TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 1015

MEDICA - ODONTOLOGICA

USE OF PRECLINICAL AND EARLY CLINICAL DATA FOR DOSE SELECTION OF A SELECTIVE ESTROGEN RECEPTOR MODULATOR TOREMIFENE IN TREATMENT OF BREAST CANCER

by

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UNIVERSITY OF TURKU
Turku 2012

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ISBN 978-951-29-5022-5 (PRINT)
ISBN 978-951-29-5023-2 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2012

To my family

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Use of preclinical and early clinical data for dose selection of a selective estrogen receptor modulator toremifene in treatment of breast cancer

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Painosalama Oy, Turku, Finland 2012

ABSTRACT

In development of human medicines, it is important to predict early and accurately enough the disease and patient population to be treated as well as the effective and safe dose range of the studied medicine. This is pursued by using preclinical research models, clinical pharmacology and early clinical studies with small sample sizes. When successful, this enables effective development of medicines and reduces unnecessary exposure of healthy subjects and patients to ineffectice or harmfull doses of experimental compounds.

Toremifene is a selective estrogen receptor modulator (SERM) used for treatment of breast cancer. Its development was initiated in 1980s when selection of treatment indications and doses were based on research in cell and animal models and on noncomparative clinical studies including small number of patients. Since the early development phase, the treatment indication, the patient population and the dose range were confirmed in large comparative clinical studies in patients. Based on the currently available large and long term clinical study data the aim of this study was to investigate how the early phase studies were able to predict the treatment indication, patient population and the dose range of the SERM.

As a conclusion and based on the estrogen receptor mediated mechanism of action early studies were able to predict the treatment indication, target patient population and a dose range to be studied in confirmatory clinical studies. However, comparative clinical studies are needed to optimize dose selection of the SERM in treatment of breast cancer.

Keywords: breast cancer, estrogen receptor, SERM, toremifene, dose

Juha Ellmén

Prekliinisen ja aikaisen kliinisen vaiheen tutkimustulosten käyttö selektiivisen estrogeenireseptorin säätelijä toremifeenin annoksen valinnassa rintasyövän hoitoon

Farmakologia, lääkekehitys ja lääkehoito, Turun yliopisto
Annales Universitatis Turkuensis, Medica-Odontologica
Painosalama Oy, Turku, 2012

TIIVISTELMÄ

Lääkekehityksessä on tärkeää pystyä ennustamaan riittävän tarkasti ja riittävän aikaisin lääkeainekandidaatin käyttöaihe eli hoidettava tauti, oikea potilasryhmä ja tehokas ja turvallinen lääkkeen annosalue. Tähän pyritään aikaisen vaiheen tutkimusmalleilla, kliinisen farmakologian ja pienen otoskoon potilastutkimuksilla. Onnistuessaan tämä mahdollistaa tehokkaan lääkekehityksen ja vähentää koehenkilöiden ja potilaiden tarpeetonta altistamista tehottomille tai haitallisille annoksille tutkittavaa lääkettä.

Toremifeeni on rintasyövän hoidossa käytettävä selektiivinen estrogeenireseptorin säätelijä (SERM). Sen kehitys lääkkeeksi aloitettiin 1980 luvulla ja käyttöaiheitten, potilasryhmän ja käytettävien annosten valinta perustui tutkimuksiin solu- ja koe-eläinmalleissa sekä ei vertaileviin kliinisiin tutkimuksiin pienillä potilasmäärillä. Alkuvaiheen jälkeen toremifeenin käyttöaihe ja annokset varmistettiin laajoilla vertailevilla tutkimuksilla. Tämän väitöskirjatutkimuksen tarkoituksena on selvittää nyt käytettävissä olevan laajan ja pitkäaikaisen kliiniseen tutkimustiedon perusteella, miten hyvin lääkekehityksen aikaisen vaiheen tutkimukset pystyivät ennustamaan lääkkeen käyttöaiheet, kohdepopulaation ja annokset.

Estrogeenireseptorivälitteisessä lääkkeen vaikutuksessa, aikaisen vaiheen tutkimukset kykenivät ennustamaan käyttöaiheen, potilasryhmän ja annosalueen myöhäisemmän vaiheen varmentaviin kliinisiin tutkimuksiin. Vertailevia kliinisiä tutkimuksia tarvitaan SERM:n annoksen optimoinnissa rintasyövän hoidossa.

Avainsanat: rintasyöpä, estrogeenireseptori, SERM, toremifeeni, annos

TABLE OF CONTENTS

ABBREVIATIONS

AF	Activation function
ANCOVA	Analysis of covariance
AUC	Area under the plasma concentration time curve
ALAT	Alanine amino transferase
ASAT	Aspartate amino transferase
ATIII	Antithrombin III
BMD	Bone mineral density
CL/f	Mean oral clearance
CC	Coactivation complex
CR	Complete response
CoA	Coactivator
CoRe	Corepressor
Cmax	Maximum concentration
CYP3A	Cytochrome 3A enzyme
CYP2C16	Cytochrome 2C16 enzyme
CYP2D6	Cytochrome 2D6 enzyme
CYP450	Cytochrome 450 enzyme family
DAHT	(deaminohydroxy)-toremifene
DDMT	N,N-dimethyltoremifene
DES	Diethylstilbesterol
DMT	N-demethyltoremine
DNA	Deoxyribonucleic acid
DBD	DNA binding domain
DMBA	Dimethylbenz [a] anthracene
ER	Estrogen receptor
E_1	Estrone
E_2	Estradiol
E_3	Estriol
ERE	Estrogen responsive element
EEG	Electroencephalogram

FSH	Follicle stimulating hormone
GnRH	Gonadotrophine releasing hormone
HDL	High density lipoprotein cholesterol
HER2	Human Epidermal growth factor Receptor 2
HSP	Heat shock protein
IU/L	International unit / litre
LBD	Ligand binding domain
LDL	Low density lipoprotein cholesterol
LD50	Lethal dose 50%
LH	Luteinizing hormone
MCF-7	Human breast adenocarcinoma cell line
MPA	Medroxyprogesterone acetate
μM	Micromolar concentration
NC	No change – stable disease
NS	Not statistically significant
OR	Odds ratio
P450	Cytocrome P450 enzymes
SD	Standard deviation
PD	Progressive disease
PoC	Proof of concept
PoM	Proof of mechanism
PoP	Proof of principle
PR	Partial response
P-170	P-glycoprotein 170
SHBG	Sex hormone binding globulin
SERM	Selective estrogen receptor modulator
TF	Transcription factor
TAM20	Tamoxifen 20 mg daily
TAM30	Tamoxifen 30 mg daily
TAM40	Tamoxifen 40 mg daily
TOR20	Toremifene 20 mg daily
TOR40	Toremifene 40 mg daily
TOR60	Toremifene 60 mg daily

TOR200	Toremifene 200 mg daily
TOR240	Toremifene 240 mg daily
tmax	Time to maximum concentration
t½	Elimination half life
TTF	Time to treatment failure
TTP	Time to progression
VTE	Venous thromboembolism
WHO	World health organization
Vd/f	Volume of distribution
UICC	The International Union Against Cancer
USA	The United States of America
4-HTam	4-hydroxy tamoxifen
4-HTor	4-hydroxy toremifene
4-OH-A	4-hydroxy adrostendione

LIST OF ORIGINAL PUBLICATIONS

The present work is based on the following original papers which will be referred in the text by the Roman numerals I-VI

I Ellmén J, Werner D, Hakulinen P, Keiling R, Fargeot P, Falkson G, Bezwoda W.R: Dose dependent hormonal effects of toremifene in postmenopausal breast cancer patients. Cancer Chemotherapy Pharmacol 45: 402-408, 2000.

II Ellmén J, Hakulinen P, Partanen A, Hayes D.F: Estrogenic effects of toremifene and tamoxifen in postmenopausal breast cancer patients. Breast Cancer Res Treat 82: 103-111, 2003.

III Gershanovich M, Garin A, Baltina D, Kurvet A, Kangas L, Ellmén J, Eastern European Study group: A Phase III Comparison of Two Toremifene Doses to Tamoxifen in Postmenopausal Women with Advanced Breast Cancer. Breast Cancer Res Treat.45: 251-262, 1997.

IV Gershanovich M, Hayes D.F, Ellmén J, Vuorinen J: High-Dose Toremifene vs Tamoxifen in Postmenopausal Advanced Breast Cancer. Oncology 11: 29-36, 1997.

V Pyrhönen, S, Ellmén J, Vuorinen J, Gershanovich M, Tominaga T, Kaufmann M, and Hayes D.F: Meta-analysis of trials comparing toremifene with tamoxifen and factors predicting outcome of antiestrogen therapy in postmenopausal women with breast cancer. Breast Cancer Res Treat. 1473: 1-11, 1999.

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1. INTRODUCTION

The selection of a valid dose range for a new compound intended to be used as a human pharmaceutical is an essential phase in clinical drug development. In addition to showing the efficacy and safety of the intended dose range, one must also identify and justify the lowest effective dose, and preferably also the dose beyond which efficacy is not improved or toxicity becomes unacceptable.

In preclinical, toxicological and pharmacological *in vitro* and *in vivo* models, the dose range of a candidate compound is evaluated, for example, by studying its binding to the target receptor as a proof of principle (PoP) and its capability to inhibit the target enzyme as a proof of mechanism (PoM). During preclinical research, pharmacokinetics and toxicity of a candidate compound in different animal species is studied and documented to support safety in foreseen clinical studies.

In phase I clinical studies, which are usually conducted employing healthy human subjects, single and repeated dose pharmacokinetics and tolerability will be studied with escalating doses of the candidate compound. When studying compounds with a narrow therapeutic window, such as drugs used for cancer chemotherapy, where the therapeutic doses are also toxic, it is generally accepted, based on ethical considerations, to conduct these studies only with patients who could potentially benefit from the experimental treatment. Depending on the assumed pharmacological action of the compound, one may also use pharmacodynamic variables such as biomarkers as surrogates to predict treatment efficacy already in these early studies. These biomarkers could reflect primary or secondary pharmacological actions of the drug, such as affinity or effect on the target protein, effect on serum chemistry or metabolism, electrophysiological response or cytology. Techniques to study biomarkers can be invasive, based on collection of biological samples, or noninvasive, such as electrophysiological measurements or in vivo imaging. In many disorders, one may quite well predict the therapeutic dose range based on the phase I results.

After demonstrating clinical benefit and proof of concept (PoC) in patients in a phase II clinical study, a series of studies are often needed to confirm the dose selection in target patient population. An ideal dose finding study would include a sufficient number of dosing groups to establish the lowest effective and the highest tolerated dose of the compound, a sufficient number of patients to allow reliable statistical estimates for efficacy, and sufficient duration of treatment to demonstrate long term efficacy and safety. In practice, this kind of a large dose finding study requires a high number of patients, a long treatment time and will be expensive and time consuming. After showing the PoP and PoM in preclinical studies followed by demonstration of efficacy and preliminary safety in a PoC study in patients, confirmatory phase II-III clinical studies with longer duration are initiated in order to confirm efficacy and safety of the drug within the given dose range and to obtain marketing authorizations for the new drug.

Usually, in the development of a new pharmaceutical, time and money is a limited resource and one would like to proceed stepwise from PoP to PoM and subsequently to PoC with effective investment of resources until the data is available to justify the full development until marketing authorization and clinical use by the patients and healthcare professionals.

In this setting, preclinical and phase I biomarkers and surrogate endpoints together with the early phase II studies in patients can support the selection of the appropriate dose range to be further studied and developed in the phase II dose finding and phase III studies. Obtaining this information early may reduce the time and costs of drug development, and may avoid exposing a large number of patients to toxic or ineffective drugs or doses of a drug.

Ideally, preclinical models will predict the target disease and a patient population to be studied for PoC and the dose range for clinical studies. Preclinical models, however, may suggest none or alternatively more than one potential mechanism of action, target diseases and populations for a drug candidate. Subsequently there will be a need for a number of small exploratory clinical trials with a limited number of patients in order to select the target disease. The predictive value of such small scale experimental studies for the target indication and target patient population will be confirmed later during the drug development with large scale confirmatory studies.

During the lifecycle of a drug, new indications or extensions of the existing ones may be developed. Clinical variables used to demonstrate efficacy of a drug in the original indication and the criteria used for dose selection may not be valid for the new or extended indication, and even new efficacy variables may need to be developed and used. Ultimately, this may result in new dose selection and new dosing regimens for the new indications and patient populations.

Toremifene (Fareston®) is a selective estrogen receptor modulator (SERM) previously classified as an antiestrogen, and developed for treatment of advanced breast cancer in postmenopausal women. It was the first human pharmaceutical, containing a new chemical entity, developed entirely by a Finnish drug company obtaining marketing authorizations worldwide. The development of the compound was initiated in early 1980s and the clinical studies were conducted in late eighties and erly nineties. Marketing authorizations were granted in 1995 in Japan, 1996 in Europe and 1997 in the USA.

Dose selection for the confirmatory phase III trials in advanced breast cancer was based on *in vitro* receptor pharmacology, results from preclinical pharmacokinetic and pharmacodynamic animal models, and on phase I and early phase II clinical studies. During the development for the first indication, it became evident that another SERM, tamoxifen, was effective and safe even in adjuvant treatment of early breast cancer, prolonging disease free and overall survival after the primary therapy, surgery. Subsequently, in early 1990s, studies with toremifene were initiated also in the adjuvant treatment of early breast cancer. Dose selection in the adjuvant treatment studies was

based on the dose used in phase III studies and on the results of a new phase II dose finding study, both conducted in patients with advanced breast cancer. To date, all these clinical studies have been completed and reported allowing evaluation of the long term efficacy and safety of the drug in large patient populations..

The purpose of this investigation is to retrospectively evaluate how the evaluation criteria, preclinical and early clinical data used were able to predict the target indication, target patient population and clinically effective and safe toremifene doses for the treatment of patients with malignant diseases.

2. REVIEW OF LITERATURE

2.1. Effects of estrogen in man

Estrogens are steroidal hormones and have function in female physiology and reproduction. They also have effect on the musculoskeletal system, cardiovascular system, and in the brain (McDonnell and Norris 2002). Physiological estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). E_2 is a major estrogen in women at the age of fertility, whereas E_1 is the predominant estrogen in postmenopausal women and E_3 is seen primarily in pregnant women. Estrogens are synthesized from cholesterol and androgens in the ovaries by the granulosa cells. In postmenopausal women, with nonfunctional ovaries, estrogens are produced by the aromatase enzyme converting androgens to estrogens. Aromatase is expressed in variety of organs and tissues including, blood vessels, bone, brain, breast, skin, endometrium, and fat. The human aromatase enzyme belongs to cytochrome P450 family and is the product of the CYP19A1 gene in chromosome 15 (Thompson and Siiteri 1974; Chen, Besman et al. 1988).

2.1.1. Receptors, binding and action of estrogens

Estrogens have effects on growth, development, differentiation and on regulation of action in a wide variety of tissues. Estrogen receptor (ER), the molecular target for the estrogen ligands, such as physiological estrogens or SERMs, is a protein and a nuclear transcription factor, which mediates most of the actions of estrogen. It exists as two distinct receptor forms, ERα and ERβ, which are encoded by genes related to genes coding receptors for other steroid and thyroid hormones, retinoids and other small hydrophobic molecules (Tsai and O´Malley 1994). ERα contains 595 and ERβ 530 amino acids (Gustafsson 1999).

The theory on the evolutionary relationship of the steroid and nuclear receptors is based on the strong conservation of the DNA binding domains (DBD) and less-conserved ligand binding domains (LBD), suggesting a common ancestral molecule for these receptors (Ogawa, Inoue et al. 1998). The ER receptors share 59 % of the amino acids in their LBD, the site responsible for binding of physiological estrogens and synthetic SERMs (Gustafsson 1999) (Figure 1a).

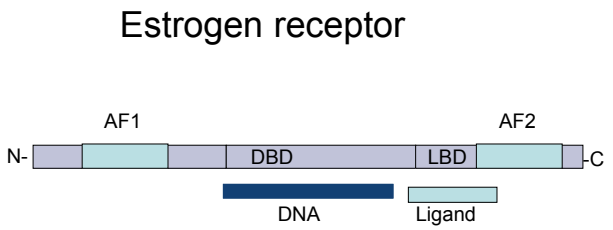

Figure 1a. Schematic representation of Estrogen receptor α structure. DNA Binding domain (DBD) and Ligand Binding Domain (LBD) The N- terminus contains the activation function 1 (AF-1) site where other transcription factors interact. The DBD contains the structure that binds to DNA. The LBD domain contains the ligand binding site and the AF-2 domain that directly interacts with coactivator proteins.

Basically, ER exist in two distinct states: on and off (Katzenellenbogen, O´Malley et al. 1996) The expression of ERα and ERβ can be cell specific, and the ligands can bind selectively either to ERα or to ERβ. The structure of the ER-ligand complex is determined by the ligand and the ER; the structure of the complex determines its ability to interact with other molecules, which may have a cell-specific expression and lead to a cell-specific action of a given ligand (Dutetre and Smith 2000). In the absence of a ligand, ERα resides predominantly within the nucleus as an inactive monomer form associated with a large inhibitory heat shock protein complex (Klinge, Brolly et al. 1997) (Figure 1b).

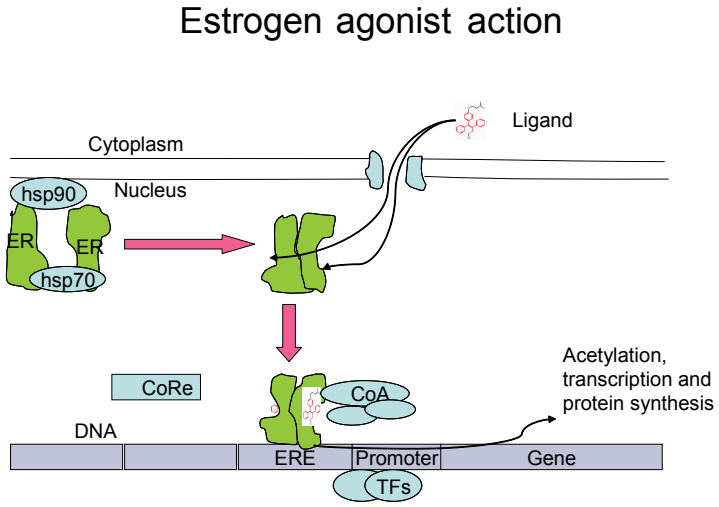

Figure 1b. Estrogen receptor (ER) and estrogen agonist ligand action within a cell and nucleus. ER is stabilized by heat shock protein 90 and 70 (hsp 90 and hsp 70) and repressed by co-repressors (CoRe) before dimerization by the ligands. ER dimer is bound to the estrogen responsive element (ERE) in DNA. Coactivator (CoA) complex acetylates DNA histones facilitating binding of general transcription factors (TFs) to promoter region followed by transcription and protein synthesis.

