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ORIGINAL ARTICLE

Synergistic cytotoxicity of a prostate cancer-specific immunotoxin in combination with the BH3 mimetic ABT-737

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Abstract

In many tumors, including prostate cancer, anti-apoptotic members of the Bcl-2 family are overexpressed and cause cell death resistance, which is a typical hallmark of cancer. Different therapeutic approaches, therefore, aim to restore the death mechanisms for enhanced apoptosis. Our recombinant immunotoxin D7(VL-VH)-PE40 is composed of the scFv D7(VL-VH) against the prostate-specific membrane antigen (PSMA) on the surface of prostate cancer cells and of the cytotoxic domain of the bacterial toxin *Pseudomonas* Exotoxin A (PE40). Since *Pseudomonas* Exotoxin A-based immunotoxins are known to preferentially inhibit the expression of the anti-apoptotic protein Mcl-1, the rationale was to test our immunotoxin in combination with the BH3 mimetic ABT-737, which specifically inhibits Bcl-2, Bcl-xl, and Bcl-w for enhanced induction of apoptosis in prostate cancer cells. The immunotoxin showed high and specific binding and cytotoxicity against PSMA expressing prostate cancer cells marked by a direct inhibition of Mcl-1. The combination of the immunotoxin with a subtoxic concentration of ABT-737 caused additive or even synergistic effects, which were based on an enhanced apoptosis induction as detected by poly(ADP-ribose) polymerase (PARP) and Caspase-3 cleavage in Western blot. Our study shows that the combination therapy of immunotoxin plus ABT-737 is a promising approach for the future treatment of advanced prostate cancer to improve therapeutic efficacy and to reduce adverse side effects.

Keywords Prostate cancer · PSMA · Immunotoxin · ABT-737 · Bcl-2 proteins · Apoptosis

Abbreviations

ABT-737	4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]-N-[4-[[(2R)-4-(dimethylamino)-1-phenylsulfanylbutan-2-yl]amino]-3-nitrophenyl] sulfonylbenzamide
Bak	Bcl-2 antagonist/killer
Bax	Bcl-2-associated X protein
BCA	Bicinchoninic acid
Bcl-2	B cell lymphoma 2
Bcl-w	Bcl-2 like 2
Bcl-xl	B cell lymphoma extra-large
BH3	Bcl-2 homology domain 3
CI	Combination Index
c-myc	Avian myelocytomatosis virus oncogene cellular homolog
ECL	Enhanced chemiluminescence
eEF-2	Eukaryotic elongation factor-2
Mcl-1	Myeloid cell leukemia sequence 1
PARP	Poly(ADP-ribose) polymerase
PE	*Pseudomonas aeruginosa* Exotoxin A
PSMA	Prostate-specific membrane antigen
WST-1	Water-soluble tetrazolium salt

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Introduction

With estimated 1.1 million new cases per year, prostate cancer remains the second-most frequently diagnosed cancer among men worldwide. Moreover, with expected 307,000 deaths, it represents the fifth leading cause of cancer deaths [1]. Whereas primary tumors can successfully be managed, there is no curative treatment for advanced stages. Therefore, new therapeutic options are urgently needed.

Tumor cells are characterized in that they hold cell death resistance, which is a typical hallmark of cancer [2]. Cell

death resistance can be based on impaired apoptosis signaling marked by a disturbed balance between pro- and anti-apoptotic members of the B cell lymphoma 2 (Bcl-2) protein family. In many tumors including prostate cancer, the main anti-apoptotic members Bcl-2, B cell lymphoma extra-large (Bcl-xl), and myeloid cell leukemia sequence 1 (Mcl-1) are overexpressed and promote tumor development [3, 4].

Different cancer therapeutic approaches aim to restore the death mechanisms for enhanced apoptosis and higher chemosensitivity [5]. Two main strategies to directly inhibit anti-apoptotic Bcl-2 family members comprise the use of antisense oligonucleotides and the application of BH3 (Bcl-2 homology domain 3) mimetics [6]. The latter are small molecules or modified peptides, which insert their BH3 motifs into the hydrophobic groove of the anti-apoptotic Bcl-2 proteins. This is followed by liberation of the pro-apoptotic members Bcl-2-associated X protein (Bax) and Bcl-2 antagonist/killer (Bak) and activation of the intrinsic apoptotic pathway [7]. The small-molecule BH3 mimetic ABT-737 (4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]-N-[4-[[(2R)-4-(dimethylamino)-1-phenylsulfanylbutan-2-yl]amino]-3-nitrophenyl] sulfonylbenzamide; Fig. 1a) was generated by structure-based design for binding to Bcl-xl [8]. Like the BH3-only protein Bcl-2 antagonist of cell death (BAD), ABT-737 selectively binds to Bcl-2, Bcl-xl, and Bcl-w with high affinity in the subnanomolar range, and is, therefore, also called a BAD-like BH3 mimetic (Fig. 1b) [8, 9]. Thus, in tumors with enhanced Mcl-1 expression, ABT-737 is less effective and combination with agents that degrade or neutralize Mcl-1 is preferred [10–13].

