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## Arimidex, tamoxifen alone or in combination (ATAC) adjuvant trial in post-menopausal breast cancer

J. Houghton, M. Baum. *On behalf of the ATAC Steering Committee and Investigators; UCL Medical School, CRC & UCL Cancer Trials Centre, Macdonald Buchanan Building, John Astor House, Foley Street, London W1P 8AN, UK*

**Purpose:** The ATAC trial is a double blind study, designed to compare the efficacy and safety of tamoxifen alone, Arimidex (anastrozole) alone, and the combination of tamoxifen plus anastrozole, as adjuvant treatment in post-menopausal women with early breast cancer, who have completed primary therapy. Treatment will be for 5 years or until first recurrence. The dose of tamoxifen is 20 mgs od and anastrozole 1 mg od.

**Methods:** Patients must be post-menopausal, have histologically proven operable breast cancer, have completed all primary treatment (surgery ± radiotherapy ± chemotherapy), and also be candidates for adjuvant hormonal therapy. The trial aims to enrol 7500 patients and more than 3700 had been recruited by the end of March 1998. The endpoints are time to recurrence of breast cancer, overall survival, and safety and tolerability.

Five sub-protocols will provide additional information on quality of life, bone changes, endometrial changes, lipid profiling and pharmacokinetic evaluation in each treatment arm.

**Results:** Demographic information available on the first 1255 patients recruited, showed 52% were 50–65 years of age and 42% over 65 years of age; 44% of tumours were stage I; 64% were ER+, 42% were PR+ and 5% ER−PR−.

**Conclusion:** Thus far demographic characteristics are in agreement with the patient population detailed in the protocol. The status of the main trial and subprotocols will be updated.

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## Endometrial changes caused by tamoxifen

Z. Rayter[1], M. Watt[2], S. Keay[2], J. Jenkins[2], P. Wardle[2]. [1]*Bristol Breast Unit, Bristol Royal Infirmary;* [2]*Department of Reproductive Medicine, St. Michael's Hospital, Bristol, UK*

**Purpose:** Prolonged therapy with tamoxifen gives rise to endometrial abnormalities and has been reported to increase the subsequent development of endometrial cancer six fold. This prospective study was designed to examine the time course over which endometrial abnormalities occur in an adjuvant setting.

**Methods:** Patients requiring adjuvant tamoxifen as part of their normal treatment for breast cancer underwent baseline pelvic examination, transvaginal ultrasound scanning (TVUS) to measure endometrial thickness (ET) and biopsy for histology and insulin growth factor-1 levels if ET was > 7 mm. Subsequent TVUS (and biopsy if ET > 7 mm) was performed at 1, 2, 3, 6, 12 and 24 months.

**Results:** Twenty four patients have been studied for a mean of 10 months. The mean endometrial thickness has increased from 3.2 mm before tamoxifen (0 months) to 5.1, 7.1, 5.2, 4.9, 6.2 and 6.9 mm at 1, 2, 3, 6, 12 and 24 months. After 6 months therapy with tamoxifen, 27.3% of women had an increase in endometrial thickness of >100% and this had risen to 54% of women after 12 months therapy.

**Conclusion:** Tamoxifen causes a rapid initial rise in endometrial thickness, perhaps due to oedema, but then continues to increase endometrial thickness progressively and in a larger proportion of patients with increased duration of use.

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## Adrenomedullin: A potential autocrine growth factor for human breast epithelial cells during development and carcinogenesis

J.M. Miller, A. Martinez, T. Moody, G. Jahnke, L. Smith, P. Brown, P. O'Connell, Allred, F.C. Cuttitta. *NIH/National Cancer Institutes, DCS/MD/DCCB/Intervention Section, Building 10, Room 12N226 MSD1906, 9000 Rockville Pike, Bethesda, Maryland 20892-1906, USA*

Adrenomedullin (AM) is a potent hypotensive peptide that was originally isolated from a human pheochromocytoma. AM has a variety of biological effects that include vasodilation, natriuresis, bronchodilation, anti-secretagogue, neurotransmission, and growth regulation in a variety of cells. Published data using MCF-7, a breast cancer cell line, showed both AM mRNA and protein expression, and in vitro studies showed specific growth regulation by AM, using an AM MoAb-G6 (Miller, MJ et. al., JBC 271: 23345–23351). In addition, we have also published data that demonstrates AM's role in mammary development. We show that AM mRNA and protein is present in all stages of breast development, and the milk contained within the lactating duct showed an AM-like entity by Western blot analysis (Jahnke, GD et al., J Mol Endocrinology 19: 279–289). In this study we investigate the functional role of AM in human breast tissue and in several breast cancer cell lines. We have identified AM mRNA expression in 4/4 normal and 6/6 malignant breast cell lines using RT-PCR. Immunohistichemical and in situ RT-PCR analysis of paraffin embedded tissue localized AM expression to epithelial cells in 11/11 normal specimens. On the basis of our previous in vitro studies with MCF-7 and growth inhibition with AM MoAB-G6, we investigated a similar course with an in vivo model. MCF-7 xenografts in nude mice were significantly reduced in volume by 30% after injection of AM Moab-G6. We have demonstrated that human breast milk contains an AM-like (6 kDa) entity by Western blot analysis that will be sequenced to verify it is authentic AM.