Following ligand binding, ER dissociates from the protein complex and forms a dimer of two ER proteins, which subsequently binds to the DNA (Klinge, Brolly et al. 1997) . The ligand binding to LBD subsequently changes the interaction of the ER with the specific DNA sequence, estrogen responsive element (ERE), in the target gene. ERs, or more specifically their activation functions (AFs), also interact with coactivators that facilitate transcription even without DNA binding (McKenna, Lanz et al. 1999). It has been proposed that agonist ligands of ERs, such as physiological estrogens, induce interaction between AF and co-activators whereas antiestrogens inhibit the interaction (Jordan 1994; Hanstein, Eckner et al. 1996). LBD has a "pocket" into which a ligand binds altering the conformation of the LBD and thus forming a surface for co-activator proteins to interact with. The nature of the conformational change is dependent on the bound ligand, and may allow ER interaction with co-activators in the presence of estradiol (E_2) but not in the presence of SERMs (Brzozowski, Pike et al. 1997; Shiau, Barstad et al. 1998). ER binds with high affinity to ERE, which results in DNA bending and facilitation of interactions of transcription components (Kim, DeHaan et al. 1997). In some tissues or species, a ligand can act as an agonist and in other tissues as an antagonist, which has been suggested to be caused by differences in the co-activator profiles (McKenna, Lanz et al. 1999).

Highlighting the complex action of hormone receptors, interaction of ER with DNA does not always require their mutual binding but can also be mediated by functional interactions through other pathways of transcriptional factors and DNA in the presence of ER (Webb 1995). It has been suggested that plasma membrane associated form of the ER can even facilitate rapid, membrane initiated, estrogen triggered signaling cascades (Moriarty, Kim et al. 2006), which could affect independently different enzymatic pathways or interact with, above described nuclear responses.

2.1.2. Regulation of estrogen production and action

Steroidal sex hormones such as E_2 are synthesized from cholesterol in the adrenal cortex, ovaries and testis, as well as in other tissues such as fat through aromatization of androgens. Of note, also breast cancer tissue has intrinsic aromatase activity and produces higher estrogen concentrations than healthy tissue. Two gonadotrophic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), are released from the anterior pituitary gland and stimulate growth and secretion of ovarian follicles in females and later, during the course of the menstrual cycle, ovulation and subsequently conversion of the ovarian follicle to corpus luteum. Release of LH and FSH is regulated by the hypothalamic gonadotrophin releasing hormone (GnRH) and there is a negative feedback loop between pituitary LH and FSH and GnRH secretion (Yen 1977). Circulating E_2 inhibits the secretion of GnRH, with a subsequent inhibition of synthesis and release of LH, FSH, and finally E_2 itself (Figure 2). Thus, diminishing concentrations of E_2 will decrease the activity of the inhibitory loop, and stimulate the action of hypothalamus-pituitary axis and increase the production of estradiol. The negative feedback may also take place in the pituitary gland through desensitization to GnRH by the effect of E_2 (Conn and Crowley jr 1991).

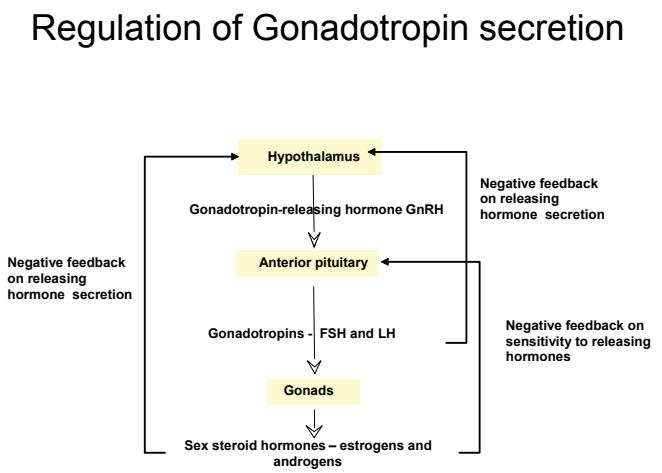

Figure 2. Negative feedback control of gonadotropin secretion and effect

In women, an increased concentration of E_2 occurs simultaneously with a period of accelerated growth and development of secondary sexual characteristics in puberty and menarche. In fertile women, ovarian follicles secrete increasing amounts of E_2 during the menstrual cycle reaching the peak at the time of the ovulation, when E_2 sensitizes the follicle further to the effects of FSH and LH leading to ovulation. E_2 from ovaries and from the corpus luteum stimulates, together with progesterone, the growth of the endometrium which becomes thick and vascular in order to support an embryo should fertilization take place. Estrogens also have an important role in non reproductive tissues such as bone, where they reduce turnover and maintain mineral density. It is also possible that estrogens protect the cardiovascular and central nervous systems. On the other hand, estrogens can act as growth promoters in some malignant tumors in the breast, endometrium and ovary. In a clinical study, estrogens combined with progestins have been shown to increase the risk of breast cancer in postmenopausal women receiving the combination for hormone replacement therapy (Writing Group for the Women's Health Initiative Investigators 2002).

2.1.3. Menopause

At the menopause, the development of new follicles and the production of E_2 in the ovaries cease. Due to the lack of negative estrogen-mediated feedback on the pituitary, the concentrations of LH and FSH in serum subsequently increase. Decreased E_2 is considered to be the main cause for menopausal symptoms like hot flushes, sleeping problems, depressive symptoms, anxiety and for long term effects such as sexual dysfunction. At least some of these symptoms are experienced by the majority of women with effects on their physical and mental well being. However, the duration and severity of these symptoms is individual, transient, and will reduce in severity and eventually disappear after menopause, whilst some women do not experience them at all.

2.1.4. Overview of estrogen dependent symptoms and disorders

Non malignant

Estrogens and their physiologically fluctuating concentrations in premenopausal women and decreasing estrogen concentration at the menopause and low concentrations after the menopause, may cause symptoms or disorders potentially requiering treatment interventions. Some of these disorders and their association with the menopausal status are presented below in Table 1.

Table 1. Disorder or symptom prevalence (percentage of subjects affected in the population) or incidence (events / year / 1000 women) associated with menopausal status in women

Symptom or disorder	Prevalence (%)	Reference
In premenopause		
Premenstrual syndrome	70 - 90	(Mishell 2005)
Mastalgia	69 -79	(Ader and Browne 1997; Oksa, Luukkaala et al. 2006)
Endometriosis	6-21	(Mahmood and Templeton 1991)
Uterine myomas	77	(Buttram and Reiter 1981; Cramer and Patel 1990)
Sexual dysfunction	38-63	(Mayer, Bauer et al. 2007)
At menopause		
Hot flushes, sweating	60	(Rödström, Bengtson et al. 2002; Rapp, Espeland et al. 2003)
In postmenopause		
Depressive symptoms	10	(Kim, McGorray et al. 2005)
Sleep complaints	61	(Kripke, Brunner et al. 2001)
Anxiety/panic attacks	18	(Smoller, Pollack et al. 2003)
Sexual problems	13 – 36	(Lindau, Schumm et al. 2007)
Urogenital atrophy	27- 36	(Pastore, Kightlinger et al. 2007)
Memory impairment	7 - 18	(Coria, Gomez de Caso et al. 1993; Larrabee and Crook 1994; Barker, Jones et al. 1995; Baum, Buzdar et al. 2003; Rapp, Espeland et al. 2003)
Decreased cognitive function	30	(Utian and Schiff 1996; Foxmann 1999; Van Geelen and Van De Weijer 2000; Rapp, Espeland et al. 2003)
Osteoporotic fractures	40 – 50	(Johnell and Kanis 2005)
	Incidence (1000 / year)	
Coronary heart disease	4.4	(Rossouw, Prentice et al. 2007)
Deep vein thrombosis	1.3	(Cushman, Kuller et al. 2004; Glynn, Ridker et al. 2007)
Stroke	2.9	(Rossouw, Prentice et al. 2007)

Malignant

Estrogens are not considered to initiate cancer, but to have a cancer growth promoting carcinogenic effect in some malignancies that originate from the reproductive organs and have hormone sensitivity. To support this theory of carcinogenic effect of estrogens, hormone replacement therapies have been shown to be associated with increased incidence of breast (Writing Group for the Women's Health Initiative Investigators 2002), ovarian (Rodriguez, Patel et al. 2001) and uterine cancer (Nelson, Humphrey et al. 2002).

Table 2. Incidence of malignant estrogen associated diseases in Finnish female population ≥ 50 years

Disease	Incidence per 1000 females / year (> 50 years of age)	Reference
breast cancer	2.9 – 3.2	(Finnish Cancer Registry 2011)
ovarian cancer	0.2 – 0.5	(Finnish Cancer Registry 2011)
uterine cancer	0.3 – 1.1	(Finnish Cancer Registry 2011)

2.1.5. Treatment of malignant estrogen dependent-diseases

The primary therapy for these malignant diseases is surgery, possibly combined with radiotherapy, with an attempt to eradicate the primary tumor. Systemic hormonal and cytotoxic therapies can be used as adjuvant after surgery, or for the treatment of inoperable, recurrent or metastatic disease. Given as systemic treatment, hormonal agents such SERMs, progestins, GnRH-analogs and aromatase inhibitors inhibit the growth of estrogen dependent malignancies. In breast cancer, SERMs are widely used as adjuvant treatment alone or sequentially with cytotoxic therapy or for the first and second line treatment of metastatic disease.

2.1.6. Endocrine treatments of postmenopausal breast cancer

Estrogens

A synthetic estrogen, diethylstilbesterol (DES), has been used for the treatment of breast cancer in postmenopausal women (Haddow, Watkinson et al. 1944) thus illustrating the paradox that hormonally active agents can promote cancer growth but also be used as a treatment of the same cancer type. With DES, the response rate tended to be dose dependent in postmenopausal patients with progressive breast cancer (Carter, Sedransk et al. 1977), and somewhat better responses were seen after the higher 1500 mg daily dose than following the lower doses down to 1.5 mg, although all doses within the range were effective. It was proposed that declining estrogen concentrations after menopause may increase the sensitivity of breast cancer cells to DES (Carter, Sedransk et al. 1977). The effect of DES on breast cancer in postmenopausal patients was comparable to that

of a new antiestrogen tamoxifen, but tamoxifen turned out to be better tolerated (Ingle, Ahmann et al. 1981). In postmenopausal breast cancer patients who had been exposed to various endocrine therapies and failed after initial response, DES at a 15 mg daily dose provided further efficacy (Lønning, Taylor et al. 2001). Again, peroral estradiol at dose level of 6 mg daily provided a clinical benefit in postmenopausal women with aromatase inhibitor resistant advanced breast cancer, but the effect was not improved by increasing the estradiol dose (Ellis, Gao et al. 2009). The mechanism behind the therapeutic effect of estrogens in breast cancer remains largely unknown. However, long term estrogen deprivation of MCF-7 breast cancer cells made them susceptible to high dose estradiol inducing cell death due to apoptosis (Song, Morg et al. 2001). Adding to the paradox of the action of estrogen was the finding, in the Women's Health Initiative Trial, that conjugated equine estrogen in postmenopausal women with hysterectomy, and subsequently with no need to oppose uterine effects by progestins, reduced breast cancer incidence (Anderson, Limacher et al. 2004; LaCroix, Chlebowski et al. 2011).

Progestins

Progestins, such as the physiological hormone progesterone and its derivatives, exert both genomic transcriptional and non-genomic effects depending on the experimental model and cell context, as well as progestin type, dose and exposure time. The progestins can elicit either proliferative or antiproliferative effects on breast epithelial cell growth (Jeng, Parker et al. 1992; Markiewicz, Hochberg et al. 1992; Gadducci and Genazzani 1997; Rabe, Bohlmann et al. 2000; Pasqualini and Chetrite 2002). Progesterone receptor (PR) is estrogen-regulated so that an estrogen effect through ER modulates PR expression. In breast cancer, ER-positive/PR-positive tumors are more common than ER-positive/PR-negative tumors.

ER and PR status can change during the course of the disease or with treatment (Hull III, Clark et al. 1983; Kuukasjärvi, Kononen et al. 1996). Studies have shown that ER levels are reduced during hormonal treatment, or are lost during tumor progression from the primary tumor to metastasis with subsequent development of resistance to endocrine therapy. During SERM therapy and development of resistance, up to a half of tumors lost PR expression, and in adjuvant setting loss was similar for both of the receptors (Gross, Clark et al. 1984). How the loss of PR predicts the disease outcome is not known, but ER-positive/PR-negative metastatic tumors have a poor prognosis for disease progression and for overall survival. Of note, hormone replacement therapy with an estrogen plus progestin combination was associated with greater breast cancer incidence and mortality (Chlebowski, Anderson et al. 2010) than unopposed estrogens (Anderson, Limacher et al. 2004), again suggesting some mechanism of interaction between these two receptors

Various progestins have been used in contraception and in hormone replacement therapy to oppose proliferative effects on the endometrium (Junod and Marks 2002; Board of Trustees of The North American Menopause Society (NAMS) 2003) but most of the experience on clinical use of progestins in the treatment of breast cancer has been gained

from medroxyprogesterone acetate (MPA) in the treatment of postmenopausal women with hormone receptor positive breast cancer. MPA has been used either alone (Muggia, Cassieth et al. 1968); (Klaassen, Rapp et al. 1976), in combination or sequentially with tamoxifen (Gundersen, Kvinnsland et al. 1990; Beexa, Roseb et al. 2006). High doses of MPA, 1000 mg, have shown better efficacy than the lower 500 mg dose administered parenterally twice weekly (Cavalli, Goldhirsch et al. 1984), and peroral administration of MPA at the same dose has shown non inferior efficacy to parenteral administration of the drug (Paridaens, Becquart et al. 1986). In comparative studies mostly including patients with ER and PR positive tumors, high doses of MPA have had a similar efficacy as SERM, but with less favourable adverse event profile (van Veelen, Willemse et al. 1986; Beexa, Roseb et al. 2006)..

Selective Estrogen Receptor Modulators (SERMs)

Antiestrogenicity as the mechanism of action in the treatment of breast cancer was suggested already in the 1960s (Herbst, Griffits et al. 1964) with the use of the triphenyl compound clomiphene, which was also used for fertility treatment. The SERM, tamoxifen, has been used clinically as an estrogen receptor modulator since 1970s for the treatment of breast cancer (Cole, Jones et al. 1971), first in the treatment of advanced breast cancer, then as an adjuvant treatment following surgery of early disease, and finally for prevention of the disease (Cuzick, Decensi et al. 2011).

The therapeutic effect of SERMs in the treatment of breast cancer is based on their antagonistic effect on ER in a cancer cell. At the same time, however, SERMs have species and tissue specific estrogen agonist actions, which are also SERM specific. The estrogen agonist action of a SERM may be beneficial by preserving bone mineral density or reducing serum cholesterol levels in postmenopausal women, or it can be also harmful by promoting blood clotting, endometrial hyperplasia or endometrial cancer growth. It has also been suggested that hypertriglyseridemia in postmenopausal women receiving hormone replacement therapy may be estrogen dependent and reversible after dose reduction (Walsh, Schiff et al. 1991).

Tamoxifen, like estrogens, has been shown to induce an increase of serum triglycerides, which was reversible after dose reduction from 20 to 10 mgs daily (Liu and Yang 2003). However, tamoxifen therapy has shown to reduce serum cholesterol concentrations (Love, Newcomb et al. 1990), which again has been explained to be related to the estrogenic effect of the drug in the liver.

Tamoxifen has been shown to have an estrogenic and antiestrogenic effects on the bone by increasing or decreasing bone mineral density, in postmenopausal and premenopausal women, respectively (Gotfredsen, Christiansen et al. 1984; Powles, Hickish et al. 1996). In the treatment of advanced breast cancer in postmenopausal women, the bone preserving effect of a SERM is not of major clinical interest. However, in long term

studies especially in adjuvant or a chemopreventive setting, the bone preserving effect in postmenopausal patient population becomes clinically meaningful.

In breast cancer patients with ER positive or ER unknown tumours, adjuvant treatment with tamoxifen has been shown to prolong disease free and overall survival (Early Breast Cancer Trialists' Collaborative Group 2005b), and at least 3 years and median of 5 years treatment was more effective than 2 years of treatment or less. When a population of breast cancer patients, with ER positive breast cancer, were re randomized after 5 years of tamoxifen therapy to either continue tamoxifen or to receive corresponding placebo no further benefit from the continuation of tamoxifen treatment over 5 years was seen (Fisher, Dignam et al. 2001). In fact, patients re-randomized to continue tamoxifen had shorter disease free survival and a trend for shorter overall survival than patients who discontinued the treatment at 5 years. However, the benefit of the 5 year treatment has been shown to be maintained at least up to 15 years (Hackshaw, Roughton et al. 2011).

Initially the clinically used dose of tamoxifen was 40 mg once daily or divided into two daily doses. At this dose level, steady state concentrations of the parent drug and the main metabolite N-desmethyltamoxifen in serum were reached after 4 and 8 weeks of treatment, respectively. However, the concentrations varied significantly among the breast cancer patients participating the study, and no correlation could be seen between the plasma levels and therapeutic response to the drug (Patterson, Settatree et al. 1980; Furr and Jordan 1984). After 20 mg daily for at least 3 months, tamoxifen plasma concentrations were in the 0.3 μM range and respective tumor concentrations three times higher. N-desmethyltamoxifen concentrations were twice as high as those of the parent drug both in plasma and in tumor tissue (MacCallum, Cummings et al. 2000). In an early clinical study with tamoxifen in 263 postmenopausal patients with advanced breast cancer, 20 and 40 mg in two daily doses did not differ significantly regarding response rate or response duration (Bratherton, Brown et al. 1984), suggesting that there may not be further clinical benefit obtained by increasing the dose beyond the effective antiestrogenic dose. Today, a 20 mg daily tamoxifen dose has been widely accepted as the standard for breast cancer treatment or even as preventive therapy for breast cancer in women at high risk for the disease (Cuzick, Decensi et al. 2011). It has been further suggested that lower doses of tamoxifen down to 1 or 5 mg daily, based on breast cancer proliferation and blood estrogenic biomarkers, could be effective in breast cancer treatment (Descensi, Robertson et al. 2003).