In our laboratory, the recombinant immunotoxin D7(VL-VH)-PE40 was generated for the targeted treatment of prostate cancer. The binding domain consists of the scFv (single chain variable fragment) D7(VL-VH), which is specific for the prostate-specific membrane antigen (PSMA), a transmembrane protein on the surface of prostate cancer cells [14, 15]. The 40 kDa truncated form of *Pseudomonas aeruginosa* Exotoxin A (PE), called PE40, was used as the toxin domain. It enables the immunotoxin to permeate cellular compartments to reach the cytosol and to inhibit ribosomal protein biosynthesis by ADP-ribosylation of the eukaryotic elongation factor 2 (eEF-2). This is followed by induction of apoptosis of the target cell [16]. Since PE-based immunotoxins are known to preferentially inhibit the Mcl-1 expression [17–20], the rationale of the present study was to test the immunotoxin D7(VL-VH)-PE40 in combination with ABT-737 for enhanced induction of apoptosis in prostate cancer cells.

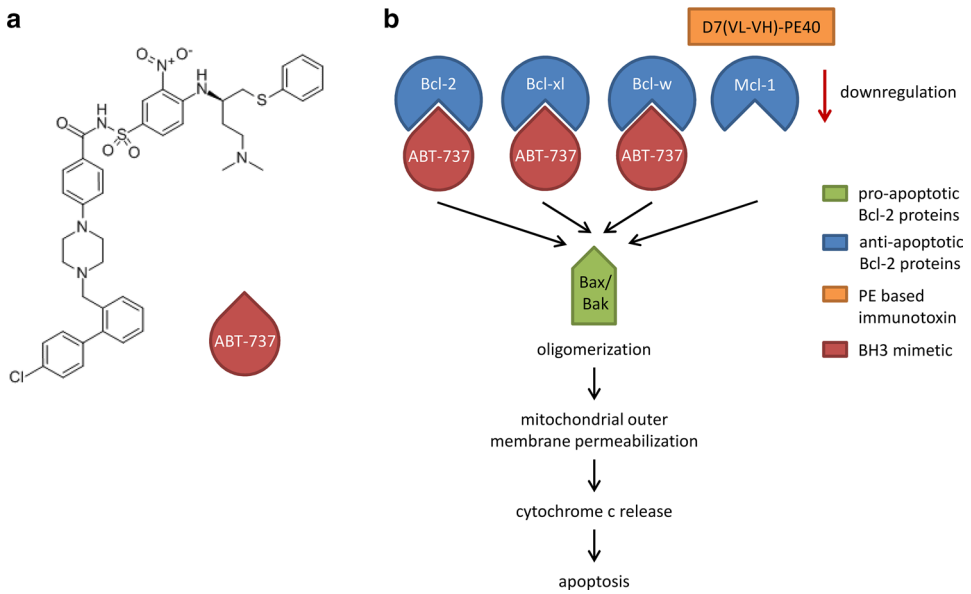


Fig. 1 Chemical structure and mode of action of the BH3 mimetic ABT-737 in combination with the immunotoxin D7(VL-VH)-PE40. **a** Chemical structure of the BH3 mimetic ABT-737 (4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]-N-[4-[[(2R)-4-(dimethylamino)-1-phenylsulfanylbutan-2-yl]amino]-3-nitrophenyl] sulfonylbenzamide). **b** Induction of apoptosis by combination of ABT-737 and the immunotoxin D7(VL-VH)-PE40. ABT-737 inhibits the anti-apoptotic proteins Bcl-2, Bcl-xl, and Bcl-w, but not Mcl-1, by binding. The immunotoxin downregulates Mcl-1 by inhibition of protein biosynthesis. This leads to the induction of the intrinsic apoptotic pathway via oligomerization of the released pro-apoptotic proteins Bax and Bak, mitochondrial outer membrane permeabilization and cytochrome c release

