Sheet and Informed Consent Form.

14.5.2 Confidentiality

Individual patient medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited.

Samples will be transferred to the GCLP facility and will be identifiable by the patient's unique trial number only. Consent forms sent to the LCTC as part of the registration process may contain patient identifiers for the purpose of monitoring as described in the trial risk assessment. Such information will be stored separately from clinical data in secure, locked cabinets.

14.5.3 Quality Assurance and Quality Control of Data

Systems of quality assurance, including all elements described in this protocol have been/will be implemented within relevant institutions with responsibility for this trial. Quality control is applied to each stage of data handling to ensure that data are accurate, reliable and processed correctly.
The EMERALD trial centres, facilities, laboratories and all data (including sources) and documentation must be available for GCP audit and inspection by competent authorities or REC. Such audits/inspections may take place at any trial centre where the trial related activity is taking place (the Sponsor, trial centres, LCTC, or at any investigator's centre including laboratories, pharmacies etc.).

The trial centre staff should assist in all aspects of audit/inspection and be fully cognisant of the LCTC communication strategy for multicentre trials. This includes management systems such as the Green Light Process which conforms to the total Quality Management System currently operating within the LCTC.

14.6 Record Retention

The investigator at each investigational site must make arrangements to store the essential trial documents, (as defined in Essential Documents for the Conduct of a Clinical Trial (ICH E6, Guideline for Good Clinical Practice)) including the Investigator Trial File, until the LCTC informs the investigator that the documents are no longer to be retained. In addition, the investigator is responsible for the archiving of all relevant source documents so that the trial data can be compared against source data after completion of the trial (e.g. in case of inspection from authorities).The investigator is required to ensure the continued storage of the documents, even if the investigator, for example, leaves the clinic/practice or retires before the end of required storage period. Delegation must be documented in writing.

The LCTC undertakes to store original documents completed for the trial research e.g. ICF for the same period, except for source documents pertaining to the individual investigational site, which are kept by the investigator only.
At the point where it is decided that the trial documentation is no longer required; the investigator will be responsible for the destruction of all site trial specific documentation and

the Sponsor/LCTC will be responsible for the destruction of all trial related materials retained by the Sponsor/LCTC.

Verification of appropriate informed consent will be enabled by the provision of copies of patients' signed informed consent/assent forms being supplied to the LCTC by recruiting centres. This requires that name data will be transferred to the LCTC, which is explained in the PISC. The LCTC will preserve the confidentiality of patients taking part in the study and the University of Liverpool is a Data Controller registered with the Information Commissioners Office.

15 INDEMNITY

EMERALD is sponsored by The University of Liverpool and co-ordinated by the LCTC in the University of Liverpool. The University of Liverpool does not hold insurance against claims for compensation for injury caused by participation in a clinical trial and they cannot offer any indemnity. As this is an investigator-initiated study, The Association of the British Pharmaceutical Industry (ABPI) guidelines for patient compensation by the pharmaceutical industry do not apply. However, in terms of liability, NHS Trust and Non-Trust Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical trial, and they are legally liable for the negligent acts and omission of their employees. Compensation is therefore available in the event of clinical negligence being proven.

Clinical negligence is defined as:
"A breach of duty of care by members of the health care professions employed by NHS bodies or by others consequent on decisions or judgments made by members of those professions acting in their professional capacity in the course of their employment, and which are admitted as negligent by the employer or are determined as such through the legal process".

Indemnity for legal liability
The potential legal liabilities of the sponsor, of individuals contracted to undertake duties on behalf of the sponsor and of personnel involved in the EMERALD study are provided for through the NHS indemnity scheme.

16 FINANCIAL ARRANGEMENTS

This is a non-commercial trial, and no direct payments are available to cover the costs associated with patient recruitment, treatment administration, follow-up visits, data collection or reasonable travel expenses. The trial is endorsed by Cancer Research UK, consequently having automatic endorsement from the National Cancer Research Network (NCRN) and UK Clinical Research Network (UKCRN). These organisations will be responsible for providing local investigators with the necessary research infrastructure.

Enobosarm is supplied free of charge by GTx, Inc. for the duration of the EMERALD study.

A per patient payment will be made to cover the cost of AnR testing, eligibility checks, translational blood sample processing and collection of FFPE material.

A per test payment will be made for AnR testing on patients who are AnR negative (not eligible for the trial) or who are AnR positive but do not give informed consent to join the trial.

17 TRIAL OVERSIGHT COMMITTEES

17.1.1 Trial Management Group (TMG)

A Trial Management Group (TMG) will be formed comprising the Chief Investigator, other lead investigators (clinical and non-clinical) and members of the LCTC (e.g. Trial Coordinator, Trial Statistician, Data Manager and Monitor). The TMG will be responsible for the day-to-day running and management of the trial and will be in frequent contact.

