Inhibitors of apoptosis proteins (IAPs) are key negative regulators of programmed cell death. Their frequent deregulation in most cancer types contributes to tumor cell survival and resistance to cancer therapy, making IAPs attractive therapeutic targets. Debio 1143 (aka AT-401/SM4-401), a new potent orally-available monovalent SMAC mimetic, targets multiple IAP members and is currently in clinical trials for cancer treatment. In this study, pharmaco-imaging was used to evaluate the effects of Debio 1143 on cell death and metabolism in the triple negative breast cancer cell line (TNBC) MDA-MB-231.

### Material and Methods

**In vitro evaluation of Debio 1143 on cell proliferation and induced-apoptosis**

Breast cancer cells: The MDA-MB-231 cell line obtained from ECACC was investigated in this study. Cells were cultured as adherent monolayer in RPMI 1640 medium supplemented with 10% fetal bovine serum. The effects of Debio 1143 on MDA-MB-231 cell proliferation were determined by MTS assay, IC50 was determined using XFe96 software, in vitro apoptosis induction by Debio 1143. The effects of increasing concentrations of Debio 1143 on apoptosis were assessed using the Annexin V-FITC / 7-AAD kit (Beckman Coulter) and the PE Active Caspase-3 Apoptosis kit (Becton Dickinson). In vivo evaluation of Debio 1143 effects in subcutaneous MDA-MB-231 tumor-bearing mice Experimental model: Animal experiments were performed according to ethical guidelines of animal experimentation (1) and were approved by Oncode’s internal ethical committee (Oncosign). Five million MDA-MB-231 cells with matrigel were implanted subcutaneously in female CB-17 SCID mice 72h after a whole body irradiation (80 Gy). In vivo evaluation of apoptosis induction by Debio 1143 (3µM and 10µM) in mice with palpable subcutaneous tumors of comparable size (mean volume 340 mm3). In vivo tumor evaluation of apoptosis induction by Debio 1143 (99mTc-Annexin V) was detected at 24 hours post-treatment. In vivo evaluation of apoptosis induction by Debio 1143 (99mTc-Annexin V) was evaluated by 18F-FDG (fluorodeoxyglucose) PET/CT (4) after 1 and 2 weeks of treatment.

**Dose-response evaluation of cytotoxicity of Debio 1143 and Paclitaxel on MDA-MB-231 cell line.** IC50 were determined using XLfit® software. In vivo evaluation of debio 1143 effects in subcutaneous MDA-MB-231 tumor-bearing mice Experimental model: Animal experiments were performed according to ethical guidelines of animal experimentation (1) and were approved by Oncode’s internal ethical committee (Oncosign). Five million MDA-MB-231 cells with matrigel were implanted subcutaneously in female CB-17 SCID mice 72h after a whole body irradiation. In vivo evaluation of apoptosis induction by Debio 1143 (3µM and 10µM) in mice with palpable subcutaneous tumors of comparable size (mean volume 340 mm3). In vivo tumor evaluation of apoptosis induction by Debio 1143 (99mTc-Annexin V) was detected at 24 hours post-treatment. In vivo evaluation of apoptosis induction by Debio 1143 (99mTc-Annexin V) was evaluated by 18F-FDG (fluorodeoxyglucose) PET/CT (4) after 1 and 2 weeks of treatment.

**Antitumor activity of Debio 1143 in subcutaneous MDA-MB-231 tumor bearing mice**

Debio 1143 displayed a significant antitumor activity as soon as 7 days after treatment initiation (optimal T/C: 95% on day 8). Paclitaxel was not active at this suboptimal dose.

**Conclusions**

Debio 1143 induced apoptosis in TNBC model MDA-MB-231, which can be detected by 99mTc-Annexin V imaging in vivo. Debio 1143 displayed a significant antitumor activity in the MDA-MB-231 tumor model, associated with a significant decrease in tumor glucose metabolism visualized by 18F-FDG PET/CT imaging. This study supports the clinical evaluation of Debio 1143 in TNBC and the use of pharmaco-imaging techniques as non-invasive biomarker to follow the compound activity.

**Clinical trial**

Debio 1143 is currently being evaluated in a Phase I study of combination with Carboplatin and Paclitaxel in Patients With Squamous Non-Small Cell Lung Cancer (GSLC), Platinum-refractory Ovarian Cancer, and Breast-like/CLAUD-Low Triple Negative Breast Cancer (NCT01930292).