using an internal *β-Actin* gene standard as previously described [5].

2.5. Reporter gene assays

ER transactivation was determined 36 h post-transfection by measuring ERE-Tk-Firefly luciferase activity against an internal TK- Renilla-luciferase standard using a commercial assay (Promega, Southampton, UK).

2.6. Cell proliferation studies

Cell proliferation responses to steroids and antisense ODNs were evaluated over 7–14 days by direct counting of viable cells [6]. All treatments were replenished every 2 days.

3. Results

Chimeric MP-PO antisense oligonucleotides at concentrations of 0.1–1 μM had limited efficacy, reducing ER protein expression and transactivation in MCF-7 cells by 15–20%. At the higher concentrations (2–10 μM) required for effective ER gene inhibition, these ODNs lost specificity with both antisense and scrambled ODNs substantially reducing ER levels. At concentrations of below 10 μM these ODNs also failed to reduce MCF-7 basal growth. Constitutively expressed full length and truncated ER antisense RNAs reduced ER protein, mRNA and transactivation by 50, 70 and 25%, respectively over wild-type MCF-7 and pCDNA3 stably transfected MCF-7 controls, but had no effect on *actin* or G418-resistant gene expression. MCF-7 ER antisense stable transfectants exhibited no alterations in their steroid sensitivity when compared with wild-type or pCDNA3 stably transfected MCF-7 cells. In transient transfection experiments constitutive expression of an AF-2 compromised ER mutant (*DNER-1*) repressed ER transactivation in a dose-responsive manner by 70–80% in both MCF-7 and in wild-type ER transfected ER-negative COS-7 cells. The apparent trans-dominant effect of *DNER-1* on wild-type ER activity was not reflective of a general inhibition of gene transcription since in similar dose–response curves the mutant ER failed to block transcription from either the basic ERE deleted *Tk-Luc* reporter gene construct or a TPA response element bearing the *Tk-Luc* construct.

4. Conclusions

In contrast to antisense oligonucleotides, constitutively expressed ER antisense RNAs and ER dominant negative mutants appear feasible alternatives to current ER ligand derivatives as a means of selectively downregulating oestrogen/ER actions *in vitro*.

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Identification of women at high risk of developing endometrial cancer on tamoxifen

M. Berlière [a], G. Radikov [a], C. Galant [b], Ph. Piette [b], E. Marbaix [b], J. Donnez [a,*]

[a] *Department of Gynaecology, Catholic University of Louvain, Ave. Hippocrate, 10 — B-1200 Brussels, Belgium*
[b] *Department of Pathology, Catholic University of Louvain, Ave. Hippocrate, 10 — B-1200 Brussels, Belgium*

* Corresponding author. Tel.: +32-2-764-9502; fax: +32-2-7649507.
E-mail address: donnez@gyne.ucl.ac.be (J. Donnez).

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Table 1
Results expressed in person-years

	Group I (patients without initial endometrial lesions)	Group II (patients with initial endometral lesions)
Incidence benign lesions	55/425 (12.9%)	15/85 (17.6%)
Incidence atypical lesions	3/425 (0.7%)	10/85 (11.7%)*

*Statistically significant ($P < 0.0001$).

Uterine side-effects of tamoxifen have been extensively detailed in recent years. Prospective and retrospective studies have shown a higher incidence of endometrial cancer in long-term users. The aims of this study were to determine if a group of high risk women could be identified by pretreatment screening and if conservative treatment of benign lesions could prevent the subsequent development of endometrial cancer. The conclusions of our observations may prove useful in the determination of recommendations for the gynaecological follow-up of patients on tamoxifen.

Tamoxifen has been found to be associated with various endometrial pathologies, such as benign polyps and atypical lesions [1,2]. With regard to endometrial carcinomas, molecular mechanisms associated with endometrial carcinogenesis remain the subject of controversy and study [3–5]. In order to determine whether a group of high risk women could be identified by pretreatment screening, we initiated a prospective study [6].

Between January 1993 and January 1998, 575 postmenopausal patients with an intact uterus and a diagnosis of histologically proven breast cancer, who were to receive tamoxifen as adjuvant therapy, were enrolled. Of these, 510 patients are currently evaluable. Prior to tamoxifen treatment, all the patients underwent a gynaecological examination and vaginal ultrasound. In the case of an abnormal ultrasound (endometrial thickness more than 5 mm), an outpatient hysteroscopy with endometrial biopsy was carried out. The lesions taken into account were all endometrial polyps. On the basis of this screening, two groups of patients were identified: 425 (83.3%) patients without initial lesions (group 1), and 85 (16.7%) patients with initial lesions (group 2). Endometrial polyps (without atypia) were removed by hysteroscopy prior to tamoxifen treatment. All the women on tamoxifen, 20 mg daily, were followed up and, in the absence of any gynaecological symptoms, the basal screening was repeated annually. During tamoxifen treatment, the therapeutic modalities of endometrial lesions were the same: operative hysteroscopy for benign lesions, and in the case of atypical lesions, hysterectomy. Because the duration of follow-up was variable (between 1 and 5 years), data were expressed in person-years (see Table 1).

Beyond 2 years of follow-up, the incidence of atypical lesions was significantly higher ($P < 0.0001$) in the group with initial lesions (IR 0.018) than in the group without initial lesions (IR 0.001). Moreover, the severity of the lesions was greater in the group with initial lesions. The conclusions of this study are, firstly that a group of women exhibiting increased sensitivity to the oncogenic potential of tamoxifen can be identified by a pretreatment uterine evaluation; secondly, that endometrial resection does not protect against the subsequent development of endometrial cancer; and finally, that many endometrial lesions develop while on tamoxifen, which can be atypical but totally asymptomatic.

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