17.1.2 Trial Steering Committee (TSC)

The Trial Steering Committee will consist of an independent chairperson, independent experts in the field of medical oncology, an independent biostatistician, up to seven Principal Investigators and members of the TMG. Dr Adrienne Morgan of Independent Cancer Patient's Voice (ICPV) will also sit on the TSC as a patient representative. The role of the TSC is to provide overall supervision for the trial and provide advice through its independent chairperson. The ultimate decision for the continuation of the trial lies with the TSC. The membership of the Trial Steering Committee is available in the Trial Master File, in Section 17 Oversight in the TSC Charter.

17.1.3 Independent Safety and Data Monitoring Committee (ISDMC)

The Independent Data and Safety Monitoring Committee (IDSMC) consists of an independent chairperson, an independent expert in the field of breast cancer and an independent expert in medical statistics. The membership of the ISDMC is recorded in the ISDMC Charter which is available in the Trial Master File, Section 17 Oversight.

The IDSMC will be responsible for reviewing and assessing recruitment, interim monitoring of safety and effectiveness, trial conduct and external data. The IDSMC will first convene before the trial opens and will then define frequency of subsequent meetings (at least annually). Details of the interim analysis and monitoring are provided in section 10.

The IDSMC will provide a recommendation to the Trial Steering Committee concerning the continuation of the study.

18 PUBLICATION

The results from different centres will be analysed together and published as soon as possible. Individual clinicians must undertake not to submit any part of their individual data for publication without the prior consent of the Trial Management Group.

The Trial Management Group will form the basis of the Writing Committee and advise on the nature of publications. The Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org/) will be respected. All publications shall include a list of patients, and if there are named authors, these should include the trial's Chief Investigator, Statistician(s) and Trial Coordinator involved at least. If there are no named authors (i.e. group authorship) then a writing committee will be identified that would usually include these people, at least. The ISRCTN allocated to this trial should be attached to any publications resulting from this trial.

The members of the TSC and IDSMC should be listed with their affiliations in the acknowledgements/appendix of the main publication.

External organisations and collaborators can apply for access to data generated by LCTC operational activity. Data sharing is encouraged to promote research and maximise potential benefits of these data, although a limited period (defined in consultation with lead applicant/CI) of exclusive use of data for primary research is reasonable.

Applications to share should be addressed to the CI outlining requirements. Applications will be reviewed by the LCTC in partnership with the CI via the trial adoption committee. LCTC will ensure that new studies that result from data sharing meet the high standards (quality, ethical and financial) maintained by LCTC. New studies must also add recognisable value to the original dataset. LCTC should receive full recognition if data are shared for the purposes of secondary research. LCTC will make data available for sharing for a minimum of five years following the end of a research grant.

Data may be shared under the following conditions:
- The data are to be used for research purposes
- The research is supported by CRUK
- Projects that transform or link pre-existing datasets

LCTC istaff are all trained in the principles of Data Protection and are responsible for the security and transmission of all shared data. The IS team will determine the best method and format for transfer. Metadata will be provided to describe the data and associated format. Data will be encrypted prior to any transmission. Any data sent using the Internet will be encrypted during transfer using SSL. A Material Transfer Agreement will be put in place prior to any data transfer.

All personal data will be anonymised and the necessary permissions (ethical, legal and institutional) must be obtained prior to sharing. Commercial confidentiality will be maintained when publishing or sharing data.

19 PROTOCOL AMENDMENTS

19.1 Version 1 19/Sep/2016

Original Approved version.

19.2 Version 2 08/Dec/2016

Updated inclusion/exclusion to clarify males are excluded and only postmenopausal females included
Specified temperature to be taken as part of physical examination in Section 6.1
Change of version and date of protocol

19.3 Version 3 13/Jan/2017

Change of Trial Statistician, removal of named Data Manager and Lab Technician
Clarification of trial objectives, endpoints and outcome measures
Clarification of trial design and end of trial definition
Removal of references to blood samples being taken at the Follow-up Visit
Removal of PSA, LH and FSH from trial procedures
Change of version and date of protocol
Changes to statistical analysis method, interim analysis and minor and major violations
Update to inclusion criteria
Minor error corrections (mistypes, spelling, etc.) including clarification of Visit and Day
Change of use of the word 'subject' to 'patient'
Update to drug compliance section in line with pharmacy procedures
ISRCTN number, REC name and CTA number added
Addition of abbreviations
Updated reference safety information section (this does not change the reference safety information itself)

19.4 Version 4 30/Aug/2017

Version and date of the protocol updated
Director of Liverpool Cancer Trials Unit on signature page updated in line with staff change
Updated contact details for some staff members
Sample size changed to 147
Changed bullet points to numbered bullets on the inclusion/exclusion criteria
Duration of trial updated from 2 years to 14 months
Minor corrections and clarifications
Changed Mid-Treatment Visit to a phone call and removed assessments not possible over the phone
Updated visit numbers (Surgery Visit becomes Visit 2 and Follow-Up Visit becomes Visit 3)
Changed the Follow-Up Visit from Day 28 (+4 days) to Day 21-28 (+4 days) in line with pre-scheduled follow-up visits
Added caveat that patients can be recruited on 12 and 13 days before surgery at the discretion of the Chief Investigator
Added caveat that if a patient's surgery is delayed for any reason that treatment may continue until the surgery at the discretion of the Chief Investigator added