In adjuvant setting, treatment duration extends from 6 months as the treatment duration in metastatic disease (Study V) up to 5 years (Early Breast Cancer Trialists' Collaborative Group 1998) with different adverse event profiles. Long term tamoxifen treatment has been associated with increased incidence of endometrial cancer (Fornander, Rutqvist et al. 1989; Fisher, Costantino et al. 1994; Van Leeuwen, Benraadt et al. 1994), thromboembolic and cerebrovascular events (Deitcher and Gomes 2004), and treatment discontinuations due to the poor tolerability of the treatment (Morandi, Rouzier et al. 2004). In the ATAC trial (ATAC Trialists' Group 2002), more cerebrovascular and deep-

venous thromboembolic events and gynaecological complications including endometrial cancers were seen in the tamoxifen treated group compared to the group treated with the aromatase inhibitor anastrozole. As expected, musculoskeletal disorders and bone fractures were more common in the anastrozole treated group probably due to less bone mass preserving estrogen effect when compared to tamoxifen (Amir, Seruga et al. 2011).

Several SERMs with reduced agonist profile have been developed in order to reduce or avoid the estrogen agonist effect, and thus improve the efficacy and safety profile of SERMs. One of the ways to improve the therapeutic profile of a SERM has been to modulate its structure. Tamoxifen and most of the SERMs studied can be chemically divided to non steroidals, such as trimethylethylene derivatives, and benzothiapines or steroidals, such as a pure antiestrogen ICI 182780 or fulvestrant. The side chains of the triphenylethylene structure have been modulated in compounds such as toremifene, idoxifene and droloxifene, or triphenylethylene ring has been altered in compounds such as benzothiapine raloxifene (Figure 3)

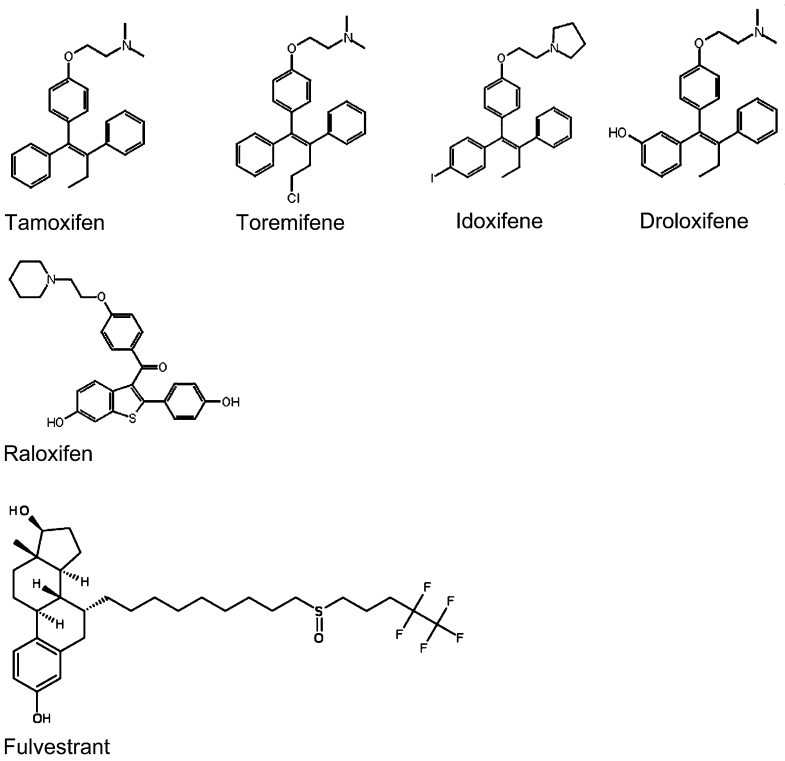

Figure 3. Major nonsteroidal selective estrogen receptor modulators (SERMs) and that of a pure antiestrogen fulvestrant

The binding of a SERM to the ligand binding domain of the ER results in a conformational change of the receptor which, due to different side chain or "fixed ring" structure, is different from that induced by estrogen, (Brzozowski, Pike et al. 1997; Shiau, Barstad et

al. 1998). Conformation of the ER, liganded by different agonists, may produce agonist specific estrogenic/antiestrogenic ratio of action, which also may be tissue specific and may depend on the status of co-activators and co-repressors in the particular cell and tissue (Klinge 2000).

Droloxifene or 3-hydroxytamoxifen has clearly higher binding to ER, better growth inhibition of breast cancer cells in vitro and less estrogenic action in the rat uterus and shorter half-life than tamoxifen (Hassman, Rattel et al. 1994) . In bone it has an estrogenic, bone mineral density preserving effect (Löser, Seibel et al. 1985; Kawamura, Mizota et al. 1993; Ke, Simmons et al. 1995). In phase II clinical trials in patients with advanced breast cancer, droloxifene produced objective treatment responses in tamoxifen naïve and refractory patients (Rauschning and Pritchard 1994). In phase III studies, the drug, however, was less effective than tamoxifen and the development of the drug was discontinued (Buzdar, Hayes et al. 2002).

Idoxifene demonstrated increased affinity to ER, reduced uteretrophic effect compared to tamoxifen and a better effect in cancer cell xenograft growth in vivo (McCague, Leclerq et al. 1989; Chandler, McCague et al. 1991). However, in phase II and III clinical studies in first line therapy of advanced breast cancer, the drug did not show improved efficacy nor safety profile over tamoxifen and development of the drug was discontinued (Arpino, Nair Krishnan et al. 2003; Johnston, Gumbrell et al. 2004).

The benzothiopene derivative raloxifene is structurally quite different from the triphenylethylene compounds (Figure 3). It was shown to bind to the ER and displace estradiol, inhibiting its uterotrophic effect in immature rats, whilst drug given alone had only a minimal uterotrophic effect (Black, Jones et al. 1983). In a preclinical rat tumor model, the drug was able to block estrogen binding similarly to tamoxifen, but was unable to prevent mammary tumor growth to a similar extent as tamoxifen (Gottardis and Jordan 1987; Delmas, Bjarnason et al. 1997), and development of the drug for breast cancer treatment was discontinued. However, due to its ability to prevent breast cancer in the rodent model, to maintain bone density and to inhibit tamoxifen induced endometrial cancer growth, it was developed for the prevention of osteoporosis (Jordan, Phelps et al. 1987; Gottardis, Ricchio et al. 1990). Clinically, raloxifene has been shown to preserve bone mineral density and the drug has been used for treatment of osteoporosis in postmenopausal women (Delmas, Bjarnason et al. 1997; North American Menopause Society 2006; Vogel, Costantino et al. 2006; Cuzick, Decensi et al. 2011).

Pure antiestrogens

SERMs have partial-agonist activity through ER when they antagonise the trophic action of estrogens and at the same time they have intrinsic estrogen-like agonist activity *in vivo*. The net effect is a balance between agonist and antagonist activity. Thus the SERMs have complex organ, cell and gene-specific actions(Furr and Jordan 1984; Jordan and Murphy 1990; Wakeling 1995; Macgregor and Jordan 1998). It has been suggested, that reduction

or removal of estrogen agonist activity would possibly improve the clinical efficacy of SERMs by increasing growth control of ER positive breast cancer (Wakeling 1993). The first compound, which could be called "a pure antiestrogen" was ICI 164384 (Wakeling and Bowler 1987), and further development led to a more potent pure antioestrogen, ICI 182780 fulvestrant (Wakeling, Dukes et al. 1991). Fulvestrant administred as intramuscular injections has demonstrated therapeutic efficacy in advanced tamoxifen resistant breast cancer . Also a number of other pure antioestrogens such as RU 58668 based on steroid structure have been reported (Levesque, Merand et al. 1991; Nique, Van de Velde et al. 1994; Howell, DeFriend et al. 1995) and non-steroidal molecules such as ZM 189154, (Dukes, Chester et al. 1994), EM-800, (Gauthier, Caron et al. 1997), and dichlorotriarylcyclopropanes (Day, Magarian et al. 1991). Fulvestrant (Figure 3) has been shown to be effective in the treatment of advanced breast cancer in postmenopausal women at intramuscular monthly doses of 250 mg, and the effect was superior to that of aromatase inhibitor anastrozole in regard to time to disease progression but with no difference in overall survival (Robertson, Osborne et al. 2003; Howell, Pippen et al. 2005). When increasing the fulvestrant dose up to 500 mg monthly with an additional dose of 500 mg after two weeks of treatment initiation, the efficacy was again similar to that of anastrozole for objective responses and long lasting disease stabilization. However, the time to progression variable favored statistically significantly the fulvestrant treatment arm (Robertson, Llombart-Cussac et al. 2009). Lacking the estrogen agonist action of the drug one would expect to see adverse events related to the estrogen deprivation such as osteoporosis and thromboembolic complications especially in long term follow-up. Indeed, there were no major differences in adverse event profiles between fulvestrant and anastrozole gastrointestinal disturbances and postmenopausal symptoms such as hot flushes being the most prominent.

Aromatase inhibitors

Due to the adverse event profile and partial estrogen agonist activity of SERMs, reduction of estrogen production by aromatase inhibition, instead of the ER modulation, was proposed as a therapeutic target (Schwarzel, Kruggel et al. 1973; Brodie, Schwarzel et al. 1977; Macedo, Sabnis et al. 2009). Initially aminoglutethimide was developed as an anti-epileptic drug, but was found to inhibit cytochrome P450 enzymes and suppress adrenal steroid production. Subsequently use of aminoglutethimide was started as a medical adrenalectomy for the effective treatment of breast cancer (Santen, Brodie et al. 2009). The key treatment effect of aminoglutethimide was the inhibition of the aromatase enzyme with subsequent reduction of estrogen concentrations. Unfortunately, aminoglutethimide also inhibited CYP11 enzyme and decreased cortisol concentrations. For this unspecific enzyme inhibition and the following poor tolerability profile, aminoglutethimide has not been widely used in breast cancer treatment.

4-Hydroxy-androstenedione (4-OH-A) was the first and most potent aromatase inhibitor (Brodie, Schwarzel et al. 1977; Santen, Brodie et al. 2009), acting as a competitive inhibitor and inactivator of the enzyme (Brodie, Garrett et al. 1981). Subsequently, 4-OH-

A reduced estrogen concentrations and caused an antitumor effect in a rat mammary tumor model, and in comparison to tamoxifen was more effective without intrinsic estrogenic effects (Brodie, Schwarzel et al. 1977). 4-OH-A was the first selective aromatase inhibitor used successfully in the treatment of breast cancer (Brodie, Garrett et al. 1981; Santen, Brodie et al. 2009) and was ,subsequently, renamed formestane.

Today, aromatase inhibitors can be classified into steroidal and non-steroidal by their molecular structure. Steroidal, or type I, aromatase inhibitors such as formestane and exemestane have similar structures to androstenedione, a substrate to aromatase on which the inhibitors bind covalently and irreversibely. Non-steroidal or type II inhibitors, such as fadrozole, vorozole, rogletimide, letrozole and anastrozole, bind non-covalently to the enzyme preventing binding of androgens (Chen, Besman et al. 1988; McDonnell 1999).

Formestane, fadrozole, vorozole, and rogletimide have shown activity in the treatment of breast cancer (Brodie, Garrett et al. 1981; Santen, Brodie et al. 2009; Burstein, Prestrud et al. 2010) and were followed by new aromatase inhibitors, such as anastrozole, letrozole, and exemestane, with improved oral bioavailability and better tolerability profile (Smith and Dowsett 2003) in the treatment of metastatic breast cancer and in an adjuvant treatment setting in postmenopausal women. Inhibition of aromatase causes a reduction of the estrogen concentration in postmenopausal women by at least 97% (Brodie and Njar 1996).

Recent trials have shown that aromatase inhibitors improve disease free survival more than conventional tamoxifen treatment in postmenopausal patients with ER positive breast cancer (Goss, Ingle et al. 2003; Coombes, Hall et al. 2004). In one of these trials (ATAC Trialists' Group 2002) with 9366 postmenopausal women with early breast cancer with and without lymph node metastases, estimates for breast cancer recurrence rates at 3 years were 11 and 13 % in the anastrozole, and tamoxifen groups respectively. In a third group, where both of the drugs were given combined, the recurrence rate was 13%, practically the same than in the tamoxifen alone group. A follow-up analysis of the study (ATAC Trialists' Group 2008) indicated that the statistically significant superiority of the aromatase inhibitor 13 % vs 16 % over tamoxifen was maintained. It is not understood why the combination of tamoxifen and aromatase inhibitor was inferior to an aromatase inhibitor alone in the ATAC study. Steroidal aromatase inhibitors such as exemestane have been also shown to be superior to tamoxifen in extending disease free survival, administered alone or in combination with or after tamoxifen (Jonat, Gnant et al. 2006; Coombes, Kilburn et al. 2007; Rintasyöpäryhmä 2007).

The aromatase inhibitor letrozole has been shown, subsequent to 5 years tamoxifen therapy, to decrease bone mineral density after 24 months of treatment in femoral neck and in lumbar spine in postmenopausal breast cancer patients, when compared to placebo. Similarly, the bone biomarkers serum alkaline phosphatase, serum C-telopeptide and urine N-telopeptide indicated significantly increased bone turnover at 24 months (Perez, Josse et al. 2006). In the whole patient population bone fractures were observed in 5.3

% of patients in the letrozole group and 4.6 % in the placebo group after 30 months of follow-up (Goss, Ingle et al. 2005). In an adjuvant study comparing anastrozole to tamoxifen, after a median follow-up time of 68 months, bone fractures were seen in 11% of patients in the anastrozole group and in 7.7 % in tamoxifen group (Howell, Cuzick et al. 2005).

2.1.7. Treatment of breast cancer

In Finland the annual number of breast cancer cases in females have increased from 704 in the years from 1957-1961 to 4090 between 2005-2007, and it is predicted to increase up to 5119 by the year 2020 (Finnish Cancer Registry 2011). This indicates that one out of 11 women in Finland will have the disease at some point during her life. According to the National Cancer Institute statistics, the death rate due to breast cancer in the USA peaked in 1980s and started to decrease thereafter (Ries, Eisner et al. 2002; Horner, Ries et al. 2009). A similar age adjusted trend can be seen in the Finnish Cancer Registry Statistics (Finnish Cancer Registry 2011). Individual clinical trials usually do not have enough patients and statistical power to confirm survival benefit of any single breast cancer treatment, and the treatments were considered mainly palliative. However, in 1990s meta-analyses of available clinical studies in adjuvant treatment of breast cancer were able to show that hormonal and cytotoxic treatments indeed prolonged the overall survival of breast cancer patients when given in adjunct to surgery and radiotherapy (Early Breast Cancer Trialists' Collaborative Group 1992; Early Breast Cancer Trialists' Collaborative Group 1998; Finnish Cancer Registry 2011). Along with the introduction of new therapeutic agents, evidence is accumulating that also in the treatment of advanced or metastatic stage of breast cancer, systemic treatments may have not only a palliative effect but also provide survival benefit (Nabholtz, Senn et al. 1999; Slamon, Leyland-Jones et al. 2001; O'Shaughnessy, Miles et al. 2002; Giordano, Buzdar et al. 2004).

The diagnosis and treatment of breast cancer is based on the histopathological examination of the suspect primary tumor and the adjacent lymph nodes with possible metastases (Singletary, Allred et al. 2002). Primary therapy of early breast cancer is surgery, whith either partial or total resection of the breast tissue and with or without sentinel node biopsy and further evacuation of the axillary lymph nodes depending on the size and histological grade of the primary tumor and the extension of spreading of the possible metastases (Mirsky, O'Brien et al. 1997; Morris, Morris et al. 1997; McCready, Holloway et al. 2005; Rintasyöpäryhmä 2007). Surgery is followed by radiotherapy of the breast and/or to adjacent areas such as the axilla to eradicate the possibly remaining tumor cells in the operation field and in the lymph nodes not evacuated by surgery (Early Breast Cancer Trialists' Collaborative Group 2005a). After breast-conserving surgery, radiotherapy to the conserved breast halves the rate at which the disease recurs and reduces the breast cancer death rate by about a sixth (Early Breast Cancer Trialists' Collaborative Group 2011a).

Adjuvant treatment of breast cancer

The aim of the adjuvant treatment of breast cancer is to prevent or at least prolong the time to disease recurrence and subsequently to prolong breast cancer spesific and the overall survival of the patients. Depending on the individual prognostic and predictive factors for risk of breast cancer recurrence, surgery and radiotherapy is followed by systemic adjuvant drug treatment. The duration of such an adjuvant treatment can be many years, its nature depending on the age and menopausal status of the patient, on the histological grading of the tumor and presence of specific prognostic and predictive markers such as hormone receptors, proliferation markers and oncogenes (Early Breast Cancer Trialists' Collaborative Group 1992; Early Breast Cancer Trialists' Collaborative Group 1998; Harvey, Clark et al. 1999; Goldhirsch, Wood et al. 2007; Rintasyöpäryhmä 2007).

The majority of breast cancer tumors express ER and/or PR, and the majority of breast cancers in premenopausal women responded to reduction of estrogen through oophorectomy in early experiments (Beatson 1896; Love and Philips 2002) and in later studies through adrenalectomy and hypophysectomy (Love and Philips 2002). Today, adjuvant treatment of breast cancer in patients with hormone receptor positive breast cancer is also hormonal, usually with SERMs or aromatase inhibitors as monotherapy or sequentially. Tamoxifen (20 mg/day), given for five years, is a good choice (Early Breast Cancer Trialists' Collaborative Group 1992; Fisher, Dignam et al. 2001; Rintasyöpäryhmä 2007) but aromatase inhibitors have been introduced and are the first choice in patients with high risk of recurrence (ATAC Trialists' Group 2008). In a clinical trial comparing tamoxifen with the aromatase inhibitor anastrozole, the latter provided longer disease free survival with less gynecological, menopausal and thromboembolic adverse events, but with increased incidence of musculoskeletal disorders and bone fractures (ATAC Trialists' Group 2002; Baum, Buzdar et al. 2003; ATAC Trialists' Group 2008). Long term follow up studies indicate that the effect of aromatase inhibitors may also be evident several years after the completion of the treatment and even with modest overall survival benefit (Coombes, Kilburn et al. 2007; ATAC Trialists' Group 2008). Five years of adjuvant tamoxifen safely reduces 15-year risks of breast cancer recurrence and death. According to a recent publication, the ER status was the only recorded factor importantly predictive of the proportional reductions. Overall non-breast-cancer mortality was little affected, despite small absolute increases in thromboembolic and uterine cancer mortality (both only in women older than 55 years), thus all-cause mortality was substantially reduced (Early Breast Cancer Trialists' Collaborative Group 2011b). Tamoxifen still remains a cheap and highly effective alternative for the adjuvat treatment of breast cancer (Hackshaw, Roughton et al. 2011).

