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The corepressor-associated protein Tab2 as a new target to revert resistance to antiestrogens in breast cancer

S. Reineri[1], S. Cutrupi[2], A. Panetto[3], L. Ricci[3], A. Lykkesfeldt[4], M. De Bortoli[1]. [1]*University of Turin, Oncological Sciences, Candiolo Torino, Italy;* [2]*University of Turin, Human and Animal Biology, Torino, Italy;* [3]*Bioindustry Park Silvano Fumero, Biolima, Colleretto Giacosa Torino, Italy;* [4]*Inst. Cancer Biology DCS, Dept. of Breast Cancer Research, Copenhagen, Denmark*

Pharmacological resistance is a serious challenge for all kind of cancers. In hormone-dependent breast cancer, the problem of resistance to endocrine treatments is alleviated by availability of different classes of drugs (SERM, aromatase inhibitors, pure antiestrogen). Nonetheless, understanding the mechanisms of resistance would significantly improve our ability to cure this disease. At the cellular level, the efficacy of antiestrogenic drugs consists primarily in contrasting the transcriptional action of estrogen-activated estrogen receptors (ER). The correct balance of transcriptional coactivators and corepressors in cancer cell nuclei plays a major role in antiestrogenic drug response and, in fact, resistant cells often display aberrant corepressor localization or coactivator overexpression. The Tab2 protein was recently reported in complex with the NCoR corepressor. In response to interleukin, MEKK1 phosphorylates TAb2 and, as a consequence, Tab2 shuttles NCoR to the cytoplasm. This was shown sufficient to convert a steroid hormone antagonist to agonist (Zhu et al. 2006, Cell 124:615−29).

Using a series of Tamoxifen (TAM) resistant cells derived from MCF7 by continuous exposure to the drug, we show here that downregulation of Tab2 using a double-strand interfering small RNA restores the antiproliferative response to TAM, indicating that Tab2 is in the pathway leading to TAM resistance in these cells. Interestingly, a similar result was obtained also using the BT474 cell line, which shows amplification and overexpression of the ERBB2 gene. Tab2, together with NCoR, is recruited at estrogen target genes by interaction with the N-terminal domain of ERα (Zhu et al., 2006). By in vitro interaction studies with recombinant proteins, we mapped the interaction of ERα to the central domain of Tab2. In addition, a mimic peptide derived from the N-terminus of ERα, but not a mutated version carrying A/L substitutions, was able to compete for Tab2/ERα interaction. On this basis, we designed a cell permeable peptide, composed of the ERα sequence fused to the Tat minimal carrier domain. Colture of TAMR cells in the presence of the ERα-Tat peptide, but not the mutated version, led to a 40−60% recovery of the antiproliferative effect of TAM, depending on the cell line or clone. In conclusion, we have identified the Tab2/ERα interaction as a potential drug target for overcoming TAM resistance in breast cancer.

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The gastric modifier esomeprazole did not affect the bioavailability and tolerability of CS-7017 in an open-label, phase I, two-way crossover clinical study in healthy subjects

R. Scheyer[1], H. Zahir[1], R. Luo[1], L. He[1], R. Noveck[2], K. Liu[3]. [1]*Daiichi Sankyo Pharma Development, Translational Medicine, Edison, USA;* [2]*MDS Pharma Services, DMPK and Drug Safety Assessment, New York, USA;* [3]*Daiichi Sankyo Pharma Development, Biostatistics, Edison, USA*

Background: CS-7017 is a novel, highly selective peroxisome proliferator activated receptor gamma agonist (PPARγ) agonist with anticancer activity in preclinical models. The aim of this phase I clinical study was to evaluate the effect of the gastric pH modifier, esomeprazole, on the bioavailability and safety of CS-7017 in healthy subjects.

Methods: Healthy male subjects aged 22−44 years were eligible for enrolment in this open-label, randomized, single-dose, two-treatment, two-period, two-way crossover study. Subjects were randomized to either treatment sequence AB or BA. In Treatment A, subjects received single oral dose of 0.5 mg CS-7017 (2 × 0.25 mg tablets) on the morning of Day 5. In Treatment B, subject received 40 mg once daily oral doses of esomeprazole on Days 1−5 and a single oral dose of CS-7017 on the morning of Day 5. Treatment periods were separated by 5 days. Pharmacokinetic (PK) measurements were taken on Day 5 of each period. The primary endpoint was the mean ratio of the PK parameters of CS-7017 co-administered with esomeprazole compared to CS-7017 administered alone. The number and severity of adverse events were a secondary endpoint.