Clarification regarding the physical examination procedures
Removal of use of LCTU MACRO Pharmacovigilance database by sites
Postmenopausal definition in inclusion criteria updated
Inclusion criteria missed from protocol summary added (acceptable risk of bleeding)
Exclusion criteria (Evidence of bleeding diathesis) removed as it is a duplication of an inclusion criteria
Clarifications to the Statistical Considerations Section
Updated Trial Treatment Section and references to IMP/Pharmacy procedures following removal of mid-treatment visit (one dispensation of IMP)
Removed stipulation of physical examination within 7 days of starting treatment
Updated screening timelines to allow 28 days for core biopsy, scans and tumour measurement
Removal of 18 hour stoppage time for enobosarm before surgery
Clarification of sample requirements
Stipulation that a core biopsy of the resected tumour tissue should be used for FFPE block at surgery (ideally)
Pharmacy procedures have been updated to remove mid-treatment visitbottles of IMP at baseline.
Classification of a serious AE/AR f) removed
Changes in libido and note regarding reasons included in the adverse events inclusion list added
REC number added
Clarification on how data queries should be returned and completed

19.5 Version 5 30/Mar/2018

Exclusion criteria updated to Previous use of oestrogen implants within the last 12 weeks (from at ANY time)
Inclusion of neoadjuvant chemotherapy or endocrine therapy patients to the trial
Where surgery has been referred to for tissue collection, research core biopsy has been added as a method of tissue collection for neoadjuvant chemotherapy and endocrine therapy patients
Where surgery has been referred to as the first standard of care treatment neoadjuvant chemotherapy and endocrine therapy have been added
Updated trial schema
Endpoints and outcome measures changed from based on serum levels instead of plasma levels.
Minor typos and phrases corrected, updated lab contact details, updated version date and number
Changed name of Visit 2 from Pre-surgical/Surgery Visit to Tissue Collection Visit – updated in Section 7.2 Schedule of Trial Procedures and Section 7.2.1 Trial Schedule
Added clarifications to the schedule of trial procedures for the research core biopsy and surgery tissue collection
ECG is no longer a required trial procedure. Changes to Section 7.3.3 ECG and Section 7.2 Schedule of Trial Procedures
Updated Follow-Up Visit schedule to allow for patients who will not routinely have a hospital visit to have a phone call follow-up instead. Removed the requirement for follow-up blood investigations if the blood results from Tissue Collection Visit were normal.
Section on Research Core Biopsy added to Section 7.3.6
Updated Section 8 to include information regarding research core biopsy and collecting samples

Caveat not to report adverse events related to neoadjuvant chemotherapy or endocrine therapy (Sections 11.3.2 and 11.3.4)
Added the risks regarding the research core biopsy and the additional patient visits to the ethical considerations section.

20 REFERENCES

Adair, F. E., and J. B. Herrmann. 1946. 'The use of testosterone propionate in the treatment of advanced carcinoma of the breast', *Ann Surg*, 123: 1023-35.

Baselga, J., M. Campone, M. Piccart, H. A. Burris, 3rd, H. S. Rugo, T. Sahmoud, S. Noguchi, M. Gnant, K. I. Pritchard, F. Lebrun, J. T. Beck, Y. Ito, D. Yardley, I. Deleu, A. Perez, T. Bachelot, L. Vittori, Z. Xu, P. Mukhopadhyay, D. Lebwohl, and G. N. Hortobagyi. 2012. 'Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer', *N Engl J Med*, 366: 520-9.

Baum, M., A. Buzdar, J. Cuzick, J. Forbes, J. Houghton, A. Howell, T. Sahmoud, and Atac Trialists' Group. 2003. 'Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early-stage breast cancer: results of the ATAC (Arimidex, Tamoxifen Alone or in Combination) trial efficacy and safety update analyses', *Cancer*, 98: 1802-10.

Birrell, S. N., J. M. Bentel, T. E. Hickey, C. Ricciardelli, M. A. Weger, D. J. Horsfall, and W. D. Tilley. 1995. 'Androgens induce divergent proliferative responses in human breast cancer cell lines', *J Steroid Biochem Mol Biol*, 52: 459-67.

Bohl, Casey E., Wenqing Gao, Duane D. Miller, Charles E. Bell, and James T. Dalton. 2005. 'Structural basis for antagonism and resistance of bicalutamide in prostate cancer', *Proceedings of the National Academy of Sciences of the United States of America*, 102: 6201-06.