In patients with increased risk of recurrence, hormonal therapy can be combined with cytotoxic chemotherapy. The best known factors increasing the risk for recurrence are metastatic spread to ipsilateral axillary lymph nodes, large and or poor histological differentiation of the primary tumor, negative or unknown hormonal receptor status of the

tumor, young age, and HER2 positivity (Goldhirsch, Wood et al. 2007; Rintasyöpäryhmä 2007). In these patients, combined chemo-hormonal adjuvant treatment has been shown to be more effective than single treatment modalities (Early Breast Cancer Trialists' Collaborative Group 1992; Gershanovich, Moiseyenko et al. 1997; Early Breast Cancer Trialists' Collaborative Group 2005a; Early Breast Cancer Trialists' Collaborative Group 2012)

In a population with ER negative tumors, the adjuvant treatment usually consists of only chemotherapy employing cytotoxic agents including antimetabolites, anthracyclines and taxanes (Goldhirsch, Wood et al. 2007; Rintasyöpäryhmä 2007). Tamoxifen has little or no effect on breast cancer recurrence or mortality (Early Breast Cancer Trialists' Collaborative Group 2011b), and is not recommended.

In tumors overexpressing human epidermal growth factor 2 receptor (HER2) there are extra copies of the HER2 gene in the nucleus, and as a result, excess HER2 protein on the surface of the cancer cell. These tumors are considered to posses very high recurrence risk and therefore treatment with HER2-receptor monoclonal antibody, trastuzumab, in combination with chemotherapy is the main stay of treatment (Piccart-Gebhart, Procter et al. 2005) (Slamon, Leyland-Jones et al. 2001; Marty, Cognetti et al. 2005; Joensuu, Kellokumpu-Lehtinen et al. 2006). However, patients with a very high quantitative tumor HER2 content may benefit less from the trastuzumab treatment than those with moderate content of the receptor (Goldhirsch, Wood et al. 2007; Joensuu, Sperinde et al. 2011).

In premenopausal patients, ovarian suppression by chemo- or hormonal therapy by GnRH-analogs or by ovarian ablation will reduce the risk of recurrence after surgery (Stenbygaard, Herrstedt et al. 1993; Early Breast Cancer Trialists' Collaborative Group 1996; Goldhirsch, Wood et al. 2007; Rintasyöpäryhmä 2007). Adjuvant drug treatment most often consists of chemotherapy given in repetitive cycles during four to six months (Early Breast Cancer Trialists' Collaborative Group 1992), plus tamoxifen in receptor positive cases.

Treatment of advanced breast cancer

In recurrent or initially metastatic breast cancer, the aim of the treatment is to slow progression of the disease, to palliate and to prolong survival. Advanced receptor positive breast cancer can be treated with hormonal agents, such as GnRH analogs and SERMs in premenopausal patients, and with SERMS, progestins or aromatase inhibitors in postmenopausal patients (Dombernowsky, Smith et al. 1998; Fossati, Confalonieri et al. 1998; Bonneterre, Thürlimann et al. 2000; Nabholtz, Buzdar et al. 2000; Mouridsen, Gershanovich et al. 2001; Jonat, Gnant et al. 2006; Rintasyöpäryhmä 2007). If the cancer is ER negative or if the disease progresses with symptoms during the selected hormonal treatment, cytotoxic chemotherapy can be successfully introduced (Norton 1994; Fossati, Confalonieri et al. 1998; Colomer 2004). The treatment is usually

continued until disease progression or until development of intolerable adverse events warranting discontinuation or change of the treatment regimen. Quite often the new treatment regimen is again effective and several treatment regimens can be introduced subsequently during the course of the disease. These chemotherapy treatments are usually given in cycles at intervals of a few weeks and hormonal therapy is given continuously for months, usually until disease progression. The concomitant use of cytotoxic chemotherapy and hormonal therapy is not recommended due to increased risk of adverse events, particularly thromboembolic complications. Palliative surgery or radiotherapy can also be used to treat symptomatic metastases such as those in the brain or bone (Rintasyöpäryhmä 2007) .

In patients with ER positive primary tumors, or if the receptor status was unknown, SERMs, tamoxifen and toremifene, have been equally effective in the treatment of metastatic breast cancer (Pyrhönen, Valavaara et al. 1997). In this patient population anastrozole and tamoxifen were equally effective regarding response rate and time to progression and both were active after failure of the other one suggesting that there was no cross resistance (Bonneterre, Buzdar et al. 2001; Thürliman, Hess et al. 2004). However, in some studies, aromatase inhibitors have been more effective than tamoxifen in the first line treatment and more effective than progestins in the second line treatment (Dombernowsky, Smith et al. 1998; Bonneterre, Buzdar et al. 2001; Pritchard 2003). If the patient has received SERMs as adjuvant treatment, the first line treatment of metastatic disease is usually aromatase inhibitors such as anastrozole, letrozole and exemestane (Dombernowsky, Smith et al. 1998; Bonneterre, Thürlimann et al. 2000; Nabholtz, Buzdar et al. 2000; Mouridsen, Gershanovich et al. 2001; Paridaens, Dirix et al. 2003; Thürliman, Hess et al. 2004).

Again, fulvestrant, a pure antiestrogen, has shown at least comparable efficacy to anastrozole in the treatment of advanced breast cancer in postmenopausal women (Robertson, Osborne et al. 2003). It is currently indicated as second-line therapy for the treatment of postmenopausal women with hormone receptor-positive advanced breast cancer who have progressed following prior endocrine therapy.

After failure of hormonal treatment, or if the tumor is hormone receptor negative, having initially poor prognosis or if symptoms require urgent response, cytotoxic therapy with regimens containing anthracyclines, taxanes, vinorelbine, capecitabine and gemcitabine, cyclophosphamide and fluorouracil can be used (Gralow 2005). In case breast cancer has HER2-gene amplification, trastuzumab is usually combined with the selected hormonal or cytotoxic treatment regimen (Slamon, Leyland-Jones et al. 2001).

Hormonal treatment resistance in breast cancer

Meta-analysis of the adjuvant studies (Early Breast Cancer Trialists' Collaborative Group 1998) indicated that five years duration of tamoxifen treatment was more effective than less than 3 years. However, when the treatment was continued beyond

five years the efficacy was not improved, and even the patients treated with placebo after the initial five years period in the comparator group, performed better (Fisher, Dignam et al. 2001). It indeed may be possible that the inhibitory effect of tamoxifen on breast cancer growth lasts no longer than 5 years (Fisher, Dignam et al. 2001). Breast cancer is considered to be either intrinsically resistant to SERMs or the resistance is acquired during the treatment but the mechanisms for tamoxifen resistance are not properly understood. Acquired tamoxifen resistance is not always due to the loss of ER expression (Encarnación, Ciocca et al. 1993; Johnston, Saccani- Jotti et al. 1995; Robertson 1996) or loss of ER binding to DNA (Johnston, Lu et al. 1997), although a high proportion of recurrent breast cancers may have lost the the positive hormone receptor status of the primary tumor and have subsequently poor response to endocrine therapy (Kuukasjärvi, Kononen et al. 1996). Availability of tamoxifen to the cancer cells (Johnston, Haynes et al. 1993; Dowsett 1996; MacCallum, Cummings et al. 2000) or altered metabolism of the drug to more estrogenic compounds (Wolf, Langan-Fahey et al. 1993; Osborne, Jarman et al. 1994) may not be the primary reasons for the development of resistance. Surprisingly, tamoxifen may even stimulate breast cancer growth (Osborne, Coronado et al. 1991; Wolf and Jordan 1993). Another finding is that an estrogen, diethylstilbestrol, at high and low doses, is effective in the treatment of metastatic breast cancer resistant to aromatase inhibitors (Beethambaram, Ingle et al. 1999; Lønning, Taylor et al. 2001; Ellis, Gao et al. 2009).

It is indeed likely that the intrinsic estrogenic effect of a drug is not a prerequisite for the development of drug resistance, as breast cancer has shown also to develop resistance against pure antiestrogen without any measurable intrinsic estrogen agonist action (Howell, DeFriend et al. 1995; Osborne, Coronado-Heinsohn et al. 1995). It has been proposed that different ER variants expressed by breast tumors could be one of the reasons for tamoxifen resistance (Fuqua 1994; Shi, Dong et al. 2009; Rao, Jiang et al. 2011; Wu, Subramaniam et al. 2011) and several genes have been shown to be associated with the resistance through ER activation and the receptor protein phosphyrolation (Holm, Rayala et al. 2006; van Agthoven, Sieuwerts et al. 2009) or through ER – HER2/neu cross-talk (Shou, Masserweh et al. 2004) or other mechanisms (Dorssers, van der Flier et al. 2001).

Finally, it has been suggested that concentratios of tamoxifen metabolites, 4-hydroxytamoxifen, endoxifen (4-hydroxy-N-desmethyltamoxifen), and their isomers are significant for a treatment response (Osborne, Coronado et al. 1991; Schroth, Goetz et al. 2009) and the polymorphisms and activity of tamoxifen metabolizing enzymes CYP2D6 and 2C19 have a significant effect on the tamoxifen treatment outcome in hormone receptor positive breast cancer (Schroth, Antoniadou et al. 2007; Kiyotani, Mushiroda et al. 2010; Madlensky, Natarajan et al. 2011). Several drugs used to treat various common diseases are CYP2D6 inhibitors and avoidance of these potent inhibitors has been recommended (Sideras, Ingle et al. 2010), although an observational study did not find any effect of concomitant CYP2D6 inhibitor use on treatment

outcome (Dezentjé, van Blijderveen et al. 2010). Depending on a CYP2D6 genotype of a breast cancer patient or on concomitant administration of inhibitors of the enzyme the patient may be good, intermediate or poor tamoxifen metabolizer with subsequent high, intermediate or low concentreations of endoxifen (Jin, Desta et al. 2005; Pritchard 2010). Thus, it has been suggested that patients with low CYP2D6 activity could benefit from increase of tamoxifen dose (Irvin, Walko et al. 2011).

2.2. Toremifene

Toremifene is a SERM developed originally for treatment of advanced breast cancer in postmenopausal women. The development of the compound was initiated in early 1980s and the clinical studies were conducted in late eighties and early nineties. Dose selection and proof of concept in advanced breast cancer was based on, *in vitro* pharmacology, nonclinical animal models, and on phase I and on small noncomparative, nonblinded phase II clinical studies.

2.2.1. Preclinical data

Pharmacology

In the *in vitro* studies, interaction with ER was the basis to support the presumed mechanism of action as PoP and to estimate the concentration range of the presumed pharmacological activity. In these studies it was shown that toremifene was a competitive ligand to estradiol and bound to ER with similar affinity than did tamoxifen. Toremifene was able to translocate ER from cytoplasm to nucleus as a toremifene-ER complex. The effect was seen after a 1.0 mg/kg intraperitoneal (i.p.) injection and after five 1.0 mg/kg daily injections. The effect was similar to that of tamoxifen and superior to that of estradiol. In a receptor binding assay, a 0.5 μM concentration of toremifene was sufficient for ER binding, and was able to displace 50 % of estradiol from the ER-complex (Kallio, Kangas et al. 1986).

The hormonal, estrogenic and antiestrogenic activity of toremifene turned out to be species specific. It mainly had an intrinsic estrogenic effect in mouse and dog, while it was predominantly antiestrogenic in rats and humans. In the immature rat uterus intrinsic estrogenic action was weak, but in mice it was clear and dose dependent. In mice the maximum estrogen-like uterotrophic effect was achieved with a 1.0 -10 mg/kg single subcutaneous (s.c.) dose and when toremifene was administed simultaneously with estradiol the maximum antiestrogenic effect, was reached at a 3.0 mg/kg single dose (Kallio, Kangas et al. 1986). At these dose levels, the effects of toremifene were similar to those of tamoxifen (Data on file, Orion Pharma). In addition, daily treatment for 30 days with toremifene at 3-30 mg/kg doses per orally significantly reduced the uterine weight of sexually mature female rats. Thus as a PoM, it can be concluded that toremifene had antiestrogenic effects in the presence of exogenous or endogenous

estrogens (Kallio, Kangas et al. 1986). In immature female rats, when assessed by the effect on uterine weight, toremifene and tamoxifen had similar intrinsic estrogen agonist activity at a 50 mg/kg p.o. dose. However, at smaller doses, ≤ 10 mg/kg, tamoxifen was 40 times more estrogenic than toremifene. In the presence of estradiol both compounds were equally antiestrogenic at the 10 and 50 mg/kg dose, but in lower doses a 10 times higher dose of toremifene was required for the same antiestrogenic effect. Importantly, with both of the compounds, the antiestrogenic effect increased up to the highest 50 mg/kg dose tested. In this test model, toremifene at 10 mg/kg or lower doses had more antiestrogenic potency in relation to its estrogenic potency when compared to the same dose range of tamoxifen, suggesting more favorable antiestrogenic to estrogenic ratio for toremifene in the treatment of estrogen dependent diseases (Di Salle, Zaccheo et al. 1990). In ovariectomised rats, toremifene 0.3, 3.0 or 30.0 mg/kg/day inhibited the decrease of trabecular bone thickness (Karlsson, Mäntylä et al. 1999), and the maximum effect was reached at the 3.0 mg/kg dose level.

Whilst studying the inhibition of cancer growth *in vitro,* a toremifene concentration range of 0.1 -5.0 μM, was shown to inhibit cell growth in a dose dependent manner and induced cell death in an ER positive MCF-7 human breast cancer cell line and in fresh human tumor specimens *in vitro* taken from gynecological cancer tissues (Kangas, Nieminen et al. 1986). Providing preclinical PoC of the antiestrogenic activity *in vivo*, toremifene decreased the number of new tumors and inhibited the growth of existing tumors *in vitro* in a dimethylbenzanthracene (DMBA) induced rat mammary cancer model, after 7 days p.o. treatment with a 1.0 mg/kg dose. The effect was similar to that of tamoxifen at a 1.0 – 7.5 mg/kg dose range and was not improved significantly by increasing the dose. Surprisingly, and not in an agreement with the hypothesis of ER mediated action, a high dose of toremifene treatment, ≥100 mg/kg for 5 days, demonstrated cytolytic efficacy also in ER negative mouse uterine sarcoma and in human desmoid tumor (Kangas, Nieminen et al. 1986). In another study employing the DMBA-induced mammary adenocarcinoma model in rat, toremifene significantly decreased emergence of new ER positive and hormone sensitive tumors and improved the ratio of regressing and growing tumors at the daily p.o. dose levels of 200 and 800 μg. The effect was similar to that seen with tamoxifen 200 μg (Robinson, Mauel et al. 1988). Increasing the dose up to 8000 μg did not improve efficacy, although the concentration of toremifene and its main metabolite N-desmethyltoremifene were 2.5 and 18 μM, corresponding to the 5 x 10 μM concentration with cytolytic activity on MCF-7 cells in vitro (Kangas, Nieminen et al. 1986; Robinson, Mauel et al. 1988). At the 800 μg dose level toremifene showed antitumor activity in the rat tumor model, where its concentration and that of N-desmethyltoremifene were at 0.05 and 0.4 μM, respectively. These were similar to the 0.1 – 1.0 μM concentrations with growth inhibitory activity that were also seen in MCF-7 cells (Kangas, Nieminen et al. 1986). In cell lines that had developed resistance to cytotoxic agents, toremifene increased their cytotoxic activity and reversed the developed resistance (DeGregorio, Ford et al. 1989) by inhibiting P-glycoprotein (P-170). This Multi Drug Resistance (MDR) reversal effect of toremifene was concentration

dependent, and the sensitizing activity could be detected at the clinically achieved 8 and 30 μM toremifene and N-demethyltoremifene steady state concentrations, respectively. The increased expression of P-170 after exposing tumour cells to doxorubicin has been used as a model of MDR, and P-170 is suggested to act as an efflux pump for toxic agents and its over expression is proposed to be one of the mechanisms in MDR (Juliano and Ling 1976). However, it has been reported (Mahvi, Carper et al. 1996) that toremifene can also overcome heat shock protein 27 (hsp27) induced drug resistance in breast cancer cells indicating that P-170 inhibition may not be the only mechanism of action by which toremifene may overcome MDR *in vitro.*

Preclinical toxicity

Toremifene was well tolerated in mice and in rats after oral dosing, with LD_{50} values > 2000 mg/kg and > 1550 mg/kg, respectively, and gastric dilatation was the primary cause of death in rats. In mice, apparently the estrogenic effect at high doses caused endometrial gland hyperplasia in the uterus and absence of corpora lutea in the ovaries. In rat, toremifene had predominantly antiestrogenic effect based on cellular hypertrophy of uterine luminal epithelium (Data on file, Orion Pharma)

In mice, rats, dogs and monkeys, the main effects after repeated oral dosing of toremifene up to doses of 48 mg/kg/day were hormonal, estrogenic and antiestrogenic. Other, non hormonal, findings were not considered relevant (Data on file, Orion Pharma). In dog, toremifene was estrogenic causing ovarian atrophy, endometrial hyperplasia, myometrial hypertrophy and vaginal hypertrophy. Estrogenic effects were also seen in the mouse uterus. In monkey, both antiestrogenic and estrogenic effects such as reduction in endometrial stromal cellularity and endometrial hyperplasia, respectively, were seen.

In a mouse carcinogenicity study, toremifene at 1, 3, 10 and 30 mg/kg daily for 104 weeks (Data on file, Orion Pharma) induced estrogenic effects at all dose levels. Neoplasia, similar to those caused by estradiol were seen in both sexes and it was concluded that, within the dose range, toremifene acts as an estrogen in mice. On this basis, the findings in mice were considered to be of limited relevance for the safety in human, where toremifene acts primarily as an antiestrogen.

When toremifene was administrated to rats with daily oral doses of 0.12, 1.2, 5 and 12 mg/kg for 104 weeks, antiestrogenic effects were seen (Data on file, Orion Pharma). Incidences of pituitary tumours in both sexes, mammary tumours in females and testicular interstitial cell tumours in males were reduced and there was no increase in the incidence of any tumour, when compared to controls, suggesting that toremifene is not carcinogenic in rat. Toxicities of toremifene up to 48 mg/kg and tamoxifen up to 45 mg/kg have been studied in four 52 week comparative studies in female rats. Tamoxifen but not toremifene induced hepatocellular carcinomas, cataracts and endometrial metaplasia and infiltrating endometrial squamous cell carcinoma (Hirsimäki, Hirsimäki et al. 1993). Tamoxifen's hepatocarcinogenicity was not considered to be due to its hormonal effects,

as toremifene and tamoxifen had similar hormonal effects in rat liver (Kendall and Rose 1992). However, induction of cytochrome P450 by tamoxifen may produce genotoxic metabolites of the drug, which may contribute to tamoxifen hepatocarcinogenesis (White, Davies et al. 1993). Again, tamoxifen is more potent than toremifene to promote liver tumours in a two-stage model of hepatocarcinogenesis (Dragan, Vaughan et al. 1995). However, there is no mechanistic theory as to how tamoxifen causes cataracts and endometrial neoplasia in rat. Toremifene was not mutagenic or clastogenic in standard genotoxicity tests (Data on file, Orion Pharma), and it was significantly less potent than tamoxifen in causing DNA adduct formation and hepatocellular carcinomas in rats (Hard, Iatropoulos et al. 1993; Davies, Martin et al. 1995; Dragan, Vaughan et al. 1995; Hemminki, Widlak et al. 1995; White, Martin et al. 1997).