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## A pure antiestrogen, ICI 182,780, stimulates the growth of tamoxifen-resistant KPL-1 human breast cancer cells in female nude mice

J. Kurebayashi[1], S. Yamamoto[1], T. Otsuki[2], H. Sonoo[1]. [1]*Department of Breast & Thyroid Surgery;* [2]*Department of Hygiene, Kawasaki Medical School, Kurashiki, Okayama 701-01, Japan*

Antiestrogen-resistance frequently occurs during the treatment of breast cancer. The critical mechanism responsible for this resistance has not yet been elucidated. We established a human breast cancer cell line, KPL-1, derived from a patient with a recurrent disease which had appeared under tamoxifen administration. Our previous study suggested that this cell line is estrogen receptor (ER)-positive but tamoxifen-resistant (Br J Cancer 71: 845–853, 1995). The effects of a pure antiestrogen, ICI 182,780, were investigated in this study. Although tamoxifen inhibited neither cell growth nor estradiol-stimulated transcriptional activity, ICI 182,780 significantly inhibited both of these. Tamoxifen and ICI 182,780 were administered to female nude mice bearing KPL-1 tumors. Tamoxifen had no effect on tumor growth, but ICI 182,780 unexpectedly stimulated tumor growth ($P = 0.022$). In addition, estradiol administration tended to inhibit tumor growth ($p = 0.198$). To the best of our knowledge, this KPL-1 cell line is the first breast cancer cell line to be growth-stimulated by ICI 182,780 and growth-inhibited by estradiol in vivo. To explore the possible causes of these novel phenotypes, the mRNA levels of ER-$\alpha$, ER-$\beta$, some growth factors, and their receptors in KPL-1 cells were compared with those in three other ER-positive human breast cancer cell lines (MCF-7, T-47D, and KPL-3C) by a semiquantitative RT-PCR. Fibroblast growth factor (FGF)-1 was overexpressed only in the KPL-1 cell line. Taken together with the results of our previous study suggesting that overexpression of FGF-1 or -4 induces breast cancer progression (Breast Cancer Res Treat 31: 153–165, 1994), paracrine interaction between tumor cells and stromal cells mediated by growth factors, such as FGF-1, might be a key factor to explain the unique hormone-responsiveness of KPL-1 cells.

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## Androstenedione (A) conversion in lymphocytes infiltrating breast cancer (BC) tissue

L.M. Berstein, T.E. Poroshina, T.S. Zimarina, A.A. Larionov, A.V. Uporov. *Prof. N. N. Petrov Research Institute of Oncology, St. Petersburg, 189646, Russia*

Estrogens are involved in breast carcinogenesis. Previously we described 3-component model of estrogenic pool formation in BC tissue which suggested the participation of lymphocytic-macrophagal infiltrate in the process (Berstein, Santen, Santner: Med. Hypotheses, 1995). In this work we isolated lymphocytes from tumor tissue, TIL (enzymatic digestion), evaluated in them conversion of A ($^3H_2O$-release assay) and compared data with aromatase (Arom) activity in the whole tissue. Material for study included 32 samples of BC tissue. In the part of samples isolated lymphocytes (because of their small number) were combined. Totally 18 TIL samples were analyzed (5 from pts with preserved menstrual cycle/I/ and 13 from pts in menopause/II/). Number of lymphocytes isolated from tumors and percent of tumor cells in lymphocytic suspension were higher in I than in II. On the contrary, A conversion in tumor lymphocytes (fM/mg prot or fM/l mln cells) was higher in group II. Positive correlation between A conversion

in TIL and Arom activity in BC tissue was revealed and no correlation was discovered between A conversion in TIL and percent of tumor cells in lymphocytic suspension. Thus, TIL possess ability to convert A with $^3H_2O$ release. Molecular-genetic studies are in process now to proof presence of Arom in these cells.

**Acknowledgement:** To Prof. R.J. Santen for fruitful discussions.

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### 11β-hydroxysteroid dehydrogenase activity in human breast cancer cells: Characterization and effect of hormonal manipulations

F. Arcuri, S. Sestini, A. Carducci, F. Manzoni, K. Schürfeldt, S. Parigi, M. Cintorino. *University Siena, Pathologic Anatomy and Histology, Le Scotte V. le Bracci, 1-53100 Siena, Italy*

**Purpose:** 11beta-hydroxysteroid dehydrogenase (11b-HSD) is the enzyme responsible for the interconversion of biological active glucocorticoids (GC) and their inactive 11-oxo metabolites. Up to date, two isoforms have been cloned, a low affinity NADP+ dependent oxoreductase (type 1) and a high affinity NAD+ dependent dehydrogenase (type 2).