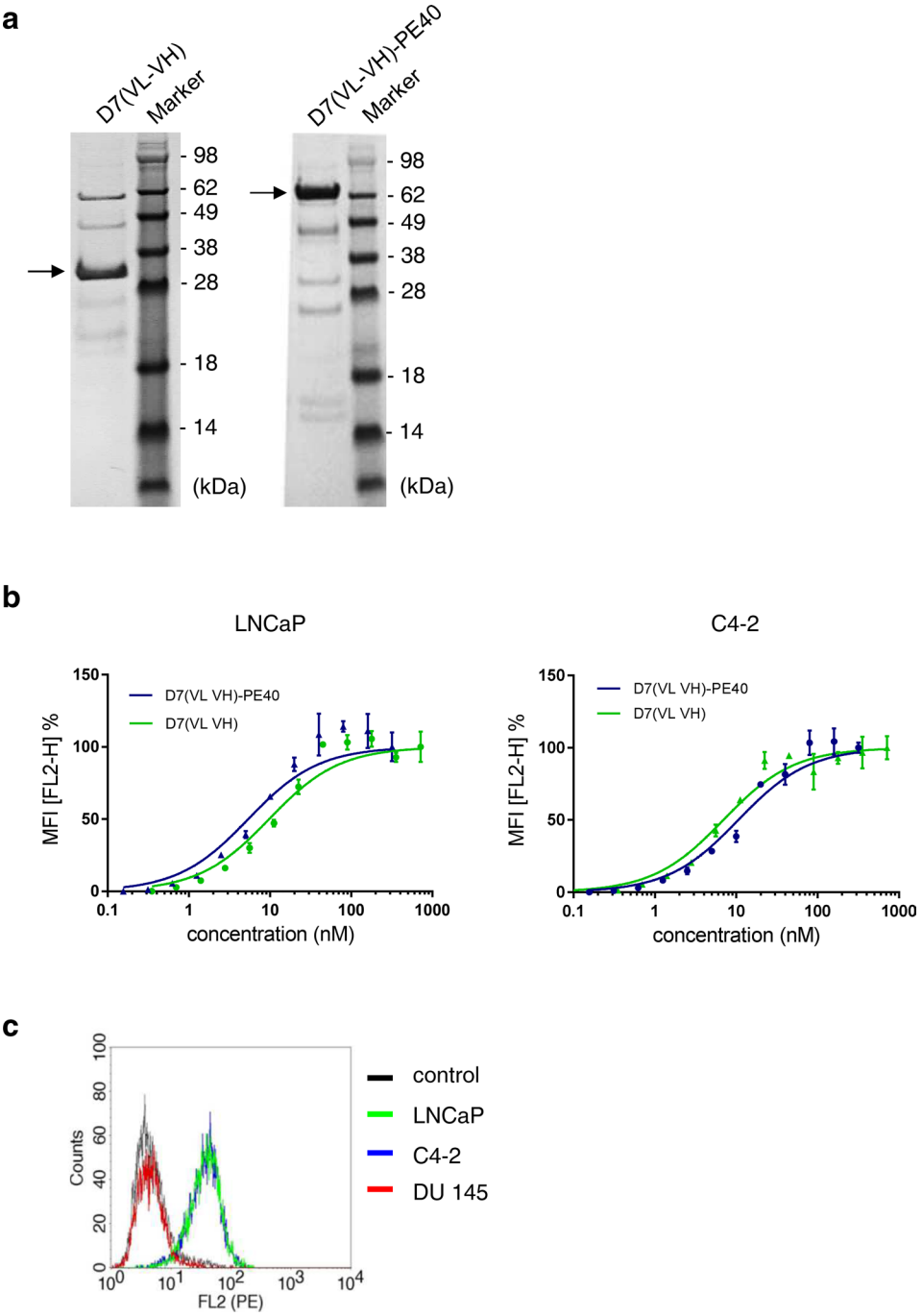
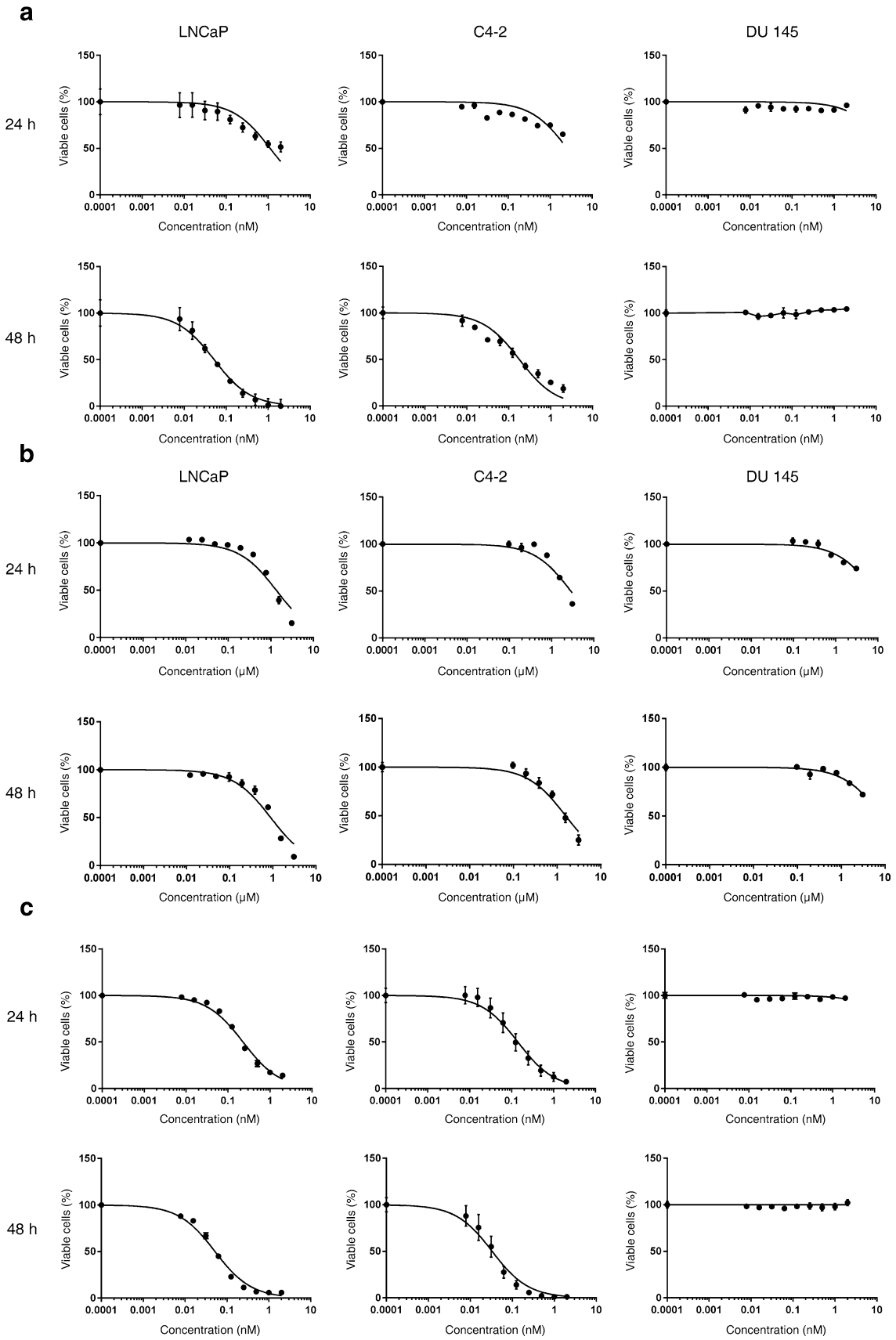