Boni, C., M. Pagano, M. Panebianco, A. Bologna, N. M. Sierra, R. Gnoni, D. Formisano, and G. Bisagni. 2014. 'Therapeutic activity of testoterone in metastatic breast cancer', *Anticancer Res*, 34: 1287-90.

Bonneterre, J., A. Buzdar, J. M. Nabholtz, J. F. Robertson, B. Thurlimann, M. von Euler, T. Sahmoud, A. Webster, M. Steinberg, Committee Arimidex Writing, and Members Investigators Committee. 2001. 'Anastrozole is superior to tamoxifen as first-line therapy in hormone receptor positive advanced breast carcinoma', *Cancer*, 92: 2247-58.

Buchanan, G., S. N. Birrell, A. A. Peters, T. Bianco-Miotto, K. Ramsay, E. J. Cops, M. Yang, J. M. Harris, H. A. Simila, N. L. Moore, J. M. Bentel, C. Ricciardelli, D. J. Horsfall, L. M. Butler, and W. D. Tilley. 2005. 'Decreased androgen receptor levels and receptor function in breast cancer contribute to the failure of response to medroxyprogesterone acetate', *Cancer Res*, 65: 8487-96.

'Cancer Research UK. Cancer Stats Key Facts Breast Cancer.'. 2013.

Carey, L. A., C. M. Perou, C. A. Livasy, L. G. Dressler, D. Cowan, K. Conway, G. Karaca, M. A. Troester, C. K. Tse, S. Edmiston, S. L. Deming, J. Geradts, M. C. Cheang, T. O. Nielsen, P. G. Moorman, H. S. Earp, and R. C. Millikan. 2006. 'Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study', *JAMA*, 295: 2492-502.

Castellano, I., E. Allia, V. Accortanzo, A. M. Vandone, L. Chiusa, R. Arisio, A. Durando, M. Donadio, G. Bussolati, A. S. Coates, G. Viale, and A. Sapino. 2010. 'Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers', *Breast Cancer Res Treat*, 124: 607-17.

Chen, J., J. Kim, and J. T. Dalton. 2005. 'Discovery and therapeutic promise of selective androgen receptor modulators', *Mol Interv*, 5: 173-88.

Chia, S., W. Gradishar, L. Mauriac, J. Bines, F. Amant, M. Federico, L. Fein, G. Romieu, A. Buzdar, J. F. Robertson, A. Brufsky, K. Possinger, P. Rennie, F. Sapunar, E. Lowe, and M. Piccart. 2008. 'Double-blind, randomized placebo controlled trial of fulvestrant compared with exemestane after prior nonsteroidal aromatase inhibitor therapy in

postmenopausal women with hormone receptor-positive, advanced breast cancer: results from EFECT', *J Clin Oncol*, 26: 1664-70.

Clarke, R. B., I. J. Laidlaw, L. J. Jones, A. Howell, and E. Anderson. 1993. 'Effect of tamoxifen on Ki67 labelling index in human breast tumours and its relationship to oestrogen and progesterone receptor status', *Br J Cancer*, 67: 606-11.

Cole, M. P., C. T. Jones, and I. D. Todd. 1971. 'A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI46474', *Br J Cancer*, 25: 270-5.

Collins, L. C., K. S. Cole, J. D. Marotti, R. Hu, S. J. Schnitt, and R. M. Tamimi. 2011. 'Androgen receptor expression in breast cancer in relation to molecular phenotype: results from the Nurses' Health Study', *Mod Pathol*, 24: 924-31.

Coombes, R. C., P. Goss, M. Dowsett, J. C. Gazet, and A. Brodie. 1984. '4-Hydroxyandrostenedione in treatment of postmenopausal patients with advanced breast cancer', *Lancet*, 2: 1237-9.

Coss, CC., A. Jones, and JT. Dalton. 2014. 'Selective androgen receptor modulators as improved androgen therapy for advanced breast cancer', *Steroids*, 90: 94-100.

Coss, Christopher C., Matt Bauler, Ramesh Narayanan, Duane D. Miller, and James T. Dalton. 2012. 'Alanine Aminotransferase Regulation by Androgens in Non-hepatic Tissues', *Pharmaceutical Research*, 29: 1046-56.

Crawford, Jeffrey, Mary Ann Johnston, Ryan P. Taylor, James T. Dalton, and Mitchell S. Steiner. 2014. 'Enobosarm and lean body mass in patients with non-small cell lung cancer', *J Clin Oncol (Meeting Abstracts)*, 32: 9618-.

Dalton, J. T., K. G. Barnette, C. E. Bohl, M. L. Hancock, D. Rodriguez, S. T. Dodson, R. A. Morton, and M. S. Steiner. 2011. 'The selective androgen receptor modulator GTx-024 (enobosarm) improves lean body mass and physical function in healthy elderly men and postmenopausal women: results of a double-blind, placebo-controlled phase II trial', *J Cachexia Sarcopenia Muscle*, 2: 153-61.

