Debio 1143, an oral antagonist of the inhibitor of apoptosis proteins, synergistically enhances the effects of multiple standard of care agents in human lung cancer models

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Summary

- **The oral monovalent IAP antagonist Debio 1143 displays single agent activity across lung cancer PDX 3D cultures of different histotypes, where small-cell, large-cell and squamous samples were more sensitive than adenocarcinoma-derived samples.**
- ***In vitro* high-throughput drug combination screening identified synergy of Debio 1143 in combination with NSCLC SOC compounds (taxanes, vinorelbine, gemcitabine).**
- **Strong synergy of Debio 1143 with the NSCLC SOC compound docetaxel was confirmed *in vivo* in mouse NSCLC xenograft models, where the combination caused marked anti-tumour activity that was superior to either monotherapy.**
- **Insensitivity of adeno NSCLC cell lines to Debio 1143 monotherapy *in vitro* is not observed *in vivo*, where Debio 1143 displayed single agent activity against adeno NSCLC xenografts.**
- **Combination therapy sensitized adeno NSCLC cell lines to Debio 1143.**
- **These findings underline the feasibility of using *in vitro* high-throughput screening for the discovery of novel drug combinations with increased anti-tumour efficacy *in vivo* and provide a rationale for the combination of the SMAC mimetic Debio 1143 with NSCLC SOC compounds in ongoing clinical trials in several cancer types (ClinicalTrials.gov Identifier: NCT01930292).**

Background / Aim of the study

Debio 1143 is a monovalent IAP antagonist facilitating induction of apoptosis

Drug resistance is a major problem in cancer therapy. The combination of drugs targeting simultaneously multiple critical nodes of the signaling networks controlling growth and survival of cancer cells is necessary to achieve long-lasting responses (1, 2). The members of the Inhibitor of apoptosis protein (IAP) family are key negative regulators of programmed cell death. Their frequent overexpression in most cancer types contributes to tumor cell survival and resistance to cancer therapy making IAPs attractive therapeutic targets (3).

The oral monovalent IAP antagonist **Debio 1143** (a.k.a. SM406 and AT406) inhibits multiple IAP proteins thus facilitating cell death via both the intrinsic and extrinsic apoptosis pathways by interfering with X-linked IAP (XIAP) and cellular IAP1 and 2 (cIAP1/2), respectively. Debio 1143 is currently in clinical development for cancer treatment. The **aim of the study** was to evaluate the activity of Debio 1143 alone and in combination with SOC agents in in vitro and in vivo lung cancer models of different histotypes and to identify novel synergistic combination partners with Debio 1143 in non-small cell lung cancer (NSCLC).

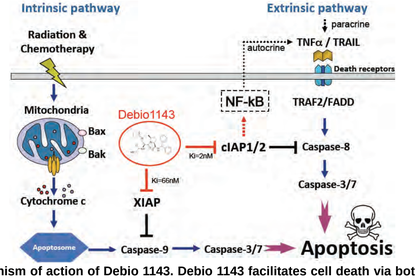


Figure 1. Mechanism of action of Debio 1143. Debio 1143 facilitates cell death via both the intrinsic and extrinsic apoptosis pathways by interfering with XIAP and c-IAP1/2, respectively.

Results

Debio 1143 displays differential anti-proliferative activity in *in vitro* lung cancer PDX models of different lung histotypes

Using clonogenic assays the anti-proliferative activity of Debio 1143 was assessed on a panel of 104 human patient-derived xenograft (PDX) 3D cultures, which included a total of 49 lung cancer models representing small, large, adeno and squamous lung cancer histotypes. Increased activity of Debio 1143 was observed in small-cell, large-cell and squamous histotypes, whereas most adenocarcinoma-derived PDX samples were less sensitive as compared to the mean activity across the full PDX panel (Fig. 2).

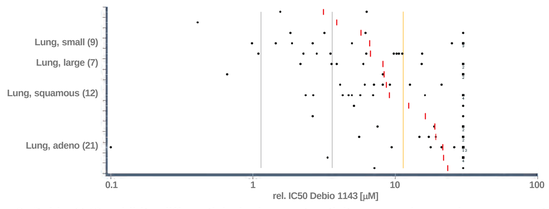


Figure 2. Debio 1143 exhibits differential single agent activity across human lung cancer PDX of different histotypes in clonogenic assays. Yellow line: geometric mean relative IC50 across all samples (11.37 µM); red lines: geometric mean relative IC50 within each histotype.

Debio 1143 synergizes with NSCLC SOC compounds to inhibit growth of human NSCLC cell lines *in vitro*

In line with the PDX results, moderate activity of Debio 1143 was observed also in human adenocarcinoma NSCLC cell lines (data not shown). To test if sensitivity of these cell lines to Debio 1143 can be increased by combination therapy, an *in vitro* high-throughput combination screen (cHTS) was performed using the 6 human adenocarcinoma NSCLC cell lines shown in Fig. 4. Pairwise combinations of Debio 1143 with 128 oncology compounds were assessed in a cell viability assay.