Fig. 2 Purity and cell binding characteristics of the anti-PSMA scFv D7(VL-VH) and the immunotoxin D7(VL-VH)-PE40. **a** SDS-PAGE of the scFv D7(VL-VH) and the immunotoxin D7(VL-VH)-PE40. **b** Binding of the scFv D7(VL-VH) and the immunotoxin D7(VL-VH)-PE40 to PSMA expressing LNCaP and C4-2 prostate cancer cells as measured by flow cytometry. **c** Compared to LNCaP and C4-2 cells, no binding of D7(VL-VH)-PE40 was seen on PSMA-negative DU 145 cells at saturation concentration. Control: without immunotoxin

Materials and methods

Cell lines, immunotoxin and reagents

The PSMA-positive prostate cancer cell line LNCaP, its androgen-independent subline C4-2, and the PSMA-negative cell line DU 145 were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). Cells were propagated in RPMI 1640 medium (Gibco, Invitrogen, Karlsruhe, Germany) containing 10% fetal calf serum (Biochrom, Berlin, Germany) and penicillin/streptomycin (100 U/ml, 100 mg/l) at 37 °C and 5% CO_2. Cell line identity was verified using short tandem repeat (STR) analysis (CLS GmbH, Eppelheim, Germany). The anti-PSMA immunotoxin D7(VL-VH)-PE40 was prepared as described earlier and stored at − 20 °C [14]. ABT-737 (Abcam, Cambridge, UK) was dissolved in DMSO at a stock solution of 20 mM and stored at 4 °C.


a
LNCaP
C4-2
DU 145
24 h
48 h
Viable cells (%)
Concentration (nM)
b
LNCaP
C4-2
DU 145
24 h
48 h
Viable cells (%)
Concentration (μM)
c
24 h
48 h
Viable cells (%)
Concentration (nM)

◂**Fig. 3** Cytotoxicity of the anti-PSMA immunotoxin D7(VL-VH)-PE40 in combination with the BH3 mimetic ABT-737. **a** Anti-PSMA immunotoxin D7(VL-VH)-PE40, **b** ABT-737, and **c** D7(VL-VH)-PE40 in combination with 100 nM ABT-737 on prostate cancer cells was measured by WST-1 viability assay. The graphics represent mean values ± SEM of three independent experiments

Flow cytometry

Flow cytometric analyses were performed to evaluate the cell binding of the immunotoxin as described previously [14]. In brief, prostate cancer cells were incubated with different concentrations of D7(VL-VH)-PE40 for 1 h on ice. After washing with ice-cold PBS, mouse anti-human c-myc (Avian myelocytomatosis virus oncogene cellular homolog) monoclonal antibody (Roche Diagnostics, Mannheim, Germany) and goat anti-mouse Ig-R-PE (Becton Dickinson, Mountain View, CA, USA) were each added for 40 min on ice. After resuspension in ice-cold PBS containing 3% FBS, 0.1% sodium azide and 2 µg/ml propidium iodide, MFI values of stained cells were determined with help of a FACSCalibur flow cytometer and the software CellQuest Pro (BD Biosciences, Heidelberg, Germany).

Cytotoxicity assay

The WST-1 (water-soluble tetrazolium salt) cell viability assay was used to examine cytotoxic effects (Roche Diagnostics, Indianapolis, IN, USA). For this, 1.5×10^4 cells/well were seeded in a 96-well plate and cultivated overnight. Then, the immunotoxin and ABT-737 were added either alone or in combination. After 24 and 48 h, WST-1 reagent was added and plates were incubated until the absorbance at 450 nm reached values of about 1.5–2.5 OD. IC_{50}-values were defined as drug concentrations leading to a reduction of 50% cell viability. They were calculated by non-linear regression [log (inhibitor) vs. response (three parameters)] (GraphPad Prism 6 Software, Inc., San Diego, CA). Combination Index (CI) was determined for quantitative definition of synergism (< 1.0), additivity (1.0–1.2) or antagonism (> 1.2) of the two active substances [21].

Cell killing assay

Dead cells were determined via trypan blue assay (Logos Biosystems, Germany). 1.5×10^5 cells were seeded in a 6-well plate, incubated overnight and treated with subtoxic concentrations of the immunotoxin alone (50 pM for LNCaP and 190 pM for C4-2 and DU 145 cells), ABT-737 alone or with the combination of both. After 24 or 48 h, the cells were trypsinized and stained with 0.4% trypan blue solution. The populations of dead and viable cells were counted by LUNA Automated Cell Counter (Logos Biosystems, Germany) and represented as percent of dead cells. Significance of the data was estimated with Student's *t* test (unpaired, parametric with Welch's correlation, GraphPad Prism 6 Software).

