Identification of synergistic drug combinations with the oral HSP90 inhibitor Debio 0932 in non-small cell lung cancer and renal cell cancer

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Summary

- **Debio 0932 is a second generation oral HSP90 inhibitor causing the degradation of potent oncogenic HSP90 client proteins.**
- **Synergy was identified *in vitro* between Debio 0932 and standard of care (SOC) agents in both non-small cell lung cancer (NSCLC) and renal cell carcinoma (RCC).**
- **Debio 0932 displays anti-tumor activity in monotherapy and synergizes with the lung cancer SOC paclitaxel in *in vivo* NSCLC models.**
- **Debio 0932 synergizes with renal cancer SOC everolimus in *in vivo* RCC xenografts.**
- **These preclinical data support the clinical development of Debio 0932 in combination with paclitaxel and everolimus in NSCLC and RCC, respectively.**

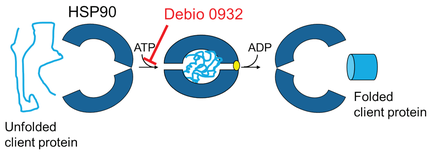
Background

Debio 0932 is an oral inhibitor of HSP90, a chaperone of many essential oncoproteins

Drug resistance is a major problem in cancer therapy which could be addressed by simultaneously targeting multiple critical nodes of the signaling networks controlling growth and survival of cancer cells (1, 2). One such approach is to target heat shock protein 90 (HSP90), a chaperone of many critical oncogenic drivers involved in proliferation, survival, invasion, metastasis and angiogenesis. Pharmacologic inhibition of HSP90 results in the proteasomal degradation of client oncoproteins, thereby eliminating their oncogenic activity (Fig.1).

The oral HSP90 inhibitor Debio 0932 displays favorable pharmacologic features and is in clinical evaluation for different cancer indications.

The goal of this study was to identify novel synergistic drug combinations for Debio 0932 in non-small cell lung cancer (NSCLC) and renal cell cancer (RCC) which would support the clinical development of Debio 0932 in those two indications.



HSP90 clients include:	
Akt	mTOR
Bcr-Abl	B-Raf
EGFR^{mut}	STAT3
HER2, 3	TGFβR
FGFR3, 4	VEGFR2
Flt3	Bcl-2
IGF1R	β-catenin
JAK1	HDAC6
MEK	c-IAP1

Figure 1. Mechanism of action of Debio 0932. Debio 0932 blocks ATP-dependent folding of HSP90 client proteins, which include many essential oncogenic drivers.

Results

Debio 0932 synergizes with NSCLC SOC agents and mTOR/Akt inhibitors to inhibit growth of human NSCLC cell lines *in vitro*

An *in vitro* high-throughput combination screen (cHTS) was performed using 6 human NSCLC cell lines bearing various genetic alterations commonly seen in NSCLC patients (Fig. 3). Pairwise combinations of Debio 0932 with 128 oncology compounds were assessed in a cell viability assay. In order to identify potential synergy the dose-response curves of single agents and combinations were analyzed by quantification of the area under the curve (AUC). Among several supra-additive combinations, Debio 0932 showed consistent synergy with microtubule and mTOR/Akt inhibitors across all 6 cell lines (Fig. 2; only combinations with positive AUC score are shown).

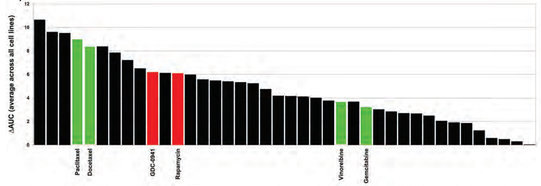


Figure 2. cHTS on 6 NSCLC cell lines identifies synergy between Debio 0932 and SOC agents (green) and mTOR/Akt inhibitors (red) as assessed by AUC-based analysis of dose-response curves. The higher the bar, the greater the synergy.

Cell line	Genotype
A549	KRAS mut, CDKN2A mut
H1975	EGFR mut (L858R, T790M resistance mutation)
H2228	EML4-ALK fusion gene, TP53 mut, CDKN2A mut, RB1 mut
H2030	KRAS mut, TP53 mut
H1650	EGFR mut (E746-A750del), TP53 mut, CDKN2A mut
H820	EGFR mut (E746-A749 del, T790M resistance mutation)

Figure 3. Genotype of the 6 human NSCLC cell lines used in the cHTS.