2.2.2. Pharmacokinetics

Preclinical pharmacokinetics

In laboratory animals oral toremifene was well absorbed in such a way that the bioavailability could be considered complete. High distribution volumes in mouse, rat, dog and monkey, indicated an extensive tissue distribution of the drug. After a single dose of the radiolabelled drug, the distribution half-life of total radiactivity was about four hours in most tissues and enterohepatic circulation was evident. In the rat, the tissue distribution pattern of unchanged toremifene and total radioactivity were similar after single and repeated dosing. Concentrations were highest in the lungs, followed by the adrenals, kidneys, liver, spleen, pancreas and ovaries, and lowest in the plasma, eyes, bone, whole blood, hypothalamus and cortex. Distribution into the target tumours was well demonstrated; the radioactivity was about 15-fold when compared to plasma concentrations. Toremifene was also strongly bound to serum protein in monkeys, dogs and rats (Data on file, Orion Pharma).

P-hydroxylation and N-demethylation and subsequent oxidation of aminoethoxy side chain, first to alcohols and finally to carboxylic acids are the main pathways of toremifene metabolism. Hydroxylated metabolites can be further conjugated. However, the concentrations and pharmacokinetics of single metabolites in serum varied among species, although there are no qualitative species specific differences (Data on file, Orion Pharma). Several metabolites of toremifene, especially N-demethyl, N,N-didemethyl and 4-hydroxytoremifene, had hormonal activity like the parent drug (Kangas 1990). In serum, N-demethyltoremifene was the main metabolite and the concentrations of other metabolites were clearly lower (Data on file, Orion Pharma). Serum kinetics of unchanged toremifene could be described by a two-compartment model both after i.v. and p.o. administration. The elimination half-life in mice was 2.5-4 hours, in rats 5-17 hours, in dogs 23-33 hours, and in monkeys 7-11 hours (Data on file, Orion Pharma). About 70 % of toremifene was excreted as metabolites in faeces, and in the rat a mean of 48 % of the given dose was excreted to the bile during two days, suggesting significant enterohepatic circulation of the drug and the metabolites (Sipilä, Kangas et al. 1990).

Clinical pharmacology in healthy subjects

In man, a single dose oral dose of toremifene was well absorbed. Peak maximum concentration (C_{max}) in serum was achieved in 4 hours (Anttila, Valavaara et al. 1990; Kohler, Hamm et al. 1990). Food prolonged the time to maximum concentration (t_{max}), but did not affect the total absorption (Data on file, Orion Pharma). Toremifene pharmacokinetics followed the two-compartment model, with a mean distribution half-life of 4 hours and a mean elimination half-life (t½) of 5 days (Anttila, Valavaara et al. 1990). At the dose range of 11-680 mg, C_{max} and area under the plasma concentration time curve (AUC) of the drug were dose dependent, however, the time to maximum concentration (t_{max}) and t½ were not dependent on the dose used. Thus it was concluded that toremifene exhibited linear kinetics in relation to the dose. The main pharmacokinetic variables of toremifene after a single dose administration are summarized inTable 3.

Table 3. Mean pharmacokinetic variables of toremifene in healthy subjects after single dose administration. (Data on file, Orion Pharma). C_{max}= maximum concentration, t_{max}= time to maximum concentration, AUC = area under the plasma concentration time curve, $t_{½}$= elimination half life

Dose (mg)	n	C_{max} (µg/mL)	t_{max} (h)	AUC (µgh/mL)	t½ (days)	Reference
11-680	37	0.03-1.93	3.3	1.8-125	5.4	(Data on file)
60	18	0.32	2.8	14.8	4.0	(Data on file)
60	12	0.28	3.0	11.5	3.3	(Data on file)
60	12	0.19	2.3	8.5	4.1	(Anttila, Valavaara et al. 1990)

Due to the slow elimination of toremifene and the main hormonally active metabolite N-demethyltoremifene in serum, a significant accumulation of the compounds took place and steady-state concentrations were reached only after 4-6 weeks of therapy (Anttila, Valavaara et al. 1990). The mean oral clearance (CL/f) and the mean apparent volume of distribution (Vd/f) were calculated after oral intake of toremifene as 4.5-7.0 L/h and 580-1222 L, respectively, indicating a slow drug elimination and large tissue distribution (Anttila, Valavaara et al. 1990; Wiebe, Benz et al. 1990). Toremifene and N-demethyltoremifene bound extensively to serum proteins and at the concentrations of 0.1 – 12.3 µM toremifene binding was 99.7 % and that of the metabolite even higher (Sipilä, Näntö et al. 1988).

In human faeces and urine, a total of 11 toremifene metabolites have been found (Watanabe, Irie et al. 1989). In faeces, 4-hydroxylated side-chain alcohol, 4-hydroxylated side-chain carboxylic acid, and an unknown carboxylic acid were present at the highest concentrations. In human urine, four unconjugated and three conjugated metabolites have been detected (Watanabe, Irie et al. 1989). In serum, the number of metabolites detected has been four, and two of them, the main metabolite, N-demethyltoremifene, and the minor metabolite, (deaminohydroxy)toremifene, were found in all subjects

(Anttila, Valavaara et al. 1990). The mean elimination half-life of the main metabolite was longer than that of the parent drug, 7-11 days. The chemical structures of the parent drug toremifene and those of the main metabolites are presented in Figure 4.

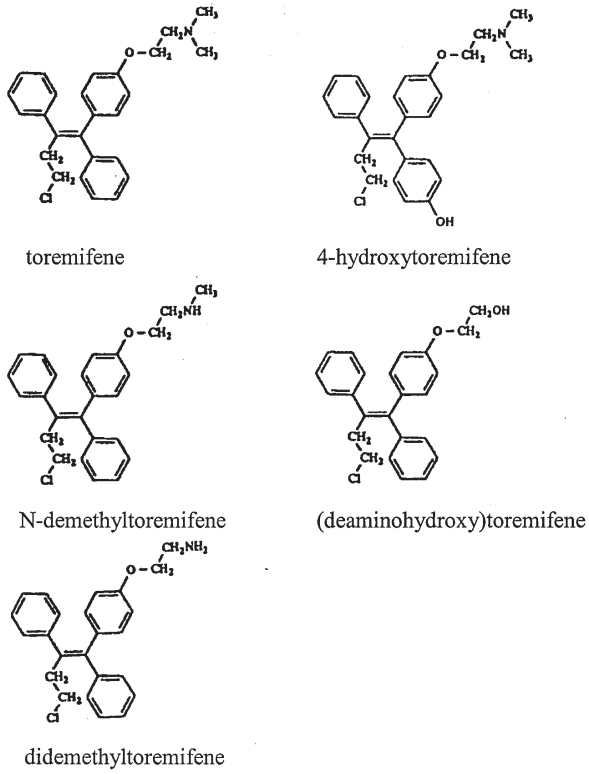

Figure 4. Chemical structures of toremifene and the main metabolites

In humans, the cytochrome P450 3A enzyme system (CYP3A) in liver seems to be the major metabolic pathway for toremifene (Berthou, Dreano et al. 1994). CYP3A concentration and formation of N-demethyltoremifene tend to be correlated and the formation of this metabolite can be inhibited by other competing substrates of CYP3A.

Steady-state plasma concentrations of toremifene and N-demethyltoremifene were reached dose dependently between 1 and 4 weeks. At doses of 200-400 mg daily, steady state concentrations were seen after 1 – 2 weeks, but at daily doses of 20 mg or below 3-4 weeks was required (Kohler, Hamm et al. 1990; Wiebe, Benz et al. 1990). At steady-state, AUCs of toremifene and N-demethyltoremifene were dose dependent (Wiebe, Benz et al. 1990). The average minimum steady-state level of toremifene in serum was 0.3, 0.7, 0.8, 2.2, 3.5, and 8.5 μM when using the doses of 10, 20, 40, 60, 200 and 400 mg/day (Anttila, Valavaara et al. 1990; Wiebe, Benz et al. 1990). The concentration of N-demethyltoremifene was two or three times higher than that of the parent drug and 4-hydroxytoremifene was only detectable after high, ≥ 200 mg, daily doses of toremifene

(Wiebe, Benz et al. 1990). In patients with impaired kidney function, the toremifene pharmacokinetics were not significantly changed (Anttila, Laakso et al. 1995). However, the elimination rate of toremifene and the main metabolite was significantly increased in patients with activated liver function and decreased in patients with impaired liver function, resulting in decreased and increased terminal half-lives, respectively. A significantly longer elimination half-life was seen in the apparently healthy volunteers, aged from 55 to 87 years, compared to younger healthy subjects, aged from 19 to 23 years (Data on file, Orion Pharma). However, there were no significant differences between the two groups regarding bioavailability of the drug.

2.2.3. Clinical toremifene data before phase III

Toremifene dose selection for confirmatory phase III studies was based on open, non comparative, phase I – II studies either with healthy subjects or with postmenopausal women with advanced breast cancer or with other advanced malignancies. Preclinical data, human pharmacokinetics, tolerance, biological and clinical effects of the drug guided the dose selection.

Clinical pharmacology in patients

In patients with advanced cancers who were treated with toremifene doses from 10 to 400 mg daily, steady state concentrations of the parent drug and the main metabolite were seen in plasma after one to four weeks, depending on the dose, so that the lower doses reached the steady state later. The concentrations were dose dependent, although no clear differences could be seen between the concentrations after 20 and 40 mg daily doses (Wiebe, Benz et al. 1990). Already with the lowest 10 mg daily dose, the plasma concentrations of the parent drug toremifene alone, or especially when combined with those of the pharmacologically active main metabolite N-demethyltoremifene, were well within the concentration range shown to inhibit cancer growth in experimental models *in vitro* and *in vivo* (Kangas, Nieminen et al. 1986; Robinson, Mauel et al. 1988).

Hormonal effects and tolerability

Five days administration of toremifene doses from 22 to 680 mg daily in postmenopausal women reduced LH, FSH and increased sex hormone binding globulin (SHBG) (Kivinen and Mäenpää 1990). The dose dependent antiestrogenic potency of toremifene and tamoxifen were evaluated by vaginal cytology in healthy postmenopausal women (Homesley, Shemano et al. 1993). All subjects were applied a transdermal estradiol patch, which released 100 µg of estradiol over 24 hours, twice weekly for 38 days. Toremifene or tamoxifen, were administered per orally daily for 10 days from study day 29 onwards. As a result, the toremifene dose of 10 mg had borderline, or no antiestrogenic activity, but doses of 20, 40 and 80 or 120 – 200 mg exerted marked antiestrogenic effects, which were, however, not statistically different from each other. Tamoxifen at doses

of 10 and 20 mg/day had antiestrogenic effects similar to toremifene at doses of 20-200 mg/day. In another study with 11 postmenopausal women, a 5 day treatment with toremifene (68 mg) or tamoxifen (60 mg) daily had a significant antiestrogenic effect on estradiol primed vaginal epithelium. In the same study, a toremifene dose of 20 mg daily for 10 days indicated a similar, although not statistically significant, trend, (Mäenpää, Söderström et al. 1990).

In a phase I study, 107 postmenopausal women with various difficult-to-treat malignancies received toremifene doses of 10, 20, 40, 60 200 or 400 mg daily for 8 weeks. All of these doses were generally well tolerated with no clearly dose dependent adverse events. No toremifene related ECG changes were found at any dose level. In another clinical study to evaluate toremifene effects on QTc interval by ECG in 250 male patients a dose dependent effect on QTc prolongation was observed at 80 and 300 mg dose levels (European Medicines Agency 2011). Out of 48 evaluable patients, three with breast cancer had partial objective response at the 200 mg dose level and the fourth with advanced endometrial cancer responded at the 400 mg dose level. The estrogenic effect increased dose dependently from daily doses of 20 or 60 mg upwards, as indicated by SHBG increase and antithrombin III decrease or FSH and LH decrease, respectively. The antiestrogenic effect, assessed by vaginal cell count, was seen at the 20 mg daily dose and above (Hamm, Tormey et al. 1991). This result suggested a dose dependent increase of estrogenic and antiestrogenic activity of toremifene. The estrogenic effect increased up to the 200 mg dose, but no evidence of increased antiestrogenicity above the 20 mg dose could be seen.

In another study, the maximum tolerated dose of toremifene was determined in 19 postmenopausal women with previously treated metastatic breast cancer. Escalating doses of toremifene from 200, to 300 and to 400 mg/m^2 were administered daily until unacceptable toxicity or until disease progression. Nausea, vomiting and dizziness in three out of the five patients treated were considered to be dose related adverse events, and the highest dose, 400 mg/m^2, was not considered to be sufficiently well tolerated. The 300 mg/m^2 dose was recommended to be tested in phase II efficacy and safety studies (Bishop, Murray et al. 1992). Steady state concentrations were reached from 1 to 3 weeks and the mean predose concentrations of toremifene and N-demethyltoremifene were 3.8 and 7.8 μM at 200 mg/m^2 and 5.3. and 11.0 μM at 300 mg/m^2 dose levels, respectively. Maximum concentrations were reached from 2.5 to 3.2 hours post dose and were 6.9 and 9.9 μM for the parent drug. The maximum concentrations of the main metabolite were only 25 – 30 % higher than the minimum concentrations. Due to the poor tolerability, pharmacokinetics of toremifene at the highest 400 mg/m^2, dose level could not be determined. Ten of the 19 patients had their disease stabilized at least for 8 weeks and 2 had partial objective remission of their tumours (Bishop, Murray et al. 1992).

Clinical efficacy and safety studies

In phase II clinical studies, postmenopausal patients, with metastatic or locally recurrent or advanced ER positive, unknown or ER negative breast cancer, were treated with toremifene doses of 20-400 mg/day. In these studies (Table 4), objective response rates, assessed by the IUCC criteria (Miller, Hoogstraten et al. 1981), ranged from 21 to 68 % in patients with ER positive or ER unknown tumors without previous treatment. The lowest dose tested in a study (20 mg/day), demonstrated efficacy but did not reach as high response rates as were seen in other studies using daily doses of 60, 120 and 200 – 240 mg of toremifene (Valavaara, Pyrhönen et al. 1988; Valavaara and Pyrhönen 1989; Hietanen, Baltina et al. 1990; Pyrhönen, Valavaara et al. 1990; Modig, Borgström et al. 1990a; Modig, Borgström et al. 1990b; Tominaga, Abe et al. 1993; Baltina, Hietanen et al. 1996).

Table 4. Phase II clinical studies with toremifene as first line treatment of advanced breast cancer in ER positive or unknown patients.

Dose (mg)	No. of Eval. Pt.	No. of CR + PR	Response rate (%)	SD (%)	Reference
ER positive					
20	14	0+3	21	57	(Pyrhönen, Valavaara et al. 1990)
60	46	8+17	54	26	(Valavaara, Pyrhönen et al. 1988)
ER positive or unknown					
40	29	7	24	-	(Tominaga, Abe et al. 1993)
60	29	4	14	-	
120	5	1	20	-	
240	5	2	40	-	
60	12	3+3	50	42	(Modig, Borgström et al. 1990a)
60	24	6+5	48	25	(Gundersen 1990)
240	38	10+16	68	21	(Hietanen, Baltina et al. 1990)

CR = complete response, PR = partial response, SD = stabilized disease, TTP = mean time to progression

As a second or third line treatment, after a failure of previous hormonal or cytotoxic therapy, moderate activity was achieved employing higher doses of toremifene ranging from 200 to 240 mg/day (Table 5.). In these studies, objective response rates varied from 0 to 33 %. In the largest study with 102 patients who had progressed on tamoxifen, only a 5 % response rate, with a 95 % confidence interval (CI) of 3 to 7 %, was observed suggesting relatively strong cross resistance between toremifene and tamoxifen in this patient population (Vogel, Shemano et al. 1993). A similar result was obtained in a randomized cross-over trial with 66 postmenopausal breast cancer patients comparing toremifene 240 mg to tamoxifen 40 mg and crossing after failure of the first randomised treatment to the other (Stenbygaard, Herrstedt et al. 1993). In a third study with 50 patients with ER positive primary tumors, a similar 4 % response rate, with 95 % CI of 0.5 to 14 %, was observed with toremifene 240 mg daily dose (Pyrhönen, Valavaara et

al. 1994). In a study, 21 breast cancer patients with ER negative primary tumors were treated with toremifene 400 mg daily but no objective responses were seen (Perry, Berry et al. 1995). Tolerability of the whole dose range tested was considered to be good.

Table 5. Phase II clinical studies with toremifene as second or third line treatment of advanced breast cancer in ER positive or unknown patients.

Dose (mg)	No. of Eval. Pt.	No. of CR + PR	Response rate (%)	SD (%)	Reference
Previous hormonal therapy					
200	9	0+3	33	44	(Ebbs, Roberts et al. 1990)
200	102	2+3	5	23	(Vogel, Shemano et al. 1993)
240	50	1+1	4	44	(Pyrhönen, Valavaara et al. 1994)
240	35	0+0	0	26	(Jönsson, Malmberg et al. 1991)
Previous hormonal or/and chemotherapy					
60	8	0+2	25	38	(Hindy, Juhos et al. 1990)
120	2	0+0	0	100	
300	6	0+2	33	17	
240	23	0+0	0	30	(Stenbygaard, Herrstedt et al. 1993)
200	13	0+1	8	31	(Modig, Borgström et al. 1990b)
ER-negative					
400	21	0+0	0	23	(Perry, Berry et al. 1995)

CR = complete response, PR = partial response, SD = stabilized disease, TTP = mean time to progression

To test clinically the MDR reversal hypothesis (DeGregorio, Ford et al. 1989; Kirk, Houlbrook et al. 1993), toremifene was given to patients with ovarian cancer in combination with cytotoxic treatment. The treatment was tolerated and efficacy was encouraging (Mäenpää, Sipilä et al. 1992).