Western blot analysis

Prostate cancer cells were treated with subtoxic immunotoxin concentrations (50 pM for LNCaP and 190 pM for C4-2 and DU 145 cells) alone or in combination with a subtoxic concentration of 100 nM ABT 737. After 4, 24 and 48 h, cells were harvested and incubated in lysis buffer containing 30 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Triton-X, 10% glycerol, 1 mM 1,4-dithiothreitol, 200 µM phenylmethylsulfonylfluorid supplemented with cOmplete™, EDTA-free Protease Inhibitor Cocktail (Roche Diagnostics) for 20 min on ice. Afterwards, cells were centrifuged at 4000 rpm and supernatants were collected for Western blot analysis. Protein concentration of the whole cell lysates was measured by BCA (Bicinchoninic Acid) protein assay kit (Thermo Scientific, Rockford, IL, USA).

150 µg of cell lysate per lane were separated by SDS–PAGE and blotted. Blot membranes were saturated in 5% low-fat milk/PBS-Tween 20 (0.05% v/v) for 1 h at room temperature (RT) and incubated with antibodies against human Bcl-2, Bcl-xl, Mcl-1 (Santa Cruz, Heidelberg, Germany), mouse anti-human caspase 3 (ECM Biosciences, Köln, Germany), or anti-human poly(ADP-ribose) polymerase (PARP; Cell Signaling Tech., Danvers, MA, USA) in 5% low-fat milk at 4 °C overnight. After washing with PBS-Tween 20, membranes were incubated with the secondary antibodies goat anti-mouse IgG-HRP or goat anti-rabbit IgG-HRP (Santa Cruz) in 5% low-fat milk at 4 °C overnight. β-actin was detected using a HRP-labeled mouse anti-human β-actin monoclonal antibody (Cell Signaling Tech.). Blots were developed with an enhanced chemiluminescence (ECL) system and protein bands were detected with help of Agfa device Curix 60 (Düsseldorf, Germany).

Results

The purity of the 72 kDa anti-PSMA immunotoxin D7(VL-VH)-PE40 and the 32 kDa scFv D7(VL-VH) was proved by SDS-PAGE (Fig. 2a). To verify the specificity of the immunotoxin D7(VL-VH)-PE40, binding to PSMA-positive prostate cancer cells was tested by flow cytometry. The apparent binding affinity (K_d), defined as the immunotoxin's concentration leading to half-maximal-specific binding, was

found to be 5.5 nM on LNCaP and 10.8 nM on C4-2 cells. Comparable binding affinities were calculated with the antibody fragment scFv D7(VL-VH) (LNCaP: $K_d = 10$ nM and C4-2: $K_d = 6.1$ nM), indicating that there was no influence of the PE40 toxin domain on PSMA binding (Fig. 2b). The immunotoxin was found not to bind to PSMA-negative DU 145 cells (Fig. 2c).

Next, we determined the cytotoxicity of the immunotoxin and ABT-737 to find suitable concentrations for further combinatorial testing. With D7(VL-VH)-PE40, IC_{50} values of 1.402 and 0.052 nM were reached on LNCaP cells after 24 and 48 h, respectively. On C4-2 cells, IC_{50} values of 2.56 and 0.191 nM in the same timeframes were about two to threefold higher than on LNCaP cells. No cytotoxicity was observed on DU 145 control cells (Fig. 3a; Table 1). ABT-737 was shown to elicit a 50% reduction of viable LNCaP cells at concentrations of 1.348 and 899 nM after 24 and 48 h, respectively. On C4-2 cells, IC_{50} values of 2.868 and 1.596 nM were reached. On DU 145 cells, only a weak cytotoxic activity was observed and IC_{50} values were not reached within our tested concentration range (Fig. 3b; Table 1).

We tested different immunotoxin concentrations in combination with subtoxic concentrations of ABT-737. Compared to the immunotoxin alone, a 6.1-fold enhanced cytotoxicity after addition of 100 nM ABT-737 was determined already after 24 h on LNCaP cells ($IC_{50} = 0.230$ nM) (Fig. 3c; Table 1). Addition of 200 or 400 nM ABT-737 increased the cytotoxic effects of D7(VL-VH)-PE40 6.6-fold ($IC_{50} = 0.210$ nM) and 12.7-fold ($IC_{50} = 0.110$ nM). Calculation of the combination indices (CI) of 0.25, 0.32, and 0.40 evidenced that both agents acted with a strong synergism (Table 2). The combination of D7(VL-VH)-PE40 with 100, 200, or 400 nM ABT-737 led to additive effects of the two substances after 48 h on LNCaP cells (CI values between 0.97 and 1.2) (Table 2). The reason for a missing synergistic effect at that time was the strong cytotoxicity of the immunotoxin ($IC_{50} = 0.052$ nM), which predominated the effects of the BH3 mimetic.

Table 2 Combination indices (CI) calculated for different concentrations of ABT-737 in combination with the immunotoxin D7(VL-VH)-PE40 according to Bijnsdorp et al. [21]

	Combination index (CI)			
	LNCaP		C4-2	
	24 h	48 h	24 h	48 h
D7(VL-VH)-PE40 + 100 nM ABT-737	0.25	1.20	0.086	0.230
D7(VL-VH)-PE40 + 200 nM ABT-737	0.32	0.97	nd	nd
D7(VL-VH)-PE40 + 400 nM ABT-737	0.40	1.00	nd	nd

CI < 0.8 synergism; 0.8–1.2 additivity; > 1.2 antagonism

nd not determinable

A 20.3-fold increased cytotoxicity ($IC_{50} = 0.126$ nM) was observed on C4-2 cells by addition of 100 nM ABT-737 to the immunotoxin after 24 h. A CI of 0.086 indicated the synergism of both substances. After 48 h, an IC_{50} value of 0.029 nM was reached by combination, leading to a CI of 0.23 (Fig. 3c; Tables 1, 2). This showed that the synergistic effects of the immunotoxin and the BH3 mimetic continued until 48 h on the C4-2 line, representing the androgen-independent stage of prostate cancer and showing a higher resistance against the immunotoxin and ABT-737 than LNCaP cells.