Dalton, J. T., R. P. Taylor, M. L. Mohler, and M. S. Steiner. 2013. 'Selective androgen receptor modulators for the prevention and treatment of muscle wasting associated with cancer', *Curr Opin Support Palliat Care*, 7: 345-51.

Dauvois, S., C. S. Geng, C. Levesque, Y. Merand, and F. Labrie. 1991. 'Additive inhibitory effects of an androgen and the antiestrogen EM-170 on estradiol-stimulated growth of human ZR-75-1 breast tumors in athymic mice', *Cancer Res*, 51: 3131-5.

Dobs, A. S., R. V. Boccia, C. C. Croot, N. Y. Gabrail, J. T. Dalton, M. L. Hancock, M. A. Johnston, and M. S. Steiner. 2013. 'Effects of enobosarm on muscle wasting and physical function in patients with cancer: a double-blind, randomised controlled phase 2 trial', *Lancet Oncol*, 14: 335-45.

Dowsett, M., J. Cuzick, J. Ingle, A. Coates, J. Forbes, J. Bliss, M. Buyse, M. Baum, A. Buzdar, M. Colleoni, C. Coombes, C. Snowdon, M. Gnant, R. Jakesz, M. Kaufmann, F. Boccardo, J. Godwin, C. Davies, and R. Peto. 2010. 'Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen', *J Clin Oncol*, 28: 509-18.

Dowsett, M., I. E. Smith, S. R. Ebbs, J. M. Dixon, A. Skene, C. Griffith, I. Boeddinghaus, J. Salter, S. Detre, M. Hills, S. Ashley, S. Francis, G. Walsh, and Impact Trialists. 2005. 'Short-term changes in Ki-67 during neoadjuvant treatment of primary breast cancer with anastrozole or tamoxifen alone or combined correlate with recurrence-free survival', *Clin Cancer Res*, 11: 951s-8s.

Dowsett, M., I. Smith, J. Robertson, L. Robison, I. Pinhel, L. Johnson, J. Salter, A. Dunbier, H. Anderson, Z. Ghazoui, T. Skene, A. Evans, R. A'Hern, A. Iskender, M. Wilcox, and J. Bliss. 2011. 'Endocrine therapy, new biologicals, and new study designs for presurgical studies in breast cancer', *J Natl Cancer Inst Monogr*, 2011: 120-3.

Early Breast Cancer Trialists' Collaborative, Group, C. Davies, J. Godwin, R. Gray, M. Clarke, D. Cutter, S. Darby, P. McGale, H. C. Pan, C. Taylor, Y. C. Wang, M. Dowsett, J. Ingle, and

R. Peto. 2011. 'Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials', *Lancet*, 378: 771-84.

Ellis, M. J., A. Coop, B. Singh, Y. Tao, A. Llombart-Cussac, F. Janicke, L. Mauriac, E. Quebe-Fehling, H. A. Chaudri-Ross, D. B. Evans, and W. R. Miller. 2003. 'Letrozole inhibits tumor proliferation more effectively than tamoxifen independent of HER1/2 expression status', *Cancer Res*, 63: 6523-31.

Finn, Richard S., Miguel Martin, Hope S. Rugo, Stephen E. Jones, Seock-Ah Im, Karen A. Gelmon, Nadia Harbeck, Oleg N. Lipatov, Janice Maria Walshe, Stacy L. Moulder, Eric Roland Gauthier, Dongrui Lu, Sophia Randolph, Veronique Dieras, and Dennis J. Slamon. 2016. 'PALOMA-2: Primary results from a phase III trial of palbociclib (P) with letrozole (L) compared with letrozole alone in postmenopausal women with ER+/HER2- advanced breast cancer (ABC)', *ASCO Meeting Abstracts*, 34: 507.

Goldenberg, I. S. 1964. 'Testosterone Propionate Therapy in Breast Cancer', *JAMA*, 188: 1069-72.

Goldenberg, I. S., and M. A. Hayes. 1961. 'Hormonal therapy of metastatic female breast carcinoma. II. 2alpha-Methyl dihydrotestosterone propionate', *Cancer*, 14: 705-6.

Gonzalez-Angulo, A. M., K. Stemke-Hale, S. L. Palla, M. Carey, R. Agarwal, F. Meric-Berstam, T. A. Traina, C. Hudis, G. N. Hortobagyi, W. L. Gerald, G. B. Mills, and B. T. Hennessy. 2009. 'Androgen receptor levels and association with PIK3CA mutations and prognosis in breast cancer', *Clin Cancer Res*, 15: 2472-8.

GTx. 2015. "GTx - 024 IDB V14 Investigator Brochure Version 14 06 August 2015." In.