Although pharmacokinetic, biological and biochemical data suggested that a 20 mg daily dose of toremifene would have been efficacious in the treatment of advanced ER positive breast cancer, in a noncomparative and prematurely terminated study with 14 patients only a 21 % objective response rate was seen (Pyrhönen, Valavaara et al. 1990). In three other noncomparative clinical studies with a 60 mg daily dose of toremifene and with 12, 24 and 46 ER positive or unknown patients in each, about 50 % response rates were observed (Valavaara, Pyrhönen et al. 1988; Gundersen and Kvaloy 1989; Modig, Borgström et al. 1990a). In addition, in a study with 38 patients with ER positive breast cancer receiving 240 mg toremifene daily, an objective response rate of 68 % was observed.

Earlier preclinical data also suggested that high dose toremifene may have efficacy in ER negative tumors (Kangas, Nieminen et al. 1986), but no efficacy in the treatment of ER /PR negative breast cancer was observed (Perry, Berry et al. 1995). Futhermore efficacy

after failure of previous hormonal and or cytotoxic therapy was limited suggesting strong cross resistance with tamoxifen.

In an evaluation of toremifene dose effect, the response was apparently low with the 20 mg dose and somewhat higher responses were observed with the 60 mg and particularly 240 mg daily doses. On the basis of these observations, together with preclinical observations, toremifene doses of 60 and 200-240 mg were selected for confirmatory phase III studies for the first line treatment in postmenopausal breast cancer patients with ER positive or unknown primary tumors.

Based on the phase III studies toremifene dose 60 and 40 mg were authorized for clinical use globally and in Japan, respectively. The doses have been effective and safe in clinical practice since the year 1989 and with estimated exposure of over 500 000 patient years.

3. AIMS OF THE STUDY

The objective of this study was to evaluate feasibility of preclinical and early clinical data for the selection of clinically effective and safe dose and target population for toremifene treatment. A completed clinical program was used as a reference and the specific questions were addressed by the following separate investigations:

- To study feasibility and applicability of preclinical and early clinical data on the dose selection for toremifene
- To study feasibility of biochemical surrogate markers to predict safety and tolerability of different toremifene doses and tamoxifen in the target patient population.
- To study the clinical efficacy and safety of low and high doses of toremifene compared with tamoxifen in the target patient population.
- To study factors predicting efficacy of SERM therapy and the feasibility of using preclinical and early clinical data in the selection of the target patient population
- To study the dose- dependence of the concentrations of toremifene and its metabolite in human plasma, and in lung and tumor tissues for dose selection

4. MATERIALS AND METHODS

Patients

Studies I - V included a total of 2050 patients with advanced breast cancer, and in the study VI there were 18 patients with operable lung tumors. The studies were conducted in the UK, France, Italy and South Africa (Study I), in the USA and South Africa (Study II), in Russia, Estonia and Latvia (Study III), in the USA, South Africa, Russia, Latvia and Estonia (Study IV), in Finland, Sweden, Norway, Czech Republic, Poland, Hungary, Germany, Russia, Estonia, Latvia, Japan, South Africa and in the USA (study V) and in Finland (Study VI). Most of the patients in studies I-IV were postmenopausal women; but two males were included into the study III by misinterpretation of the protocol. In the study VI, 17 male and one female patient with operable lung tumours were included.

In the breast cancer studies, the primary tumour had to be histological or cytologically verified, ER positive (ER concentration $\geq$ 10 fmol/mg of cytosol protein) or the ER status was unknown at the time of diagnosis. Patients with known ER negative tumours were excluded, but it is, of course, possible that some of these patients may have been included due to unknown ER status. The patients had at least one measurable or unmeasurable but evaluable breast cancer lesion, and they had received no previous systemic drug therapy for the advanced stage of their disease. Earlier adjuvant treatment, however, was allowed if 12 months had elapsed since the treatment discontinuation. In the kinetic study VI, the patients had operable lung tumours and at least 2 months of life expectancy.

The breast cancer patients were treated at least for two months or until disease progression, and the patients with lung tumours received toremifene for 7 days before their surgery. Summary of patient numbers, age, dose and treatment duration by study are shown in Table 6.

Table 6. Summary of patient age, primary variables, study and comparative drug doses and treatment duration in different study populations

Study	n	Drug and dose mg/day	Treatment duration (weeks)	Patient age	Primary variables
I	90	TOR20[1]	12[2]	65±10[3]	RR and TTP[5]
	81	TOR40	12	66±10	
	89	TOR60	12	65±10	
II	165	TOR60	8	64 ±10	RR and TTP
	156	TOR200	8	63±10	
	148	TAM20	8	61±11	
III	157	TOR60	8	61 (38-85)[4]	RR and TTP
	157	TOR240	8	62 (35-82)	
	149	TAM40	8	60 (31-90)	
IV	369	TOR200-240	8	62	RR and TTP
	364	TAM20-40	8	61	
V	725	TOR40-60	8	-	Predictive factors for treatment response
	696	TAM20-40	8	-	
VI	18	TOR240-600	1	62 (42-70)	Tissue concentrations

[1]Toremifene 20 mg (TOR20), 40 mg (TOR40), 60 mg (TOR60), 200 mg (TOR200) or 240 mg (TOR240), tamoxifen 20 mg (TAM20) or 40 mg (TAM40) [2]At least or until disease progression, [3]Mean ± SD, [4]Median and range, Objective Response rate (RR) and Time to disease Progression (TTP)

Study drug

Tablets of the study drug toremifene were manufactred by the Orion corporation, Orion-Farmos, Turku, Finland for 20, 40,60 and 200mg doses. Tablets of Tamoxifen (20 and 40 mg)s were manufactured by the Orion Corporation, Orion-Farmos, Turku, Finland or tamoxifen 20 (Tominaga, Abe et al. 1993) or 30 mg tablets (Study V) by local suppliers in the open studies. All the studies in the meta-analysis were open, with the exception of a double blind study (Pyrhönen, Valavaara et al. 1997) where toremifene 60 mg and tamoxifen 40 mg tablets were made identical by appearance, weight and size.

Clinical efficacy variables

Response criteria used for the assessment of measurable breast cancer were based on World Health Organization criteria adopted by UICC (Miller, Hoogstraten et al. 1981) and those for unmeasurable but evaluable bone dominant disease on Eastern Cooperative Oncology Group (ECOG) criteria (Oken, Greech et al. 1982). The patients were

controlled at 2 month intervals in studies II – V, and at 4 week intervals in study I. For measurable disease, a complete response (CR) or a partial response (PR) were recorded if all signs of lesions had disappeared or at least a 50 % reduction of bidimensional area of the index lesions was observed during two consecutive visits at least 4 weeks apart, respectively. Disease progression (PD) was recorded when the bidimensional area of at least one index lesion grew at least by 25 % in one assessment or a new lesion was detected. For the bone lesions, PR was defined as a partial decrease of lytic lesions, recalcification of lytic lesions, or decreased density of blastic lesions, and again, for at least for two consecutive observations, at least four weeks apart. For the no change (NC) category, a bone lesion had to stay stable for at least eight weeks. In bone dominant disease the criteria for CR and PD were the same as for measurable disease, except no exact measure for the increase was required for the PD.

Time to disease progression (TTP) or time to treatment failure (TTF) was defined as the time from randomization to disease progression or treatment discontinuation for any reason, respectively. Survival is defined as time from randomization until death for whatever reason.

Clinical safety variables

Patients were evaluated for safety before the studies and every 8 (Studies II – V), every 4 weeks (Study I) or after 7 days (Study VI). Safety evaluation included medical history, physical and gynaecological examinations, performance status, complete blood counts and serum chemistries. Reported adverse events were assessed for their causality by the investigator and graded according to the WHO guideline (WHO Collaborating Center for International Drug Monitoring 1988).

Biochemical variables

Standard immunometric methods at each collaborating site were used for the assessment of FSH, LH, sex hormone binding globulin (SHBG), E2 and antithrombin III (AT III) in serum.

Statistics

Sample size estimations were based on the assumption of detecting 20% difference in response rate (Studies I and III) or to show statistical equivalence (Study II; (Hayes, Van Zyl et al. 1995) among the treatment regimens in the studies. Treatment regimens were compared using type I error rate (α) of 0.05 and type II error rate (β) of 0.20. Primary analyses were based on "intent to treat" principle where all randomized patients who had received the study drug were included. Response rates among the treatment groups were compared by the Chi-square test and by Fisher's exact test. The Kaplan-Meier method was used to estimate, and the Log-rank test was applied to compare the treatment regimens with regard to "survival" variables such as time to disease progression and treatment failure or overall survival. Treatment effects on the biochemical variables were assessed

with analysis of covariance for repeated measurements. In the study IV, a general fixed effects meta-analysis approach was used for estimation and testing of hypotheses over the studies. In the study, predictive factors for treatment success were assessed using likelihood ratio test and log-rank test. After individual tests, stepwise logistic regression model and Cox's regression model were utilized to determine all predictive factors that were independently associated with the efficacy variables. Descriptive statistics were used to characterize the patient populations.

Ethical and regulatory considerations

All the subjects in these studies were patients with cancer. In all studies, all patients received treatment, according to the accepted treatment principles at the time, for their disease. Study VI was a short term kinetic investigation in connection with standard treatment, but the actual study drug did not provide any potential benefit for the patients participating in the study. All studies were approved by ethical committees at each participating site and informed consents, where the risks and possible benefits were explained, were obtained from the patients before enrolment. The studies were approved by responsible health authorities in each participating country. Studies II (Hayes, Van Zyl et al. 1995) and III were pivotal in the sales licence applications of toremifene in the European Union (EU) and in the USA, and were subsequently subjects for regulatory inspections. The drug by trade name "Fareston®" was granted marketing authorisation in Japan in 1995, in the EU in 1996 and in the USA 1997 as the first new chemical entity developed in Finland to be used as human medication.

5. RESULTS

5.1. Dose dependent clinical efficacy of toremifene

Response rate

In study I, all three doses of toremifene (20, 40 and 60 mg daily) were effective and reduced the size of breast cancer metastases. In terms of response rate, no difference was seen between 40 and 60 mg doses, but the 20 mg dose did not reach the same efficacy as the 40 mg dose ($p = 0.05$, Table 7.). In study III, differences among the toremifene (60 or 240 mg) and tamoxifen (40 mg) treated groups were not statistically significantly different, although there was a trend favouring the higher toremifene dose. In the analysis comparing combined toremifene 200/240 mg group to 20/40 mg of tamoxifen group (Study IV) the 5.2 % unit difference favouring the high dose group over the standard tamoxifen approached statistical significance ($p = 0.087$). In the meta-analysis over the 5 comparative phase III studies (Study V) comparing toremifene 40-60 mg to tamoxifen 20-40 mg doses, the response rate was found to be equivalent between the groups (Table 7).

Time to disease progression

There were no differences among the toremifene doses of 20, 40 or 60 mg regarding time from randomization to breast cancer progression in study I. Similarily, no differences among toremifene 60 and 240 or tamoxifen 40 mg groups were found in study III, nor in the meta-analyses over the high dose studies (Study IV) or over the five comparative phase III studies (Study V) comparing toremifene 40-60 mg to tamoxifen 20-40 mg doses in time to disease progression or in time treatment failure (Table 6).

Survival

No survival difference among toremifene 60, 240 or tamoxifen 40 mg treatment arms was observed in Study III. In the meta-analysis of all available phase III data, survival in the toremifene 60 mg group was equivalent to that in tamoxifen 20-40 mg group (Study V). Again, no difference was found between pooled high dose toremifene data to standard dose of tamoxifen (Study IV, Table 6).

Table 7. Summary of treatment efficacy in the clinical studies

Study	n	Drug and dose mg/day	Response rate (%[1])	Time to progression (months[2])	Survival (months[2])	Statistical significance for all variables
I	90	TOR20[1]	24	7	-[7]	p = 0.01 for
	81	TOR40	40	6	-	Response rate
	89	TOR60	33	7	-	
II[4]	221	TOR60	21	6	38	NS[6]
	212	TOR200	23	6	30	
	215	TAM20	19	6	32	
III	157	TOR60	20	5	25	NS
	157	TOR240	29	6	24	
	149	TAM40	21	5	23	
IV	369	TOR200-240	23-29	6	24-30	NS
	364	TAM20-40	19-21	5-6	23-32	
V	725	TOR40-60	24	5[5]	31	NS
	696	TAM20-40	25	5[5]	33	

[1]Complete and partial response %, [2]Median , [3]Toremifene 20 mg (TOR20), 40 mg (TOR40), 60 mg (TOR60), 200 mg (TOR200) or 240 mg (TOR240), tamoxifen 20 mg (TAM20) or tamoxifen 40 mg (TAM40) [4]Clinical efficacy data from study ((Hayes, Van Zyl et al. 1995)) [5]Median time to treatment failure [6]Not significant (NS) [7] Not assessed

5.2. Dose dependent hormonal effects

FSH and LH

In studies I and II, toremifene doses of 20, 40, 60, 200 mg and tamoxifen 20 mg daily all showed an *in vivo* estrogen agonist effect on the hypothalamus-pituitary-axis by reducing FSH and LH concentrations in serum ($p < 0.01$), which approached premenopausal levels during the treatments (Figure 5). There were no statistically significant differences among the toremifene 20, 40 and 60 mg doses, but in study II, toremifene 200 mg had more potent estrogen agonist effect on the axis than the toremifene 60 mg (FSH, $p < 0.05$ and LH, $p = 0.07$) or tamoxifen 20 mg doses (FSH and LH, $p < 0.001$). Again, in study II, there was also a significant difference in FSH and LH ($p < 0.001$ and 0.07, respectively) between tamoxifen 20 mg and toremifene 60 mg, suggesting a more potent estrogenic effect of tamoxifen.

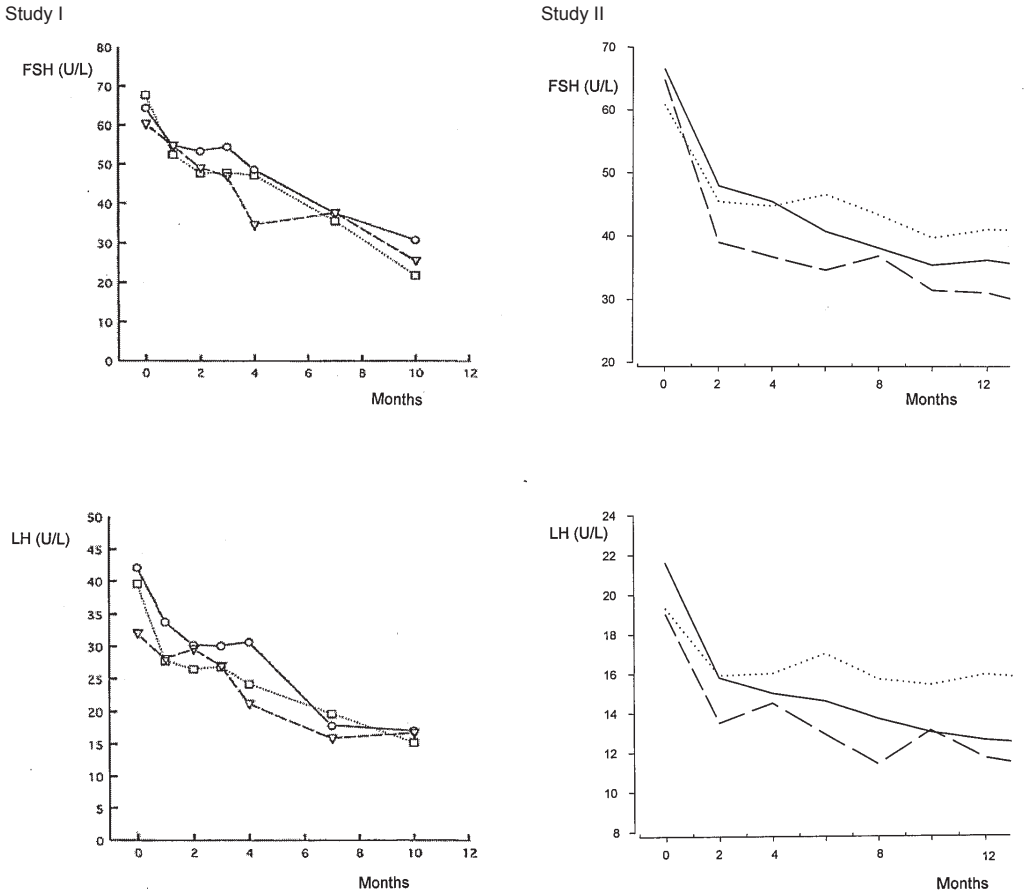

Figure 5. Mean FSH and LH concentrations in postmenopausal women with advanced breast cancer receiving toremifene 20(—○—○—), 40 (—□—□—), 60 (-▽---▽---▽- / ·····), 200 (————) or tamoxifen 20 (————) mg daily in studies I and II

Estradiol (E_2)

In study II, no statistically significant differences in overall mean serum E2 concentrations were observed among the treatment groups (P = 0.09), although after 10 months in the toremifene 200 and in the tamoxifen 20 group there was a significant increase in the concentrations (P < 0.05, Figure 6)

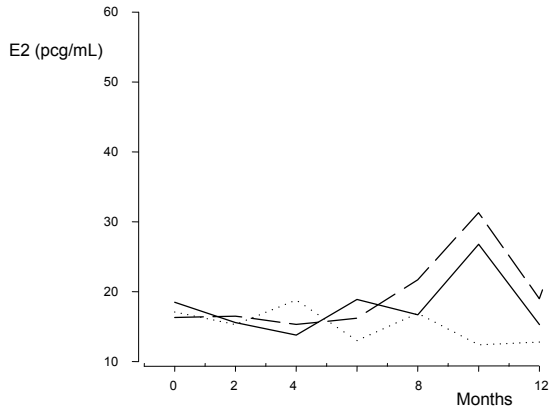

Figure 6. Mean serum estradiol concentrations in postmenopausal women with breast cancer treated with toremifene 60 (·····) or 200 mg (————) or with tamoxifen 20 mg daily (————) in study II

SHBG

In studies I and II, all toremifene doses increased SHBG levels ($P < 0.01$) in serum and the effect was dose dependent. The mean concentrations during toremifene 200 mg treatment were higher than during toremifene 60 mg ($P < 0.01$) or tamoxifen 20 mg ($P < 0.001$) treatment (Figure 7).

