

## F.1 Summary

### Antiestrogens and Endometrial Adenocarcinoma

Antiestrogenic effects on tumor growth and on protein expression of estrogen-, progesterone- and epidermal growth factor receptors – *in vitro* and *in vivo*

Endometrial carcinoma is the most common malignancy of the female genital tract. Although hysterectomy is the conventional therapy of endometrial carcinoma at present, pure antiestrogens (AE) could be useful in support or even as an alternative for the treatment. Adjuvant or palliative therapies as with AE in breast cancer, have not yet been established for endometrial cancer. The aim of this study was to establish a preclinical rationale for use of AE in the treatment of endometrial carcinoma.

Six human endometrial adenocarcinoma cell lines were analyzed for their estrogen receptor (ER) expression by an ER enzyme immunoassay. Based on these results the human endometrial carcinoma cell lines were defined as ER-positive and ER-negative cell lines. The ER-expression of ECC-1 cells was 187 fmol ER/mg protein whereas Ishikawa cells expressed 24 fmol ER/mg protein. In the human endometrial cancer cell lines MFE-296, MFE-280, MFE-319 and KLE ER protein expression was not detectable. These cell lines were further used to compare the growth inhibitory potency of nonsteroidal partialagonistic AE tamoxifen and raloxifen with steroidal pure AE ZK 191703 and ICI 182,780.

The crystal violet method was used for *in vitro* proliferation assays. For *in vivo* studies the human endometrial cancer cell lines were implanted subcutaneously in estrogen- or tamoxifen-substituted immune-deficient SCID mice. Estrogens or AE displayed effects on cell proliferation only in ER-positive cell lines. *In vitro* and *in vivo* growth stimulation by estrogens and growth inhibition by AE were more potent in ECC-1 cells than in Ishikawa cells. We found a positive correlation between ER expression and estrogenic and antiestrogenic effects. The pure AE ZK 191703 was the most potent AE *in vitro* with a  $IC_{50}$  in ECC-1 cells of  $6,6 \times 10^{-10}$  M compared to the  $IC_{50}$  of ICI 182,780 with  $1,4 \times 10^{-9}$  M. The inhibition of endometrial tumor growth *in vivo* by treatment with ZK 191703 was significantly different in comparison to the estrogen- and tamoxifen-stimulated control group. In these experiments ZK 191703, tamoxifen and raloxifen were administered orally whereas ICI 182,780 was given subcutaneously once a month. Tamoxifen stimulated the tumor growth of Ishikawa cells but inhibited the growth of ECC-1 cells. Therefore, we concluded a tumor-specific effect of tamoxifen in human endometrial adenocarcinoma.

Hematoxylin-Eosin(HE)-stained tumor sections of ER-positive estrogen-stimulated endometrial tumors showed a shift in tumor tissue/stromal proportion (TSP) in favor of the tumor tissue. In contrast HE-stained tumor sections from ER-negative endometrial tumors showed a shift in TSP in favor of the stromal tissue, when stimulated with estrogens. These results indicated an estrogen-stimulated growth of murine stromal cells as tumor growth of ER-negative endometrial carcinoma cells was estrogen-unaffected. After treatment with AE, which antagonize the estrogen effects, the TSP of ER-positive and ER-negative tumors shifted the other direction.

The ER destabilisation in human endometrial carcinomas by treatment with pure AE ZK 191703 and ICI 182,780 was demonstrated *in vitro* and *in vivo* by two independent methods: ER enzyme immunoassay and immunohistochemistry using the monoclonal antibody 1D5. ER destabilisation was dose-dependent as observed after 24 hours and persisted 5-7 days. The increased ER expression after low-dose longtime-treatment with ICI 182,780 is probably due to a delayed escape phenomenon.

In addition, the inhibition of an ER response by pure AE was shown *in vitro* and *in vivo* by a dose-dependent decrease in progesterone receptor (PgR) expression after incubation with pure AE. A correlation between ER and epidermal growth factor receptors expression was not observed in Ishikawa cells or ECC-1 cells on protein level.

Cell cycle analyses showed an 85-90% increase in ECC-1 cell fraction of G0/G1-phase after incubation with pure AE. Therefore, we concluded an arrest of human endometrial ER-positive adenocarcinoma cells in G0/G1-phase of cell cycle.

The results obtained from our preclinical studies showed a therapeutic effect of pure AE against estrogen- and tamoxifen-stimulated human endometrial carcinoma. From these data we conclude that the pure AE ZK 191703 could be more potent than ICI 182,780 in adjuvant and palliative endocrine therapies. Target groups of endocrine AE-therapy are patients with preexisting endometrial carcinoma under tamoxifen-longtime therapy, premenopausal patients with endogenous estrogen-activity and postmenopausal patients with estrogen-replacement therapy. ER sensitivity of the endometrial tumors to estrogens is a prerequisite for this therapy. The determination of ER expression in endometrial tumors allows a statement of tumor responsiveness to AE-treatment and an assessment of prognosis with regard to tumor regression. Our findings regarding ZK 191703 require further evaluation of this pure steroidal AE in clinical studies in order to determine its antiproliferative effectiveness against human endometrial adenocarcinoma.