Since no IC_{50} values were reached for both agents on DU 145 cells due to a missing or weak cytotoxicity, a CI calculation was not possible for this cell line (Fig. 3c; Table 1).

Since a reduction of cell viability can be rest upon inhibition of cell growth or cell killing, we examined the effects of D7(VL-VH)-PE40 in combination with ABT-737 in view of cell death via trypan blue assay. Addition of ABT-737 to subtoxic concentrations of immunotoxin D7(VL-VH)-PE40 significantly enhanced the percentage of dead LNCaP and C4-2 cells, whereas DU 145 control cells remained unaffected (Fig. 4). This proved that the reduction of cell viability by immunotoxin/ABT-737 combination therapy was based on the induction of cell death.

Table 1 IC_{50} values of D7(VL-VH)-PE40 and ABT-737 alone and in combination on different prostate cancer cells

	IC50 (nM)					
	LNCaP		C4-2		DU145	
	24 h	48 h	24 h	48 h	24 h	48 h
D7(VL-VH)-PE40	1.402	0.052	2.560	0.191	> 2.0	> 2.0
ABT-737	1.348	899	2.868	1.596	> 3.100	> 3.100
D7(VL-VH)-PE40 + 100 nM ABT-737	0.230	0.051	0.126	0.029	> 2.0	> 2.0
D7(VL-VH)-PE40 + 200 nM ABT-737	0.210	0.032	nd	nd	nd	nd
D7(VL-VH)-PE40 + 400 nM ABT-737	0.110	0.020	nd	nd	nd	nd