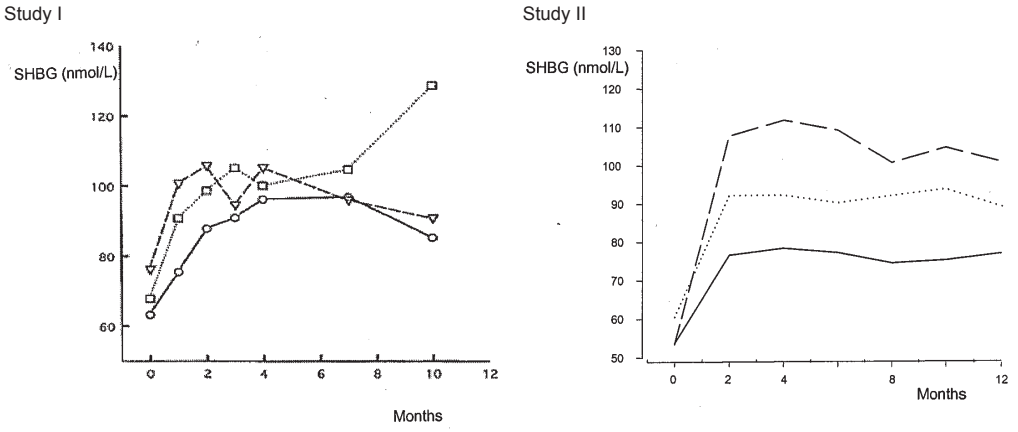

Figure 7. Mean serum SHBG concentrations in postmenopausal women with breast cancer treated with toremifene 20(—○—○—), 40 (—□—□—), 60 (-▽---▽---▽- / ·····), 200 (————) or tamoxifen 20 (————) mg daily in studies I and II

Vaginal cytology

In study II, the mean superficial cell counts increased in all treatment groups, indicating estrogen agonist action on vaginal cytology. The counts were higher in both toremifene dose groups, 60 and 200 mg, when compared to TAM20 ($p<0.05$). No difference was seen between the two toremifene doses.

Safety

A trend in study I suggests dose dependent tolerability among toremifene 20, 40 and 60 mg daily doses in postmenopausal breast cancer patients. There were less treatment emergent adverse events (P = 0.07) and less patients with those events in the 20 mg toremifene group when compared especially to the 60 mg group (Table 8). Similarily, in studies II and III there was a non significant trend, suggesting more adverse events in the high dose, 200 and 240 mg daily, toremifene groups. When in study III, moderate or severe and study drug related adverse events were considered, the trend became significant ($p < 0.05$) with 21, 12 and 11 events in the toremifene 240 mg, toremifene 60 and tamoxifen 40 groups, respectively. When pooling all available comparative data for toremifene 60 mg and tamoxifen 20 or 40 mg, no differences in the adverse event profiles could be observed (Table 8). In studies II and III, the 200 or 240 mg toremifene doses increased ASAT concentrations in serum above the reference ranges more often than toremifene 60 mg ($P < 0.05$) or tamoxifen 20-40 mg ($P < 0.01$) daily dosing. In study I, more patients with elevated ASAT concentrations in serum were seen in the toremifene 60 mg group than the 40 or 20 mg groups ($p < 0.05$). No other differences were found regarding other biochemical safety variables.

Table 8. Summary of treatment emergent adverse events in postmenopausal women with advanced breast cancer treated with toremifene or tamoxifen at different dose levels

Study	n	Treatment mg/day	Adverse events[1] (%)[2]	Abnormal[3] ASAT
I	90	TOR20[4]	17 (19)	5
	81	TOR40	19 (23)	0
	89	TOR60	28 (31), p = 0.07 vs TOR20	11, p < 0.05 vs TOR20
II[4]	215	TOR60	179 (83)	5
	207	TOR200	187 (90), NS	10, p < 0.05 vs TOR60
	203	TAM20	160 (79)	2
III	157	TOR60	28 (18)	15
	157	TOR240	36 (23), NS	27, p < 0.01 vs TOR60
	149	TAM40	25 (17)	15
V	592	TOR40-60	342 (58), NS	4, NS
	565	TAM20-40	312 (55)	5

[1]Predefined and solicited adverse events in studies II – V (hot flushes, sweating, nausea, vomiting dizziness, edema, vaginal discharge and vaginal bleeding) - in study I AEs were not predefined. [2] % of patient population, [3] % of patients above: in study II ASAT ≥100 IU/L; in study I and III , over the laboratory reference range at least 1.25 times the upper limit of the reference range (WHO grade I)
[4]Toremifene 20 mg (TOR20), 40 mg (TOR40), 60 mg (TOR60), 200 mg (TOR200) or toremifene 240 mg (TOR240), tamoxifen 20 mg (TAM20) and tamoxifen 40 mg (TAM40)

Factors predicting favourable therapeutic effect

In study V and in an univariate analysis of the pooled phase III data, seven baseline factors were found to predict good therapeutic response for toremifene and tamoxifen treatment at least in one of the efficacy variables. High tumor ER concentration, long disease free interval from the initial diagnosis until the metastatic disease and soft tissue dominant site of the metastases were statistically significant predictors for a good objective response, prolonged time to treatment failure and overall survival. Good performance status, old age, limited number of metastatic organs and no previous adjuvant tamoxifen were found to be predictors for a good outcome at least in one of the variables. Multivariate stepwise analysis of factors independently predicting the treatment outcome confirmed the findings of the univariate analysis. Only the use of previous adjuvant tamoxifen became significant predicting shorter time to treatment failure but, surprisingly not, lower response rate. The dominant site of the disease was observed in the order of visceral > bone > soft tissue. A short disease free interval from the diagnosis and low estrogen receptor concentration all predicted unfavourable treatment outcome in all efficacy measures and in all statistical analyses employed (Table 9).

Table 9. Multivariate stepwise analysis of factors predicting independently good effect of antiestrogen therapy in postmenopausal women with advanced breast cancer in study IV

Predicting factor	Efficacy variable		
	Response rate[1]	Time to treatment failure[1]	Survival[1]
High ER concentration	< 0.001	< 0.001	< 0.001
Long disease free time[2]	< 0.001	< 0.001	< 0.001
Soft tissue dominant site	< 0.001	< 0.001	< 0.001
High performance status	NS[3]	< 0.001	< 0.001
Few metastatic organs	NS	< 0.001	< 0.001
Advanced age	< 0.01	< 0.001	NS
No adjuvant SERMS	NS	< 0.05	< 0.01

[1] Statistical significance of a predictive factor for a good outcome after treatment initiation, p-value, [2]Time from the primary treatment to detection of metastatic disease [3]not statistically significant (NS)

Toremifene concentrations in human tissues

The concentrations of toremifene and its main metabolite N-demethyltoremifene (DMT) in lung and tumor, reported as μmol/kg tissue, were higher than those observed in serum, reported as μmol/L, in patients receiving toremifene 240, 480 or 600 mg daily for seven days. The concentrations of the parent drug and DMT (Table 10) as well as those of the minor metabolites, 4-hydroxytoremifene (4-HT), N, N-dimethyltoremifene (DDMT) and (deaminohydroxy)-toremifene (DAHT), were dose dependent in all tissues.

Table 10. Tissue concentrations of toremifene and the main metabolite N-demethyltoremifene (DMT)in lung cancer patients after receiving toremifene 240, 480 or 600 mg per orally for 7 days.

Dose (mg)	**Serum (µmol/L)**		**Tumor (µmol/kg)**		**Lung (µmol/kg)**	
	Toremifene	DM	Toremifene	DMT	Toremifene	DMT
240	2.5 (0.6)[1]	3.8 (1.0)	21 (26)	20 (26)	112 (23)	123 (50)
480	3.8 (0.7)	6.9 (1.0)	53 (40)	67 (20)	138 (30)	209 (12)
600	4.9 (0.8)	7.2 (1.0)	123 (170)	108 (131)	175 (22)	215 (67)

[1]Standard deviation (SD)

6. DISCUSSION

Preclinical pharmacology and dose selection of toremifene

A toremifene concentration of 0.5 μM was able to displace estradiol from the ER *in vitro*, and 1 to 3 mg/kg doses had antiestrogenic effect in the presence of estradiol and the effect increased up to the 50 mg/kg dose (Kallio, Kangas et al. 1986). The dose dependent estrogen agonist and antagonist effects of toremifene were similar to those of tamoxifen. In agreement with these findings, toremifene and tamoxifen also inhibited cell growth and induced cell death in breast cancer cell models at concentrations of 0.1 -5.0 μM (Kangas, Nieminen et al. 1986; Robinson, Mauel et al. 1988). However, within a dose range from 1 to 10 mg/kg, the estrogenic potency of tamoxifen was 40 times higher than that of toremifene, indicating less intrinsic estrogenic activity of toremifene with equivalent antiestrogenic dose (Di Salle, Zaccheo et al. 1990). Studies *in vivo* with a rat ER positive breast cancer model suggested that a 1 mg/kg dose would be sufficient for an antitumor effect, which was similar to that of tamoxifen and did not improve with an increasing dose (Kangas, Nieminen et al. 1986). Suprisingly and not in an agreement with the hypothesis of ER mediated action, toremifene was effective at a clearly higher dose level and in ER negative tumor models and in combination with cytotoxic agents cell lines also with aquired resistance to cytotoxic agents (Kangas, Nieminen et al. 1986; DeGregorio, Ford et al. 1989).

Clinical pharmacology and surrogate markers for toremifene dose selection

The plasma steady state concentrations of toremifene in patients, were 0.3, 0.7, 0.8, 2.2 to 3.5 μM, following 10, 20, 40, 60 to 200 mg daily doses (Wiebe, Benz et al. 1990). Based on the vaginal superficial cell count, toremifene 10 mg daily was only minimally antiestrogenic, but the antiestrogenic effect of 20 to 200 mg daily were similar to a 20 mg tamoxifen dose (Wiebe, Benz et al. 1990; Homesley, Shemano et al. 1993) suggesting that the 20 mg daily may be the lowest dose with clinically sufficient antiestrogenic effect. This was in line with the *in vitro* data (Kallio, Kangas et al. 1986; Kangas, Nieminen et al. 1986). At steady state following toremifene 20 and 40 mg daily treatment, the plasma concentrations of toremifene and the main metabolite N-demethyltoremifene were within the *in vitro* defined antiestrogenic concentration range (Wiebe, Benz et al. 1990.). The observed plasma and tumor concentrations (Lien, Webster et al. 1991);(Study VI) suggest that antiestrogenic concentrations of toremifene and the main metabolite are reached in tumor tissue with toremifene 20 and 40 mg daily doses.

In postmenopausal women in Study I, toremifene had dose dependent estrogenic effect on the hypothalamus–pituitary-axis and on the liver by decreasing FSH and LH and by increasing SHBG concentrations in plasma, respectively. No significant differences were seen among toremifene 20, 40 and 60 mg doses, but the change tended to be somewhat slower in the 20 mg group, possibly reflecting the longer time required to

reach the pharmacologically active steady state concentrations of toremifene and N-demethyltoremifene, as described earlier (Wiebe, Benz et al. 1990). When these variables were assessed during toremifene 60 and 200 mg and tamoxifen 20 mg daily treatment (Study II), 200 mg of toremifene was more estrogenic than the two other treatments. In line with the results of the *in vivo* model (Di Salle, Zaccheo et al. 1990), during tamoxifen 20 mg treatment, a trend for lower FSH concentrations was seen when compared with toremifene 60 mg . This suggests higher intrinsic estrogenic potential of tamoxifen 20 mg compared to toremifene 60 mg on the hypothalamus–pituitary-axis. However, in vaginal cytology both toremifene doses tended to be more estrogenic than tamoxifen, suggesting that the estrogenicity differences between the two drugs are tissue specific at these dose levels. Increases of serum SHBG were again highest with toremifene 200 mg and followed by toremifene 60 mg and tamoxifen 20 mg, the differences among the groups being statistically significant. In some of the patients in study II, some isolated serum estradiol increases were seen with the toremifene 200 mg and in tamoxifen 20 mg treatment groups. These changes may reflect the perimenopausal status of some patients in the study, which was allowed by the study protocol (Hayes, Van Zyl et al. 1995).

Conclusions for dose selection based on the surrogate markers in clinical pharmacology

The clinical pharmacokinetic and surrogate hormonal variables let us understand that the lowest dose expected to have clinical efficacy would be 20 mg daily at steady state. Increasing the toremifene dose beyond 20 mg daily would shorten the time required to achieve clinical effective antiestrogenic tissue concentrations of toremifene and the main metabolite, but at the same time the tissue specific estrogenic effects of the treatment would increase.

Clinical dose finding of toremifene in postmenopausal patients

Before selecting the doses for the phase III trials, small, non blinded and non controlled studies toremifene trials in breast cancer patients with ER positive or ER unknown tumors were performed. In these trials, objective response rate varied from 21 to 68 % with 20, 60 and 240 mg daily doses (Valavaara, Pyrhönen et al. 1988; Valavaara and Pyrhönen 1989; Gundersen 1990; Hietanen, Baltina et al. 1990; Pyrhönen, Valavaara et al. 1990; Modig, Borgström et al. 1990b; Tominaga, Abe et al. 1993; Baltina, Hietanen et al. 1996). The 21 % response rate in 14 patients with the 20 mg dose was not considered sufficient and the two higher doses were selected for further clinical investigations.

When 20, 40 and 60 mg doses of toremifene were compared in the same randomized parallel group dose finding study (Study I) , the response rate with a 20 mg dose was again lower than the response rate seen with the 40 mg daily dose ($p = 0.01$). No difference was seen between the 40 and 60 mg dose groups. This finding was similar to the early phase II studies comparing the 20 mg dose against 60 mg dose, although not in the same

study. Interestingly, no differences were seen in time to disease progression between the doses (Study I). In phase III studies, 40, 60, 200 and 240 mg of toremifene were tested against tamoxifen 20 , 30, 40 mg with no differences in objective treatment responses nor in survival between the two drugs or among the different doses (Hayes, Van Zyl et al. 1995; Pyrhönen, Valavaara et al. 1997) (Study III and V). In one comparison (Pyrhönen, Valavaara et al. 1997), tamoxifen 40 mg tended to have longer time to breast cancer progression than toremifene 60 mg ($p < 0.05$), but this was evident only in patients with unknown tumor ER status, whereas in patients with ER positive tumors no such difference was seen ($p = 0.578$). In all other comparison and in the meta-analysis over the whole phase III (Study V) no differences in time to progression or time to treatment failure were seen between different toremifene and tamoxifen doses.

For an objective response to be established, a tumor reduction of at least 50 % needs to be recorded in two consecutive assessments at 4 or 8 weeks intervals according to the IUCC criteria (Miller, Hoogstraten et al. 1981). Steady-state plasma concentrations of toremifene and the main active metabolite N-demethyltoremifene are reached in 1-2 weeks at ≥200 mg, in 2 weeks in 40-60 mg and in 3-4 weeks at 20 mg daily doses (Wiebe, Benz et al. 1990). It is possible that this would allow more time for the patients on higher doses to meet the predefined response criteria before breast cancer progression. This is supported by a finding in the study comparing all toremifene 200 and 240 mg data in comparative studies to tamoxifen and showing a trend for more responses in the toremifene treated group ($p = 0.087$), but again no difference in time to progression ($p = 0.972$) or in overall survival ($p = 0.397$; Study IV). Disease stabilization for at least for 12 months predicted equally long survival than did objective treatment response in a meta-analysis of 1157 patients with advanced breast cancer treated with toremifene 40-60 or tamoxifen 20-40 mg daily (Study V). Long term disease stabilization together with long duration objective responses, i.e. overall time to the disease progression, should be considered as the primary variable in dose finding of triphenylethylene SERMs for advanced breast cancer.

Increasing the dose up to 200 or 240 mg daily did not improve the efficacy of toremifene but increased slightly adverse event frequency (Study IV). This is in line with the earlier observations with tamoxifen (Bratherton, Brown et al. 1984) and suggests that beyond maximum antiestrogenic effect of a given SERM no further efficacy can be obtained.

Toremifene inhibits tumor growth in the ER negative mouse uterine sarcoma model at high doses by unknown mechanism (Kangas, Nieminen et al. 1986). Although a slightly higher response rate was seen in early phase II and also in phase III studies (Hietanen, Baltina et al. 1990) in patients with ER positive or ER unknown tumors, no effect was seen in patients with ER negative breast cancer (Perry, Berry et al. 1995) nor in the treatment of melanoma (Kleeberg, Engel et al. 1993). In other tumor types, such as uterine cancer, desmoids tumors and renal cell cancer potentially expressing ER, some objective efficacy was seen in early open studies with a limited number of patients (Horwath, Stendahl et al. 1990; Brooks, Ebbs et al. 1992; Gershanovich, Moiseyenko et

al. 1997). This high dose effect of toremifene, with some other mechanism than through ER, was not supported by the results of the early clinical studies nor in the confirmatory studies in breast cancer.

The MDR reversal hypothesis based on preclinical models (DeGregorio, Ford et al. 1989; Kirk, Houlbrook et al. 1993), showed some efficacy in the early study with ovarian cancer patients (Mäenpää, Sipilä et al. 1992). However, the effect could not be confirmed in patients with lung cancer in combination with ifosfamide (Salomaa, Liippo et al. 1996) or with renal cell cancer in combination with vinblastine (Braybrooke, Vallis et al. 2000). This finding suggests that although theoretically sufficient plasma and tissue concentrations of 10 µmol/L were achieved to overcome MDR (Study VI)(DeGregorio, Ford et al. 1989; Lara, Gandara et al. 1998; Wurz, Soc et al. 1998), the resistance towards the tested cancer treatments of malignancies could not be reversed clinically in these patients and the drug combinations. However, ifosfamide is not considered to be a substrate of P-170 and therefore the clinical lung cancer model used (Salomaa, Liippo et al. 1996) may not be relevant for the clinical testing of the hypothesis.

Predictive factors for a favourable outcome during SERM therapy

In an early study with 113 postmenopausal breast cancer patients (Valavaara, Tuominen et al. 1990) higher than 50 fmol/mg protein ER concentration predicted independently response duration, but not response rate following toremifene treatment. In a pooled-analysis of the confirmatory phase III studies, high tumor ER concentration, soft tissue as the main metastatic site and long duration of the disease free interval since the primary treatment were factors predicting independently favorable outcome of a SERM therapy in postmenopausal patients with advanced breast cancer (Study V). It was not studied how patient age, long duration of disease free interval and tumor ER concentration correlated with the degree of histological differentiation of the primary tumors. We presume, that well differentiated tumors have higher ER concentrations than poorly differentiated tumors and loss of ER correlates with poor prognosis of the disease (Kuukasjärvi, Kononen et al. 1996; Robertson 1996; Rintasyöpäryhmä 2007). The classification of ER concentration in the pooled analysis was < 10, 10-100 or > 100 fmol/mg cytosolic protein and the overall reponse rate was 24 %, which was clearly lower than in the early study (Valavaara, Tuominen et al. 1990) with 38 and 51 % response rate in patients with tumor ER concentration of below and above 50 fmol/mg, respectively. However, the findings in the meta-analysis (Study IV) were in agreement with the results of the early study to define the breast cancer patient population to be used in clinical studies for dose selection.