Improved efficacy of Debio 0932/paclitaxel combinations in human A549 and H1975 NSCLC xenografts

The beneficial effect of the combination of Debio 0932 and paclitaxel observed *in vitro* was also assessed *in vivo* in mouse xenografts of human A549 and H1975 NSCLC cells (4). In both models Debio 0932 displayed strong single agent anti-tumor activity which was further improved in combination with paclitaxel (Fig. 4).
No significant effects on body weight were observed (data not shown).

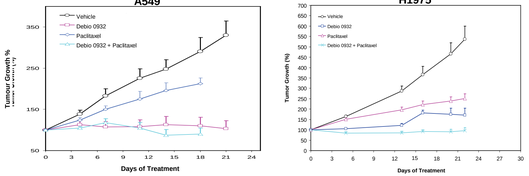


Figure 4. *In vivo* activity of Debio 0932 alone and in combination with paclitaxel in s.c. A549 and H1975 human NSCLC xenografts in nude mice. N=9 per group.

Debio 0932 in combination with everolimus inhibits growth of human RCC cell lines *in vitro*

In patient-derived and conventional RCC lines, Debio 0932 combined with the SOC agent everolimus displays synergy in cell viability assays as analyzed by the Combination Index method of Chou-Talalay (Fig. 5).

Sample	Genotype	CI Debio 0932 + everolimus
RXF 1183	BRAF N581S homozygous, HRAS Q61K homozygous	0.47
RXF 1781	VHL R210W heterozygous	0.57
RXF 486	VHL wildtype, TP53 wildtype	0.79
RXF 393	TP53 R175H homozygous	0.75
Caki-2	VHL wildtype	0.54
786-0	VHL G104fs*55 homozygous	0.8
A-498	VHL heterozygous, SETD2 mutant	1.17
SN12C	TP53 E336* homozygous	0.32

Figure 5. Combination Index (CI) of Debio 0932 and everolimus in RCC lines as assessed by the method of Chou-Talalay. Values <1 indicate synergy.

Debio 0932 in combination with everolimus inhibits growth of human RXF1183 RCC xenografts

The combination of Debio 0932 and everolimus was assessed in subcutaneous mouse xenografts of patient-derived RXF1183 RCC cells. While both Debio 0932 and everolimus displayed no anti-tumour activity as single agents in this model, the combination caused marked anti-tumour activity that was superior to either monotherapy (Fig. 6). None of the treatments had any significant effect on the body weight of animals.

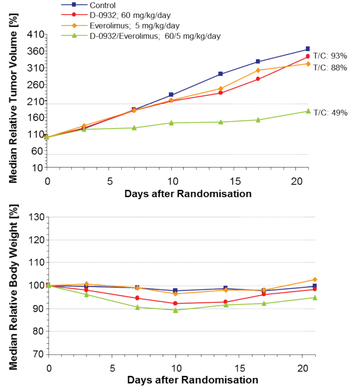


Figure 6. *In vivo* synergy between Debio 0932 and everolimus in RXF1183 human RCC s.c. xenografts in nude mice. Median relative tumor volume and median relative body weight changes upon daily oral treatment with Debio 0932 and everolimus are shown; n=10 per group.

Methods

In vitro drug efficacy testing

cHTS. Three concentrations of Debio 0932 were tested against a 5-log concentration range of 128 commercially available compounds in a 72-hr cell viability assay 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.

RCC PDX maintenance and cell viability assays. 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. Cells were plated in 96 well plates and incubated with compounds for 72h upon which cell viability was assessed by CellTiter-GLO. Combination Indexes were determined according to the Chou-Talalay method (3).

In vivo drug efficacy testing

Xenografts were performed in accordance with the guidelines for the care and use of laboratory animals.

Subcutaneous xenogratfs of NSCLC cell lines. 4×10^6 cells were injected into the right flank of CD-1 nude mice. Debio 0932 was given p.o. at 160 mg/kg every other day for 3 weeks; paclitaxel was injected i.p. at 12.5 mg/kg twice per week for 3 weeks.

Subcutaneous xenografts of patient-derived RCC tumors. Tumor fragments were obtained from RFX 1183 tumor xenografts in serial passage in NMRI nude mice. After removal from donor mice, tumors were cut into fragments (4-5 mm diameter) and placed in PBS until re-implantation. Anesthetized NMRI nude mice received unilateral, subcutaneous tumor implants in the flank. Debio 0932 was given p.o. at 60 mg/kg daily for 16 days; everolimus was given p.o. daily at 5 mg/kg/day for 16 days. Control groups were treated p.o. with vehicle (captisol) at the same time.

Tumor volume and body weight were monitored every 3-4 days.

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