The dose-response curves of single agents and combinations were analyzed for potential synergy or antagonism by quantification of the area under the curve (AUC) in order to quantify the extent of the curve shifts.
Several NSCLC SOC compounds (taxanes, vinorelbine, gemcitabine) were among the most synergistic combination partners for Debio 1143 among the 128 candidate combinations tested when looking at the average effect across all 6 cell lines (Fig. 3; only combinations with positive AUC score are shown).

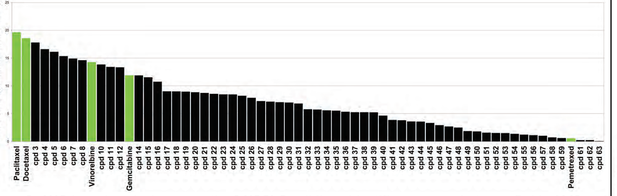
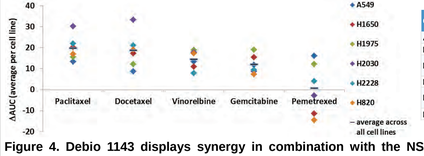


Figure 3. AUC-based analysis across the 6 NSCLC cell lines identifies synergy between Debio 1143 and several NSCLC SOC compounds. cpd: compound.

The synergy observed in the cHTS between Debio 1143 and NSCLC SOC compounds varies between the 6 adeno NSCLC cell lines. Interestingly, a pronounced combination effect is observed for both taxanes in the cell line H2030 (Fig. 4).



Cell line	Genotype
A549	KRAS mut, CDKN2A mut
H1975	EGFR mut (L858R, T790M mutations)
H2228	EML4-ALK fusion, TP53 mut, CDKN2A mut, RB1 mut
H2030	KRAS mut, TP53 mut
H1650	EGFR mut (E746_A750del), TP53 mut, CDKN2A mut
H820	EGFR mut (ΔE746-A749, T790M mutations)

Figure 4. Debio 1143 displays synergy in combination with the NSCLC SOC compounds paclitaxel, docetaxel, vinorelbine, gemcitabine and pemetrexed.

Debio 1143 displays anti-tumor activity as a monotherapy and synergizes with the lung cancer SOC compound docetaxel *in vivo*

To test whether Debio 1143 synergizes with NSCLC SOC compounds also *in vivo*, the combination of Debio 1143 and docetaxel was assessed in mouse xenografts of human A549 NSCLC adenocarcinoma cells. While both Debio 1143 and docetaxel displayed moderate anti-tumor activity as single agents, the combination caused marked anti-tumor activity that was superior to either monotherapy (Fig. 5). No significant effects on body weights were observed.
The increased Debio 1143 monotherapy efficacy observed *in vivo* may indicate involvement of host-mediated processes relevant for the Debio 1143 mechanism of action.

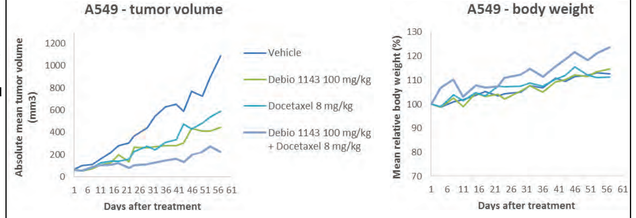


Figure 5. *In vivo* synergy between Debio 1143 and docetaxel on A549 human NSCLC xenografts in nude mice.

Methods

In vitro drug efficacy testing

PDX maintenance. PDX experiments were performed by Oncotest, Freiburg, Germany. Solid human tumor xenografts grown s.c. in serial passages in NMRI nu/nu mice were removed, disaggregated, filtered, counted and frozen in nitrogen.

Clonogenic PDX assays. A three-layer soft agar assay was used in a 24-well format: 10k cells were seeded and continuously exposed to test compounds for up to 20 days when colonies with a diameter of > 50 µm were counted. To assess synergy of drug combinations

cHTS. 3 Debio 1143 concentrations were tested against a 5-log concentration range of the candidate compounds in a cell viability assay at 72h performed in duplicates (CellTiter-GLO). Synergy was assessed using an AUC-based analysis in order to capture curve shifts, which represent increased potency and/or efficacy. The AUC score was calculated in R using the natural log of the candidate concentration range (to allow comparison of compounds despite different dosing). The ΔAUC was calculated as the difference between the AUC scores of the combination and the better of the two single agents and used as a synergy measure.

In vivo drug efficacy testing

Xenograft mouse models. Xenografts were performed in accordance with the guidelines for the care and use of laboratory animals. In brief, 5×10e6 cells were injected subcutaneously into the right flank of 4-6 week old female nude mice (BALB/c- nu/nu).

Treatment schedules: Debio 1143 was given p.o. daily 5 days a week for 3 weeks (d1-5; d8-12; d15-19); docetaxel (Taxotere; NorthCarolina Chemlabs) was injected i.v. once per week for 3 weeks (d1; d8; d15) using a dosing volume of 10 mL/kg (0.2 mL/mouse). All mice in the control group were oral gavaged with saline (0.2 mL /mouse) at the same time. Tumor volume and body weight was monitored every 3-4 days.

References

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Acknowledgements

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