Guix, Marta, Nara de Matos Granja, Ingrid Meszoely, Theresa B. Adkins, Bobbye M. Wieman, Kerek E. Frierson, Violeta Sanchez, Melinda E. Sanders, Ana M. Grau, Ingrid A. Mayer, Gary Pestano, Yu Shyr, Senthil Muthuswamy, Benjamin Calvo, Helen Krontiras, Ian E. Krop, Mark C. Kelley, and Carlos L. Arteaga. 2008. 'Short Preoperative Treatment With Erlotinib Inhibits Tumor Cell Proliferation in Hormone Receptor–Positive Breast Cancers', *Journal of Clinical Oncology*, 26: 897-906.

Harper-Wynne, Catherine L., Nigel P.M. Sacks, Karyn Shenton, Fiona A. MacNeill, Paul Sauven, Ian J. Laidlaw, Zen Rayter, Stephanie Miall, Angela Howes, Janine Salter, Margaret J. Hills, Frances M. Lowe, Roger A'Hern, Nazar Nasiri, Debbie Doody, Jhangir Iqbal, and Mitchell Dowsett. 2002. 'Comparison of the Systemic and Intratumoral Effects of Tamoxifen and the Aromatase Inhibitor Vorozole in Postmenopausal Patients With Primary Breast Cancer', *Journal of Clinical Oncology*, 20: 1026-35.

Harvey, J. M., G. M. Clark, C. K. Osborne, and D. C. Allred. 1999. 'Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer', *J Clin Oncol*, 17: 1474-81.

Hickey, T. E., J. L. Robinson, J. S. Carroll, and W. D. Tilley. 2012. 'Minireview: The androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene?', *Mol Endocrinol*, 26: 1252-67.

Honma, N., R. Horii, T. Iwase, S. Saji, M. Younes, Y. Ito, and F. Akiyama. 2012. 'Clinical importance of androgen receptor in breast cancer patients treated with adjuvant tamoxifen monotherapy', *Breast Cancer*.

Hu, R., S. Dawood, M. D. Holmes, L. C. Collins, S. J. Schnitt, K. Cole, J. D. Marotti, S. E. Hankinson, G. A. Colditz, and R. M. Tamimi. 2011. 'Androgen receptor expression and breast cancer survival in postmenopausal women', *Clin Cancer Res*, 17: 1867-74.

Jones, A, CC Coss, MS Steiner, and JT Dalton. 2013. 'An overview on selective androgen receptor modulators: focus on enobosarm.', *Drugs of the Future*, 38:309-316.

Kandouz, M., A. Lombet, J. Y. Perrot, D. Jacob, S. Carvajal, A. Kazem, W. Rostene, A. Therwath, and A. Gompel. 1999. 'Proapoptotic effects of antiestrogens, progestins and androgen in breast cancer cells', *J Steroid Biochem Mol Biol*, 69: 463-71.

Kaufman, B., J. R. Mackey, M. R. Clemens, P. P. Bapsy, A. Vaid, A. Wardley, S. Tjulandin, M. Jahn, M. Lehle, A. Feyereislova, C. Revil, and A. Jones. 2009. 'Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III TAnDEM study', *J Clin Oncol*, 27: 5529-37.

Kazmin, Dmitri, Tatiana Prytkova, C. Edgar Cook, Russell Wolfinger, Tzu-Ming Chu, David Beratan, J. D. Norris, Ching-yi Chang, and Donald P. McDonnell. 2006. 'Linking Ligand-Induced Alterations in Androgen Receptor Structure to Differential Gene Expression: A First Step in the Rational Design of Selective Androgen Receptor Modulators', *Molecular Endocrinology*, 20: 1201-17.

Kennedy, B. J. 1958. 'Fluoxymesterone therapy in advanced breast cancer', *N Engl J Med*, 259: 673-5.

Kim, J., D. Wu, D. J. Hwang, D. D. Miller, and J. T. Dalton. 2005. 'The para substituent of S-3-(phenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethyl-phenyl)-propionamid es is a major structural determinant of in vivo disposition and activity of selective androgen receptor modulators', *J Pharmacol Exp Ther*, 315: 230-9.

Lea, O. A., S. Kvinnsland, and T. Thorsen. 1989. 'Improved measurement of androgen receptors in human breast cancer', *Cancer Res*, 49: 7162-7.

Leary, Alexandra, Abigail Evans, Stephen R.D. Johnston, Roger A'Hern, Judith M. Bliss, Rashmita Sahoo, Simone Detre, Benjamin P. Haynes, Margaret Hills, Catherine Harper-Wynne, Nigel Bundred, Gill Coombes, Ian Smith, and Mitch Dowsett. 2015. 'Antiproliferative Effect of Lapatinib in HER2-Positive and HER2-Negative/HER3-High Breast Cancer: Results of the Presurgical Randomized MAPLE Trial (CRUK E/06/039)', *American Association for Cancer Research*, 21: 2932-40.