Mean values of 4–7 independent experiments in each case

nd not determinable

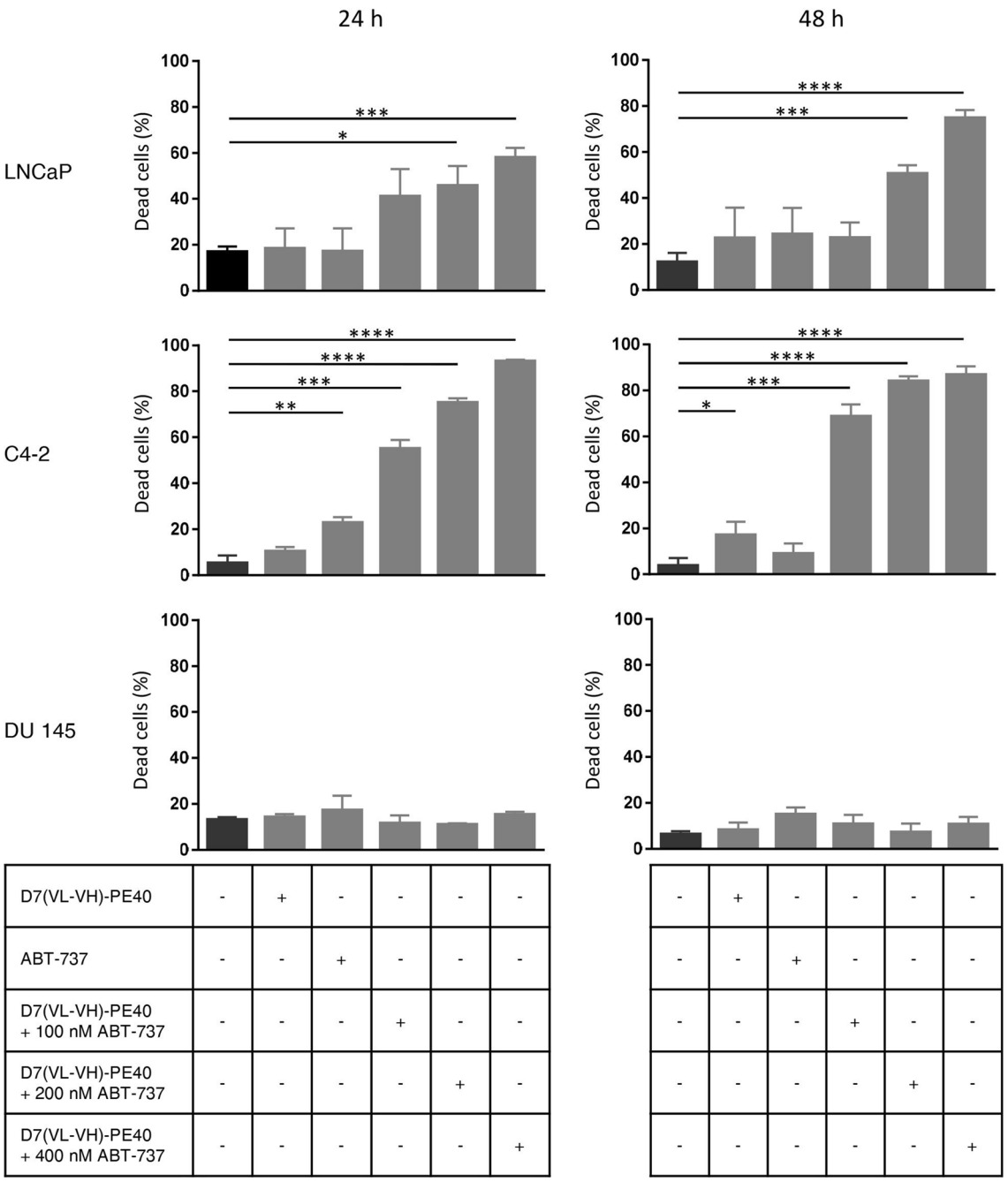


Fig. 4 Killing of prostate cancer cells by the anti-PSMA immunotoxin D7(VL-VH)-PE40 in combination with the BH3 mimetic ABT-737. The cytotoxicity of the immunotoxin alone and in combination with 100, 200 and 400 nM ABT-737 after 24 and 48 h was determined by trypan blue assay (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$)

Next, Western blot analyses were performed to explore the molecular basics for cell death. As shown in Supplementary Fig. 1, the immunotoxin led to a reduced expression of the anti-apoptotic protein Mcl-1, whereas the levels of Bcl-2 and Bcl-xl remained unaffected. As a result, subtoxic concentrations of the immunotoxin alone only slightly elicited apoptosis in LNCaP and C4-2 cells characterized by PARP cleavage and Caspase-3 activation after 48 h. In contrast, the addition of 100 nM ABT-737 led to enhanced PARP and Caspase-3 cleavage already after 24 h. Both agents did not induce apoptosis either alone or in combination in DU 145 control cells (Fig. 5).

Taken together, our results show that the combination of the anti-PSMA immunotoxin D7(VL-VH)-PE40 with

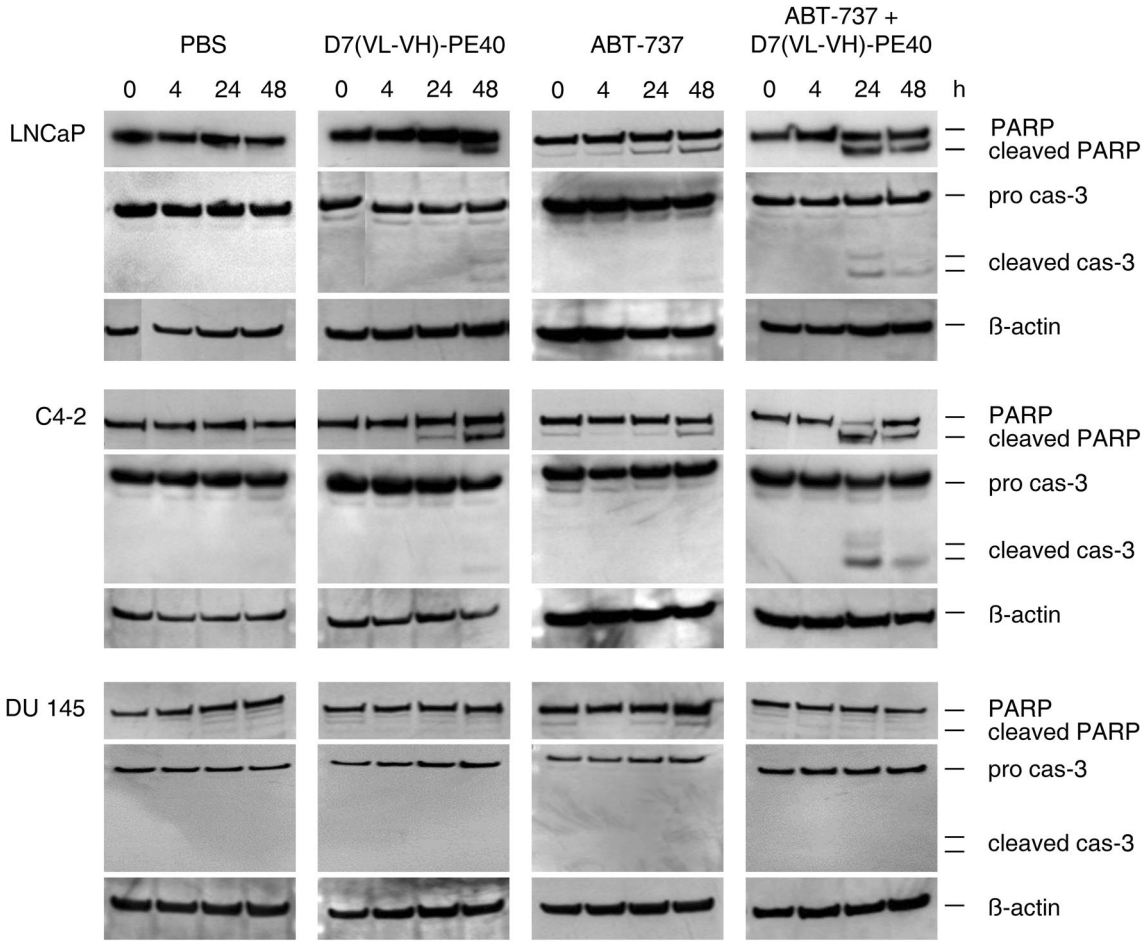


Fig. 5 Induction of apoptosis by the anti-PSMA immunotoxin D7(VL-VH)-PE40 in combination with the BH3 mimetic ABT-737. Prostate cancer cells were treated with subtoxic concentrations of D7(VL-VH)-PE40 (50 pM for LNCaP cells and 190 pM for C4-2 and DU 145 cells) and 100 nM ABT-737 alone or in combination. Cleavage of poly(ADP-ribose) polymerase (PARP) and Caspase-3 (cas-3) was detected in cell lysates by Western blot. β-actin was used as loading control

ABT-737 led to a fast, synergistic cytotoxicity on PSMA expressing prostate cancer cells based on an enhanced induction of apoptosis.