Results: A total of 22 subjects were enrolled and evaluable. Table 1 summarizes the PK parameters and treatment ratios for CS-7017 administered alone or in combination with esomeprazole. During concurrent administration with esmoprazole there was approximately a 10−15% decrease in CS-7017 exposure (based on the exposure ratios). However, both AUC and Cmax met the bioequivalence criteria (Table). Therefore, CS-7017 does not require dose modification when administered concurrently with a proton pump inhibitor eg esmoprazole. There were no deaths, serious adverse events (SAEs), discontinuations due to AEs or clinically relevant AEs in this study. In total, 7 treatment emergent adverse events (TEAEs) developed in 4 subjects (3 subjects who received the combination and 1 subject who received CS-7017 alone). Most AEs (eye irritation, ocular hyperemia, burning sensation, headache, epistaxis and pruritis) were mild except for the 2 reported headaches which were of moderate severity; none were considered related to the study treatments.

Conclusions: The results of this phase I study indicate that esomeprazole co-administration does not affect the bioavailability of CS-7017. Single-dose administration of 0.5 mg CS-7017 alone or in combination with 40 mg esomeprazole was well tolerated in healthy male subjects.

Table 1. Statistical comparison of the pharmacokinetic parameters of CS-7017 with and withoutesomeprazole co-administration

Parameter	Geometric LSM		Ratio B/A	Intra-subject
CS-7017	Single oral dose of 0.5 mg CS-7017	Oral doses of esomeprazole 40 mg plus single oral dose of 0.5 mg CS-7017	%, (95% CI)	variability, %
AUC_{last} (ng·h/mL)	347.4	314.7	90.57 (86.36, 94.99)	7.6
AUC_{0-inf} (ng·h/mL)	365.8	338.8	92.62 (87.80, 97.72)	7.8
C_{max} (ng/mL)	28.90	25.01	86.55 (80.10, 93.54)	12.4

$AUC_{0-inf} = AUC_{last} + C_{last}/\lambda_z$.; LSM, least-squares-means.

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Activation of the endothelin signaling pathway is linked with acquisition epithelial–mesenchymal transition phenotype of chemoresistant ovarian cancer cells

L. Rosanò[1], R. Cianfrocca[1], V. Di Castro[1], F. Spinella[1], A. Lucidi[2], G. Ferrandina[2], P.G. Natali[1], A. Bagnato[1]. [1]*Regina Elena Cancer Institute, Experimental Research Center, Roma, Italy;* [2]*Catholic University of the Sacred Heart, Gynecologic Oncology Unit, Roma, Italy*

Understanding the molecular mechanisms underlying chemoresistance is of utmost importance for improving the treatment of epithelial ovarian cancer (EOC). Emerging evidence suggests molecular and phenotypic association between chemoresistance and epithelial–mesenchymal transition (EMT) in cancer cells. Endothelin-1 (ET-1) and the endothelin A receptor (ET_AR) axis is implicated in the pathobiology of EOC by driving different tumor-promoting effects, including EMT. By employing as model the A2780 ovarian cancer cell line and its cisplatin- and taxol-resistant sub-lines, we demonstrated that in the resistant cells ET-1 and ET_AR are upregulated, at both mRNA and protein level, paralleled by enhanced MAPK and AKT phosphorylation and cell proliferation. To assess whether the chemoresistance in A2780 cells was associated with EMT molecular changes, we examined the expression of E-cadherin and its transcriptional repressor Snail. Enhanced Snail expression level was observed in resistant sublines, associated with a concomitant decrease in E-cadherin expression and promoter activity. Moreover, ET-1 induced a significant induction (4-fold increase) of *Snail* promoter activity in resistant cells, and the E-cadherin promoter sequences were detected bound to Snail in a time-dependent manner as demonstrated by chromatin immunoprecipitation assays, suggesting that the ET-1-dependent and sustained binding of Snail in the E-cadherin promoter might account for the EMT and chemoresistant phenotype of these cells. Moreover, the invasive capability of chemoresistant sublines was greater, with a ~2-fold increase in the number of invading cells compared with sensitive cells. Interestingly, ET_AR blockade with a specific ET_AR antagonist zibotentan (ZD4054), or its silencing with siRNA, reverted EMT, restored drug sensitivity to cytotoxic-induced apoptosis, and inhibited the invasive potential of resistant cells. In vivo, zibotentan inhibited tumor growth of sensitive and resistant A2780 xenografts, which displayed decreased expression of mesenchymal markers, and enhanced expression of epithelial markers. Finally, analysis of EOC tissues, with different responses to chemotherapy, revealed that ET_AR is overexpressed in resistant tumors and is associated with EMT marker expression. All these data indicate that blockade of ET_AR-driven EMT can overcome chemoresistance in EOC, improving the outcome of EOC patients' treatment.

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