Pharmaco-imaging study of the effects of Debio 1143, a new orally available IAP inhibitor, in a triple-negative breast cancer model

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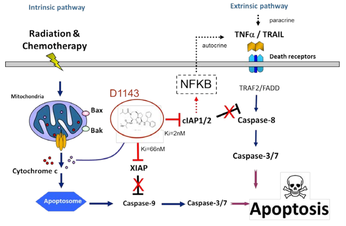
 # 2757

Introduction

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-406/SM-406), 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 tumor 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.

In vitro cytotoxicity activity of Debio 1143: The effects of Debio 1143 on MDA-MB-231 cell proliferation were determined by MTS assay. IC_{50} was determined using XLfit® 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 vitro* binding assays were performed with technetium-99m radiolabelled Annexin V (^{99m}Tc-Annexin V).

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[(1-3)] and were approved by Oncodesign's internal ethical committee (OncoMet). Five millions MDA-MB-231 cells with matrigel were implanted subcutaneously in female CB-17 SCID mice 72h after a whole body γ-irradiation.

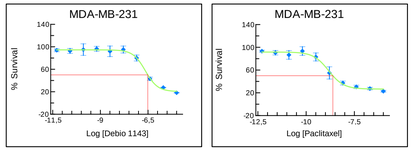
In vivo evaluation of apoptosis induction by Debio 1143 (^{99m}Tc-Annexin V): When tumors reached a mean volume of 340 mm^3, ^{99m}Tc-Annexin V SPECT/CT (Single Photon Emission Computed Tomography/Computed Tomography) imaging was performed 6 and 24 hours after a single administration of vehicle (*PO*), Debio 1143 (*PO*, 100 mg/kg), or Paclitaxel (IV, 7.5 mg/kg).

In vivo evaluation of antitumor activity of Debio 1143 by Positron Emission Tomography (PET): In a 2nd subset of mice, when tumors reached a mean volume of 150 mm^3, mice received daily *PO* administrations of vehicle, Debio 1143 (100 mg/kg, Q1Dx14) or weekly IV injections of Paclitaxel at suboptimal dose (7.5 mg/kg, Q7Dx2). Tumor metabolism was evaluated by ^{18}F-FDG (fluorodeoxyglucose) PET/CT[(4)] after 1 and 2 weeks of treatment.

(1) Principe d'éthique de l'expérimentation animale, Directive n°2010/63 CEE du 22 septembre 2010, Décret n°2013-118 du 01 février 2013. (2) NRC Guide for the Care and Use of Laboratory Animals. (3) United Kingdom co-coordinating committee on cancer research guidelines for welfare of animals in experimental neoplasia, Br. J. Cancer 2010, 102: 1555-1577. (4) Aknowledgement: PET / CT camera use was possible thanks to a French Government Grant managed by the French National Research Agency (ANR) under the program "Investissements d'Avenir" (reference ANR-10-EQPX-05-01/IMAPPI Equipex).

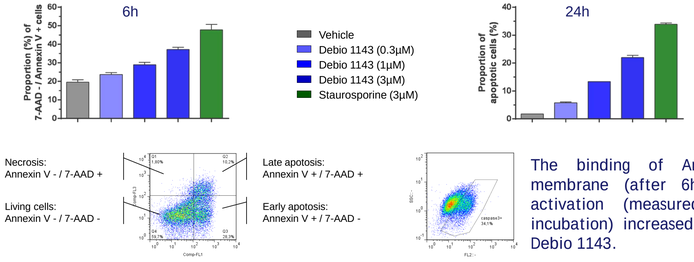
Results

In vitro evaluation of Debio 1143 cytotoxic activity



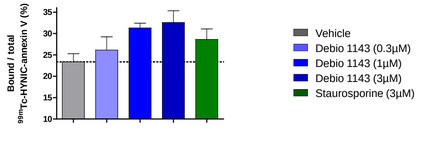
Dose-response evaluation of cytotoxicity of Debio 1143 and Paclitaxel on MDA-MB-231 cell line. IC_{50} were 2.24x10^{-7} and 5.8x10^{-9} M respectively.