Discussion

Cell death resistance in cancer cells is mainly based on the increased expression of anti-apoptotic members (Bcl-2, Bcl-xl, and Mcl-1) or on the decreased expression of pro-apoptotic members (Bax, Bak) of the Bcl-2 protein family. In an immunohistochemical study, Bcl-2 was shown to be present in 25% of cases with higher appearance in high-grade tumors (Gleason grade 8–10, 41%) and lymph node metastases (38%) than in lower-grade tumors (Gleason grade 2–7; 16%; $p < 0.05$) [4]. This is in accordance with a study of Anvari et al. in which Bcl-2 was present in 70.3% of locally advanced or metastatic tissues in association with higher Gleason scores and lower biochemical-free survival [22]. Bcl-xl and Mcl-1 were shown to be present in 100 and 86% of prostate tumor samples with more intensive staining of high grade tumors or metastases than of prostatic intraepithelial neoplasia (PIN) or low-grade tumors [4]. Investigations in prostate cancer cells showed evidence that Bcl-xl, in contrast to Bcl-2, plays a major cytoprotective role [23], which is comparable to the situation in other solid tumors [7, 24]. Mcl-1 was also shown to protect prostate cancer cells from chemotherapy and targeting of Mcl-1 was found to be promising in the treatment of the castration-resistant stage [25]. Interestingly, a decreased expression of pro-apoptotic proteins was not found in prostate cancer. Instead, Bax was shown to be expressed in most prostate cancers evaluated, with high percentages of immunopositive cells and strong immunointensity independently of tumor grade [4, 26]. Moreover, mutations of the Bak and Bax genes occur very infrequently in this tumor [26].

Taken together, inhibition of Bcl-xl and Mcl-1 should be a preferred strategy for the treatment of prostate cancer. Since Bax and Bak are usually present in a functional manner, the precondition is given that homo-oligomerization of these proteins and formation of mitochondrial pores can occur, which in turn results in mitochondrial outer membrane permeabilization, the point of no return in the commitment to apoptosis.

In our study, ABT-737 was found to induce death in prostate cancer cells with IC_{50} values in the low μM range in LNCaP and C4-2 cells, which is in line with earlier observations [27–30]. DU 145 cells were shown to be more resistant, which can be explained by the lack of the pro-apoptotic Bax protein in this cell line [28]. Since ABT-737 does not inhibit Mcl-1, the BH3 mimetic or its oral bioavailable compound of the same class, ABT-263, were combined with chemotherapeutics, 2-deoxyglucose, Pim kinase inhibitors or proteasomal inhibitors against prostate cancer cells and enhanced cytotoxic effects were noted [27–31]. However, these agents are not tumor cell specific. Therefore, to our knowledge, we examined for the first time a targeted molecule in combination with ABT-737 on prostate cancer cells.

Our anti-PSMA immunotoxin D7-(VL-VH)-PE40 showed an about 1000- to 18,000-fold higher cytotoxicity than ABT-737. An explanation for this big difference is that one immunotoxin molecule is able to catalyze the ADP-ribosylation of many eEF-2 molecules on the ribosomes, which in turn results in a fast inhibition of protein biosynthesis, especially of proteins with a short half-life. In contrast, one molecule ABT-737 can only inhibit one molecule of an anti-apoptotic protein.

We could demonstrate that subtoxic concentrations of the immunotoxin in combination with a subtoxic concentration of ABT-737 caused fast, additive or synergistic cytotoxic effects in PSMA-expressing prostate cancer cells. These effects reflect the complementary beneficial characteristics of the two agents. On the one side, the immunotoxin acts target-specific on PSMA, ensuring that the combination therapy is preferably restricted to prostate cancer cells, and markedly reduces the levels of the short-lived Mcl-1 protein by inhibition of protein biosynthesis. On the other side, ABT-737 is very effective in the specific inhibition of the Bcl-2, Bcl-xl and Bcl-w proteins. Since the therapeutic resistance of prostate cancer cells is mainly based on Mcl-1 and Bcl-xl overexpression [23, 25], both agents seem to be suitable for the future treatment of advanced prostate cancer. Moreover, the combination of both substances at subtoxic doses could lead to a reduction of side effects and to an enhanced therapeutic efficiency compared to monotherapy.

An improved cytotoxicity of ABT-737/ABT-263 in combination with PE-based immunotoxins against different target antigens was also demonstrated in cervical, melanoma, small cell lung and pancreatic cancer cells [17–20]. Moreover, combination therapy resulted in an enhanced anti-tumor activity in mice-bearing tumor xenografts [19, 20]. Future experiments will show if our combination of ABT-737 with D7(VL-VH)-PE40 will also be successful in animal models.

Conclusions

In the present study, we could show that the BH3 mimetic ABT-737, which has only limited antitumor activity as single agent, is able to lower the threshold for the induction of apoptosis by the anti-PSMA immunotoxin D7(VL-VH)-PE40 (Fig. 1b). This makes the combination therapy a promising approach for the future treatment of advanced prostate with improvement of efficacy and reduction of adverse side effects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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