Loibl, Sibylle, Berit Maria Müller, Gunter von Minckwitz, Michael Schwabe, Marc Roller, Silvia Darb-Esfahani, Beyhan Ataseven, Andreas du Bois, Annette Fissler-Eckhoff, Bernd Gerber, Uwe Kulmer, Jens-Uwe Alles, Keyur Mehta, and Carsten Denkert. 2011. 'Androgen receptor expression in primary breast cancer and its predictive and prognostic value in patients treated with neoadjuvant chemotherapy', *Breast Cancer Research and Treatment*, 130: 477-87.

Lonning, P. E., E. Bajetta, R. Murray, M. Tubiana-Hulin, P. D. Eisenberg, E. Mickiewicz, L. Celio, P. Pitt, M. Mita, N. K. Aaronson, C. Fowst, A. Arkhipov, E. di Salle, A. Polli, and G. Massimini. 2000. 'Activity of exemestane in metastatic breast cancer after failure of nonsteroidal aromatase inhibitors: a phase II trial', *J Clin Oncol*, 18: 2234-44.

Martin, L. A., G. L. Davies, M. T. Weigel, N. Betambeau, M. J. Hills, J. Salter, G. Walsh, R. A'Hern, and M. Dowsett. 2010. 'Pre-surgical study of the biological effects of the selective cyclo-oxygenase-2 inhibitor celecoxib in patients with primary breast cancer', *Breast Cancer Res Treat*, 123: 829-36.

Micello, Donata, Alessandro Marando, Nora Sahnane, Cristina Riva, Carlo Capella, and Fausto Sessa. 2010. 'Androgen receptor is frequently expressed in HER2-positive, ER/PR-negative breast cancers', *Virchows Archiv*, 457: 467-76.

Mohler, M. L., C. E. Bohl, A. Jones, C. C. Coss, R. Narayanan, Y. He, D. J. Hwang, J. T. Dalton, and D. D. Miller. 2009. 'Nonsteroidal selective androgen receptor modulators (SARMs): dissociating the anabolic and androgenic activities of the androgen receptor for therapeutic benefit', *J Med Chem*, 52: 3597-617.

Narayanan, R., SH. Ahn, MS. Steiner, and JT. Dalton. 2013. 'Nonsteroidal, Tissue Selective Androgen Receptor Modulators (SARMs) as Targeted Therapy for the Treatment of Androgen Receptor-Positive Breast Cancer', *Gordon Conference*.

Narayanan, R., C. C. Coss, M. Yepuru, J. D. Kearbey, D. D. Miller, and J. T. Dalton. 2008. 'Steroidal androgens and nonsteroidal, tissue-selective androgen receptor modulator,

S-22, regulate androgen receptor function through distinct genomic and nongenomic signaling pathways', *Mol Endocrinol*, 22: 2448-65.

Narayanan, Ramesh, Michael L. Mohler, Casey E. Bohl, Duane D. Miller, and James T. Dalton. 2008. 'Selective androgen receptor modulators in preclinical and clinical development', *Nuclear Receptor Signaling*, 6: e010.

Niemeier, Leo A., David J. Dabbs, Sushil Beriwal, Joan M. Striebel, and Rohit Bhargava. 2009. 'Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation', *Mod Pathol*, 23: 205-12.

Nieto, C. M., L. C. Rider, and S. D. Cramer. 2014. 'Influence of stromal-epithelial interactions on androgen action', *Endocr Relat Cancer*, 21: T147-60.

Osborne, C. K., and R. Schiff. 2011. 'Mechanisms of endocrine resistance in breast cancer', *Annu Rev Med*, 62: 233-47.

Overmoyer, B., P. Sanz-Altimira, AH. Partridge, M. Extermann, J. Liu, E. Winer, N. Lin, M. Hassett, L. Parker, R. Taylor, M. Hancock, S. Small, and MA. Johnston. 2014. 'Enobosarm for the treatment of metastatic, estrogen and androgen receptor positive, breast cancer. Final results of the primary endpoint and current progression free survival.', *San Antonio Breast Cancer Sympousim 2014*, P1-13-04.

Palmieri, C., D. K. Patten, A. Januszewski, G. Zucchini, and S. J. Howell. 2014. 'Breast cancer: current and future endocrine therapies', *Mol Cell Endocrinol*, 382: 695-723.

Park, S., J. Koo, H. S. Park, J. H. Kim, S. Y. Choi, J. H. Lee, B. W. Park, and K. S. Lee. 2010. 'Expression of androgen receptors in primary breast cancer', *Ann Oncol*, 21: 488-92.

Perou, C. M., T. Sorlie, M. B. Eisen, M. van de Rijn, S. S. Jeffrey, C. A. Rees, J. R. Pollack, D. T. Ross, H. Johnsen, L. A. Akslen, O. Fluge, A. Pergamenschikov, C. Williams, S. X. Zhu, P. E. Lonning, A. L. Borresen-Dale, P. O. Brown, and D. Botstein. 2000. 'Molecular portraits of human breast tumours', *Nature*, 406: 747-52.