In vitro evaluation of apoptosis induction by Debio 1143 (FACS)



The binding of Annexin-V to cell membrane (after 6h) and Caspase-3 activation (measured after 24h of incubation) increased with the dose of Debio 1143.

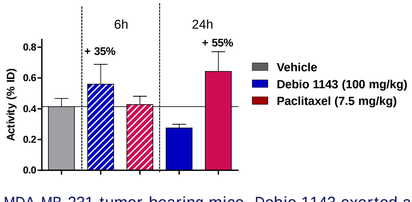
In vitro evaluation of apoptosis induction by Debio 1143 (^{99m}Tc-Annexin V)

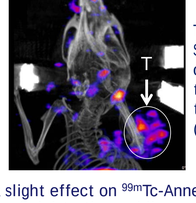


^{99m}Tc-Annexin V cell binding was increased by Debio 1143 (related to concentration) in MDA-MB-231 cell line after 24h incubation period.

^{99m}Tc-Annexin V cell binding appeared less sensitive compared to FACS analysis.

In vivo evaluation of apoptosis induction by Debio 1143 (^{99m}Tc-Annexin V)



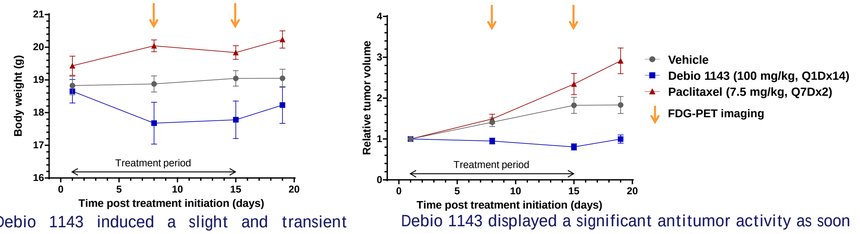


Tumor ^{99m}Tc-Annexin V SPECT/CT imaging (image obtained 24 hours after treatment in a Debio 1143-treated mouse).
(T: tumor)

In MDA-MB-231 tumor-bearing mice, Debio 1143 exerted a slight effect on ^{99m}Tc-Annexin V tumor binding with an maximal increased tumor SPECT signal 6 hours after oral administration, while paclitaxel maximum effect was detected at 24 hours post-treatment.

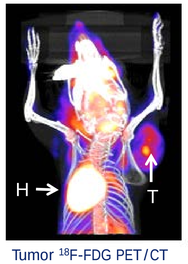
Results

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

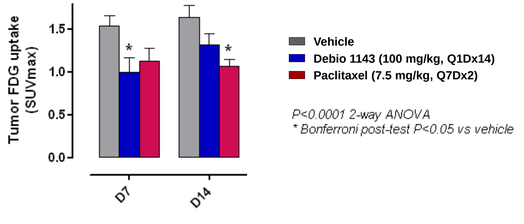


Debio 1143 induced a slight and transient decrease of body weight. Animals recovered once treatments ended.

Debio 1143 displayed a significant antitumor activity as soon as 7 days after treatment initiation (optimal T/C: 55% on D8). Paclitaxel was not active at this suboptimal dose.



Tumor ^{18}F-FDG PET/CT imaging
(T: tumor, H: heart)



^{18}F-FDG PET imaging showed that Debio 1143 induced a decrease of tumor ^{18}F-FDG uptake after one week of treatment. This effect was maintained at a lesser extent after 2 weeks of treatment.

Conclusions

- Debio 1143 induced apoptosis in TNBC model MDA-MB-231, which can be detected by ^{99m}Tc-Annexin V SPECT/CT 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 ^{18}F-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 in combination with Carboplatin and Paclitaxel in Patients With Squamous Non-Small Cell Lung Cancer (NSCLC), Platinum-refractory Ovarian Cancer, and Basal-like/Claudin Low Triple Negative Breast Cancer (NCT01930292).