Effects on serum lipids

The effect of toremifene on serum lipids was not studied in the early clinical studies. In later studies, in postmenopausal breast cancer patients or subjects at high risk for developing breast cancer, it was observed that toremifene and tamoxifen down regulated

cholesterol synthesis and reduced total and LDL-cholesterol concentrations (Gylling, Pyrhönen et al. 1995; Saarto, Blomqvist et al. 1996; Joensuu, Holli et al. 2000; Kusama, Miyauchi et al. 2004; Erkkola, Mattila et al. 2005). However, no difference in the cholesterol lowering potency between the SERMs nor between the two toremifene doses could be established.

Effects on bone

In postmenopausal breast cancer patients, tamoxifen 20 mg and toremifene 40 and 60 mg daily maintained bone mineral density during 1 and 2 years treatment (Saarto, Blomqvist et al. 1997); (Marttunen, Hietanen et al. 1998). In the 1 year study (Marttunen, Hietanen et al. 1998), urine markers for bone resorption following tamoxifen 20 mg indicated more pronounced estrogenic effect of tamoxifen on bone metabolism. In another study by the same group (Marttunen, Hietanen et al. 1999), toremifene 40 mg and tamoxifen 20 mg daily for 3 years reduced bone resorption and maintained bone mineral density similarly, although, some suggestion of a more pronounced effect of tamoxifen was seen. In healthy pre- and postmenopausal women, who were at increased risk for breast cancer and received toremifene 60 mg or placebo daily up to 5 years, no decrease in bone mineral density was seen in the postmenopausal subgroup. However, in the premenopausal subgroup, higher values of bone mineral densities were seen on placebo suggesting antiestrogenic effect of toremifene on bone in premenopausal women with own estrogen production from their ovaries (Erkkola, Mattila et al. 2005). In men with prostate cancer and receiving androgen deprivation therapy toremifene 80 mg daily decreased vertebral fractures suggesting bone preserving activity under hormone depletion (Smith, Malkowicz et al. 2011).

Adjuvant treatment of breast cancer

Preclinical and early clinical studies of toremifene were planned to support treatment of advanced breast cancer. During the course of drug development, data on adjuvant treatment with tamoxifen became available and an interest to study toremifene also in early breast cancer emerged. Based on the same mechanism of action, in treatment of advanced breast cancer and in adjuvant setting, the same doses of toremifene were studied in both of the indications.

In a clinical study and after mean follow-up of 3.4 years comparing adjuvant treatment with toremifene 40 mg to tamoxifen 20 mg for 3 years in 1480 postmenopausal lymph node positive breast cancer patients, the recurrence rates were 23 and 26 % in the whole population and 15.1 and 19.6 % in patients with ER positive tumours in toremifene and tamoxifen groups, respectively (Holli, Valavaara et al. 2000). In the same study, after a mean follow-up of 4.4 years, the recurrence rates were 22 % and 24% in the toremifene and tamoxifen groups, respectively (Holli K on behalf of the Finnish Breast Cancer Group 2002).

In another adjuvant study comparing toremifene 60 mg to tamoxifen 20 mg, both treatments combined with chemotherapy for 5 years in 1035 peri- and postmenopausal patients with lymph node positive disease, the recurrence rates after a median of 5.5 years of follow up were 28 and 31 %, in the toremifene and tamoxifen groups, respectively (International Breast Cancer Study Group 2004). Again, the difference was more pronounced in the subgroup of patients with ER positive tumours having recurrence rates of 24 and 28 % respectively.

In a most recently reported study (Lewis, Chagpar et al. 2010), 1813 peri- or postmenopausal patients with hormone receptor positive and mostly lymph node negative breast cancer received either adjuvant toremifene 60 mg or tamoxifen 20 mg daily for 5 years. After a median follow-up of 59 months there were 2 and 3 % of patients experiencing recurrences in the tamoxifen and toremifene groups, respectively, with no difference in disease free or overall survival. No statistically significant differences in efficacy between the treatment groups were seen in any of the adjuvant studies. In a meta-analysis of three randomized trials with 1890 toremifene and 1857 tamoxifen treated patients relative risk for death was higher for tamoxifen 1.07, 95% CI: 0.97–1.19, but the difference did not reach statistical difference (Zhou, Ding et al. 2011).

In a study where toremifene was combined with a steroidal aromatase inhibitor, atamestane, and compared with another aromatase inhibitor, letrozole, in postmenopausal patients with advanced breast cancer, no differences in efficacy tolerability were seen in postmenopausal patients with advanced ER-positive breast cancer (Goss, Bondarenko et al. 2007). Both toremifene doses studied in adjuvant treatment of breast cancer in postmenopausal patients were equally effective and safe and non inferior when compared to the established treatment with tamoxifen 20 mg.

SERM resistance

Early clinical trials with toremifene in postmenopausal patients with advanced breast cancer suggested low efficacy in patients with acquired tamoxifen resistance (Jönsson, Malmberg et al. 1991; Stenbygaard, Herrstedt et al. 1993; Pyrhönen, Valavaara et al. 1994). In line with these findings in Study V previous adjuvant tamoxifen predicted lower efficacy of subsequent SERM treatment in advanced disease.

Safety and tolerability

All toremifene doses used in clinical studies were well tolerated and discontinuations of treatments due to adverse events were rare, about 1 % in the 40 - 60 mg dose range (Study V) and 1 – 6 % in the high dose 240 or 200 mg groups (Hayes, Van Zyl et al. 1995)(Study III). Overall, predefined and hormonal adverse events, such as hot flashes and sweating, were common and reversible after treatment discontinuation. No differences were seen between the toremifene 40-60 mg and tamoxifen 20-40 mg dosing groups (Study V). However, adverse events and abnormal serum ASAT concentrations tended to increase in higher dose groups, where abnormal ASAT values were more common with toremifene

200-240 mg than in the tamoxifen ($p < 0.05$) or in 60 mg toremifene groups (Hayes, Van Zyl et al. 1995)(Study III). In a study with female rats, toremifene and tamoxifen had been shown to increase concentrations of ALAT and the effect was similar to that caused by diethylstilbesterol and could be considered as estrogenic effect on the liver (Kendall and Rose 1992). Similar trends in effect on the liver and in overall adverse event profile were seen among 20, 40 and 60 toremifene doses (Study I), suggesting that hormonal and adverse effects of toremifene are dose dependent even within the lower dose range. All toremifene doses were, however, well tolerated in the non comparative early clinical studies. No conclusions of small dose dependent differences in the adverse event profile were made based on these studies.

In adjuvant trials comparing toremifene to tamoxifen there were 6 (1.2%) vascular or cerebrovascular events (deep vein thrombosis, pulmonary embolism or cerebrovascular accidents) with toremifene (60 mg daily for 5 years) and 9 such events (1.8%) in the tamoxifen 20 mg group (International Breast Cancer Study Group 2004). In the study by the Finnish Breast Cancer Group employing toremifene 40 mg and tamoxifen 20 mg for 3 years, the figures were 16 (3.5%) and 26 (5.9%, $p = 0.11$), respectively. In the meta-analysis over the adjuvant studies comparing toremifene 40/60 mg to tamoxifen 20 mg a total of 74 thromboembolic events were reported in the tamoxifen groups and 52 in the toremifene groups providing odds ratio of 0.81 with 95% CIs of 0.66 – 1.01(Zhou, Ding et al. 2011).

In a gynaecological follow-up of postmenopausal breast cancer patients treated with toremifene 60 mg or tamoxifen 20 mg for 12 months in the adjuvant setting (Tomás, Kauppila et al. 1995), both of the treatments had marked estrogenic effects in the uterus and vagina, at the level of 57 % of the tamoxifen treated patients and 30 % of toremifene treated patients. In the long term follow-up of the patients in the adjuvant studies (Holli, Valavaara et al. 2000; Holli K on behalf of the Finnish Breast Cancer Group 2002; International Breast Cancer Study Group 2004), 6 endometrial cancers were found in the toremifene 40 or 60 mg treated and 4 in the tamoxifen 20 mg treated patients. In a epidemiological case control study among Finnish breast cancer patients diagnosed since 1980, an odds ratio (OR) of 2.9 (95% CI 1.8 – 4.7) was estimated for developing endometrial cancer in the tamoxifen treated patients and the corresponding OR for toremifene was 0.9 (95% CI 0.3-3.9) (Pukkala, Kyyrönen et al. 2002). In the meta-analysis, the rates of endometrial polyps and endometrial cancer between the two groups were almost the same (OR: 1.03, 95% CI: 0.61–1.73 and 0.99, 95% CI: 0.36–2.73, respectively (Zhou, Ding et al. 2011).

In studies measuring bone mineral density during long term treatment, both 60 and 40 mg toremifene doses maintained the bone mass. The effect of toremifene 60 mg was similar to tamoxifen 20 mg, but toremifene 40 mg showed less estrogen agonist effect (Saarto, Blomqvist et al. 1996; Saarto, Blomqvist et al. 1997; Erkkola, Mattila et al. 2005). In a long term follow-up trial comparing toremifene 40 mg to tamoxifen 20 mg for 3 years (Holli K on behalf of the Finnish Breast Cancer Group 2002), a

trend for more bone fractures, 13 (2.8%) vs. 5 (1.1%), was seen in the toremifene treated group.

Toremifene dose effect

Nonclinical and phase I studies suggested that toremifene at doses from 20 to 80 mg daily would be indistinquisable from tamoxifen regarding the antiestrogenic potency, although at the same time would be dose dependently less estrogenic.

The borderline dose for antiestrogenic action, 10 mg daily, was not tested in patients for efficacy, but the clinical data confirmed that by increasing the dose beyond the suggested antiestrogenic dose range did not improve efficacy. This investigation suggested also that there are no differences between toremifene 40 and 60 mg daily doses. The number of responding patients was lower in the 20 mg group, but this may be more due to set criteria for the response together with pharmacokinetics of the compound rather than due to lack of long term efficacy of the dose. The selection of a 60 mg dose, as suggested by the preclinical and early clinical data for the treatment of breast cancer is justified based on the available data. However, as with study I and the study in the adjuvant setting (Holli K on behalf of the Finnish Breast Cancer Group 2002) a lower dose, such as 40 mg daily, have also been equally effective.

Due to the slow development of the steady state concentration of the parent drug and the pharmacologically active metabolites, and the subsequent slower onset of action, a lower objective response rate may have resulted in in metastatic disease with the 20 mg daily dose. The early clinical data suggested that the antiestorgenic potency of the 20 mg dose is not inferior from those of the higher doses (Homesley, Shemano et al. 1993) and in a clinical study in advanced breast cancer a 20 mg dose provided an equally long duration of disease control than the higher doses (Study I). Confirmation of the efficacy of the 20 mg dose particularly in the adjuvant treatment of breast cancer, and effect of a higher loading dose, require further studies.

In clinical practice toremifene has been well tolerated and safety has not been a concern. However, a trend in the clinical data suggests that the incidence of adverse events may be dose dependent and this may become relevant in long term treatment. Hormonal effects, effects on bone, serum lipids and on adverse event profile indicate that toremifene possess intrinsic estrogen agonist action like tamoxifen. In some variables, however, this agonistic effect of toremifene is somewhat less than that of tamoxifen. The effect of toremifene on QTc interval has been dose dependent. In the adjuvant treatment of breast cancer, toremifene has been as effective as tamoxifen with both 40 and 60 mg daily doses. Should toremifene be developed for adjuvat treatment or for prevention of breast cancer, the use of lower doses, such as 40 or 20 mg, with potentially reduced estrogenicity, should be considered for clinical trials.

7. SUMMARY AND CONCLUSIONS

Effective and safe toremifene doses could be predicted for the proposed and established ER mediated mechanism of action in the treatment of advanced breast cancer in postmenopausal women based on the preclinical and early clinical investigations presented in this study. A comparative and randomised clinical dose finding study with additional efficacy variables would, however, have provided more information on dose selection. The suggested new mechanisms of action, based on high toremifene concentrations in the preclinical studies, were not supported by the findings in the subsequent clinical studies.

I. All toremifene doses from 20 to 60 mg daily tested in postmenopausal patients with advanced breast cancer were effective and safe. The response rate with a 20 mg dose was inferior to the higher doses, but no between-dose differences were seen in time to disease progression. No difference in efficacy or in safety was seen between 40 and 60 mg daily doses. Selection of a 60 mg daily dose of toremifene 60 was justified for phase III clinical studies and for marketing authorization applications in the treatment of postmenopausal women with advanced breast cancer. Hormonal effects, such as effects on the hypothalamus-pituitary-axis and on estrogen primed vaginal epithelium in humans, together with pharmacokinetic data from plasma and tissue were able to predict reliably the effective and safe dose range to be used in the clinical dose finding study. Early, small and noncomparative studies were able to provide a PoC for preliminary efficacy and safety and thus suggest the patient population and dose range of toremifene to be studied in confirmatory clinical trials. However, a randomized dose finding study, in an early phase of the clinical development, could have further supported defining the smallest effective dose.

II. The results of the study II were as expected on the basis of the preclinical and early clinical data for ER mediated action of toremifene. A 60 mg daily toremifene dose was as equally effective and safe as tamoxifen (20 mg daily) in the treatment of advanced breast cancer in postmenopausal women. The intrinsic estrogen agonist action of toremifene was dose dependent and the doses of 200 mg daily were more estrogenic than 60 mg daily dose. The higher incidence of adverse events and elevation of ASAT activity increased in the high dose toremifene groups may be explained by an increased estrogenic effect. Tamoxifen 20 mg had more estrogen agonist action on the hypothalamus-pituitary axis than toremifene 60 mg.

III. The results of the study III were as expected on the basis of the preclinical and early clinical data for ER mediated action of toremifene. Toremifene 60 mg daily was effective and safe in the treatment of advanced breast cancer in postmenopausal women. There was no difference in efficacy between tamoxifen 40 mg and the studied toremifene 60 mg daily dose. A further increase of the toremifene dose up to 240 mg daily did not prolong progression free or overall survival. However, there was a trend for more

responses in the 240 mg toremifene group. A higher incidence of adverse events and increased values of liver function test in the toremifene 240 mg group may be explained by the increased estrogenic effect. For the treatment of postmenopausal patients with advanced ER positive breast cancer the selected 60 mg dose is effective and safe and increasing the dose did not add clinically significant benefit.

IV. When assessing all the available data on patients receiving a high dose, 200 or 240 mg daily, and comparing it to the standard doses of toremifene and tamoxifen, a trend for superiority of the high dose treatment in the objective response rate was seen. Again, no difference was seen in time to disease progression or in overall survival. Beyond the antiestrogenic dose, no further dose dependent efficacy of toremifene could be seen in the phase III data. It should be studied further whether the short time to effective steady state concentration in serum following increased SERM doses together with the used response criteria would explain the observed increase in response rate.

V. The ER concentration of the primary tumor independently predicted a good treatment outcome with all the efficacy variables used, thus supporting the suggested mechanism of action for toremifene in postmenopausal patients with advanced ER positive breast cancer. From the interpretation of early clinical data it was possible to predict the significance of the tumor ER concentration for toremifene treatment efficacy. Together with response rate, long term disease stabilization is an important efficacy variable to be used in dose selection of a SERM for the treatment of advanced breast cancer. Patient selection has effect on the efficacy of a SERM dose supporting the use of randomized and active controlled study for a dose selection.

VI. The concentrations of toremifene and its main metabolites in serum, lung and tumor tissue were dose dependent. The concentrations in tumor exceeded those in serum and were above those expected to exert antiestrogenic action on ER and to those shown reverse MDR. In further studies it should be evaluated whether the concentrations of toremifene and its main metabolite in the tumor tissue are sufficiently high to maintain antiestrogenic effect even after lower doses, such as 20 or 40 mg, of the drug.

Based on known mechanism of action through ER, preclinical models and early clinical studies were able to provide PoC for efficacy and safety as well as to predict a dose range and patient population for a SERM to be studied in confirmatory clinical studies. Controlled clinical dose finding study would be needed to optimize the dose selection.

8. ACKNOWLEDGEMENTS

This study was performed at the Research and Development organizations of Orion Pharma and at the Department of Pharmacology, Drug Development and Therapeutics, University of Turku.

First I want to express my warmest thanks to late emeritus professor Kari Lagerspetz, PhD and emeritus professor Esko Iisalo, MD, PhD former heads of Departments of Animal Physiology and Department of Clinical Pharmacology at the University of Turku who were my first teachers and guided and encouraged me to independent scientific work.

I express my most sincere and warmest thanks to my supervisor professor Risto Huupponen, MD, PhD who initially introduced me to this project and has been utmost patient during all these years.

I am sincerely thankfull to professor Vesa Kataja, MD, PhD and to docent Erkki Elonen MD, PhD, the official reviewers, for carefull reviewing of the manuscript. Their input in the late stage of the writing was of critical importance to improve the quality of the work.

I wish to thank my friend Robert Moulder, PhD, for editing the language and making it more understandable to possible readers.

I am grateful to the group of prestigious scientists (M.Anttila, D.Baltina, W.R.Bezwoda, G.Falkson, P.Fargeot, A.Garin, M.Gershanovitch, P.Hakulinen, D.F.Hayes, L.Kangas, M.Kaufmann, A.Kurvet, R.Keiling, K.Liippo, A.Partanen, S.Pyrhönen, T.Tominaga D.Werner, J.Vuorinen, E.Vänttinen and D.Werner) for their collaboration in the studies.

I am greatly indebted to my colleagues Lauri Kangas, PhD, Alajos Hajba,MSc, Päivi Nevala, MSc, Hannu Käpylä,MSc, Kaija Halonen,MSc, Taru Karhunsaari,MSc, and Janne Komi, MD,PhD for tolerating and a walking aside me for so many years through the long and winding roads of drug development. I want to thank my former superior Risto Lammintausta, MD, PhD, for giving me the opportunity to initiate this work, already some time ago and along with other duties within Orion Pharma, and my current superior Taru Blom, MD, PhD, for letting the work to be completed.

I want to express my gratitude to the follow-up team docent Mikko Kuoppamäki, MD, PhD and to docent Pekka Kallio, PhD, for giving their advice, reviewing the manuscript, and for their continous enthusiasm to follow progress of the work.

I want to thank all my colleagues, subordinates and superiors in Orion Pharma, Research and Development for their support, understanding and patience during all these years.

I am indebted to all of those patients who voluntarily participated in these studies understanding the value of drug development for all patients suffering from the same disease.

I want to thank my late mother Annele Ellmén for her support and trust in me. Finally I wish to thank my beloved wife Marja for her love and patience and my children Mikko and Sini for being there. When this study was initiated Mikko and Sini were taking first steps in their elementary school and now we are looking forward for their graduation.

The work was financially supported by Orion Corporation, Orion Pharma, Farmitalia Carlo Erba and by Adria Laboratories.

Turku, May 2012

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