Peters, A. A., G. Buchanan, C. Ricciardelli, T. Bianco-Miotto, M. M. Centenera, J. M. Harris, S. Jindal, D. Segara, L. Jia, N. L. Moore, S. M. Henshall, S. N. Birrell, G. A. Coetzee, R. L. Sutherland, L. M. Butler, and W. D. Tilley. 2009. 'Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer', *Cancer Res*, 69: 6131-40.

Poulin, R., D. Baker, and F. Labrie. 1988. 'Androgens inhibit basal and estrogen-induced cell proliferation in the ZR-75-1 human breast cancer cell line', *Breast Cancer Res Treat*, 12: 213-25.

Qu, Q., Y. Mao, X. C. Fei, and K. W. Shen. 2013. 'The impact of androgen receptor expression on breast cancer survival: a retrospective study and meta-analysis', *PLoS One*, 8: e82650.

Robertson, J. F., A. Llombart-Cussac, J. Rolski, D. Feltl, J. Dewar, E. Macpherson, J. Lindemann, and M. J. Ellis. 2009. 'Activity of fulvestrant 500 mg versus anastrozole 1 mg as first-line treatment for advanced breast cancer: results from the FIRST study', *J Clin Oncol*, 27: 4530-5.

Simanainen, U., Y. R. Gao, K. A. Walters, G. Watson, R. Desai, M. Jimenez, and D. J. Handelsman. 2012. 'Androgen resistance in female mice increases susceptibility to DMBA-induced mammary tumors', *Horm Cancer*, 3: 113-24.

Smith, I. E., L. Johnson, M. Dowsett, J. F. R. Robertson, L. E. Robison, J. S. Kokan, A. A. Evans, C. Holcombe, K. Horgan, A. Skene, R. Prasad, M. S. Absar, R. Vidya, N. J. Bundred, C. Harding-Mackean, D. A. Wheatley, M. W. Kissin, I. F. Pinhel, L. S. Kilburn, J. M. Bliss, and On behalf of the POETIC Trialists. 2011. 'Trial of perioperative endocrine therapy: Individualizing care (POETIC)', *ASCO Meeting Abstracts*, 29: TPS117.

Tukey, John W. 1977. *Exploratory data analysis* (Addison-Wesley Pub. Co.: Reading, Mass.).

Vera-Badillo, F. E., A. J. Templeton, P. de Gouveia, I. Diaz-Padilla, P. L. Bedard, M. Al-Mubarak, B. Seruga, I. F. Tannock, A. Ocana, and E. Amir. 2014. 'Androgen receptor expression

and outcomes in early breast cancer: a systematic review and meta-analysis', *J Natl Cancer Inst*, 106: djt319.

Witzel, I., M. Graeser, T. Karn, M. Schmidt, R. Wirtz, D. Schutze, A. Rausch, F. Janicke, K. Milde-Langosch, and V. Muller. 2013. 'Androgen receptor expression is a predictive marker in chemotherapy-treated patients with endocrine receptor-positive primary breast cancers', *J Cancer Res Clin Oncol*, 139: 809-16.

Yeh, S., Y. C. Hu, P. H. Wang, C. Xie, Q. Xu, M. Y. Tsai, Z. Dong, R. S. Wang, T. H. Lee, and C. Chang. 2003. 'Abnormal mammary gland development and growth retardation in female mice and MCF7 breast cancer cells lacking androgen receptor', *J Exp Med*, 198: 1899-908.

Yu, S., C. Zhang, C. C. Lin, Y. Niu, K. P. Lai, H. C. Chang, S. D. Yeh, C. Chang, and S. Yeh. 2011. 'Altered prostate epithelial development and IGF-1 signal in mice lacking the androgen receptor in stromal smooth muscle cells', *Prostate*, 71: 517-24.

Zava, D. T., and W. L. McGuire. 1977. 'Estrogen receptors in androgen-induced breast tumor regression', *Cancer Res*, 37: 1608-10.

Zhou, J., S. Ng, O. Adesanya-Famuiya, K. Anderson, and C. A. Bondy. 2000. 'Testosterone inhibits estrogen-induced mammary epithelial proliferation and suppresses estrogen receptor expression', *FASEB J*, 14: 1725-30.

21 APPENDIX 1 – CYP3A4 INDUCERS

This list is not exhaustive or definitive, and is meant only as an aid.

- Carbamazepine
- Dexamethasone
- Ethosuximide
- Glucocorticoids
- Griseofulvin
- Phenytoin
- Primidone
- Progesterone
- Rifabutin
- Rifampin
- Nafcillin
- Nelfinavir
- Nevirapine
- Oxcarbazepine
- Phenobarbital
- Phenylbutazone
- Rofecoxib (mild)
- St John’s wort
- Sulfadimidine
- Sulfinpyrazone
- Troglitazone