Recently, the presence of 11b-HSD activity has been described in breast cancer cells and tissues however this enzyme has never been characterized. This study was aimed to evaluate the features of 11b-HSD in a human breast cancer cell line, T-47D.

**Methods:** 11b-HSD expression in T-47D cells was evaluated by standard biochemical assay and RT-PCR analysis either in cells untreated or exposed for 24 hours to estradiol (E2), estrone (E1), medroxyprogesterone acetate (MPA), dexamethasone (DEX), mifepristone (RU486) and MPA + RU486.

**Results:** Biochemical and mRNA analysis showed that 11-hydroxysteroid dehydrogenase activity of T-47D cells depends on the 11b-HSD type 2 isoform. In addition, in cells treated for 24 hours with MPA, 11b-HSD type 2 basal activity increased by mean of 10 to 12 fold whereas E1, E2 or DEX, exerted no significant effects. RU486 acted as a pure progestin antagonist, exerting no agonist effect by its own but counteracting all of MPA enhanced increase in type 2 activity.

**Conclusion:** This study demonstrated that T-47D cells express the dehydrogenase isoform of 11b-HSD suggesting a role for this enzyme in the regulation of intracellular levels of biologically active GC in breast cancer cells. Moreover, the MPA induced increase of 11b-HSD type 2 activity indicates the existence of a connection, previously undocumented in these cells, between progestins exposure and GC metabolism.

## Thursday, 1 October 1998 16:00-18:00

**EUROPA DONNA SYMPOSIUM**

# The genetic dilemma

**391** INVITED

### Inherited susceptibility to breast cancer: A psychological perspective

Ann Cull. *ICRF Medical Oncology Unit, Western General Hospital, Edinburgh EH4 2XU, UK*

New genetic knowledge offers the potential for reducing future mortality and morbidity associated with breast cancer. With media coverage growing number of individuals are seeking genetic counselling about the significance of their family history of breast cancer. In the present state of knowledge there are large margins of uncertainty around the information which people can be given about their personal risk of developing breast cancer. The number of individuals who can be offered direct gene testing remains very small and mutation searching may not be informative. The effectiveness of available strategies for prevention and early detection is unproven among younger women at increased risk. The challenge therefore lies in organising services in such a way as to provide information appropriate to the level of risk which the public can understand and use to make appropriate health choices without adverse psychological consequences. Empiric data will be reviewed to highlight the psychological issues.

**392** INVITED

### The bio-ethical dilemma

S. Spinsanti. *Istituto Giano, Via Giusti 3, I-00185 Rome, Italy*

Abstract not received.

**393** INVITED

### Inherited predispostion to breast cancer

P.A. Daly. *Department of Clinical Haematology and Oncology, St. James's Hospital and Trinity College, Dublin 8, Ireland*

Between 5 and 10% of breast cancers are thought to develop because of highly-penetrant mutations in genes conferring a major lifetime risk of developing breast and other cancers. Two genes, BRCA-1 on chromosome 17q and BRCA-2 on 13q, are sequenced. Mutations in these genes underlie many of the breast cancers developing through inherited predisposition. Current technology can identify up to 70% of mutations in BRCA-1 and 2 but this science is still not perfect. Whereas a positive test in an unaffected individual has clear implications, a positive test in unaffected family members still poses many questions in terms of life-time risks, optimal screening strategies prophylactic surgery and chemoprevention. A negative test in an unaffected individual from a family with a high cancer incidence can represent a false negative or could indicate a predisposition related to genes yet unknown. Testing unaffected family members in this setting is not appropriate. Major difficulties arise when affected members have died and testing their DNA is not possible. Where defined mutations are known to exist among populations, testing unaffected individuals may be appropriate in selected circumstances. Each society will have to approach this issue in line with prevailing social perceptions, public demand, healthcare structures and available resources. We have developed a research and development strategy based on co-operation between the National Genetics Centre and Regional Oncology Centres. Testing will be undertaken on affected individuals where there is a significant (20–30%) likelihood of a mutation being detected based on family history data. On finding a mutation predictive testing will be offered to unaffected family members along with the necessary medical and psychological support. This effort is supported by the government via the Health Research Board and rightly still remains within the sphere of clinical research.

**394** INVITED

### Genetic testing and its impact on a cohort of women and their families

W.J. Ormiston. *St. James's Hospittal and Trinity College, Department of Clinical Heamatology Oncology, Dublin, Ireland*

In Ireland a large kindred of over 550 individuals was central to the isolation of the BRCA 2 gene. When the mutation which caused cancer predisposition in this family was identified at codon S2984X in September 1996 members were offered the opportunity to avail of predictive testing. Eighteen individuals have been tested to date. During the counselling sessions pre- and post-testing it was very apparent that a negative or positive result affected not only the individuals tested but also the entire family. Indeed many women wished to have predictive testing to help determing risk to others in their nuclear family and especially their daughters. Very high anxiety levels were observed among spouses and offspring. Among the male members who tested positive four chose to keep the result to themselves and did not pass the information to their daughters.

Health professionals should be aware during pre and post counselling that genetic testing has a very powerful impact on the entire family.