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The BH3-Mimetic ABT-737 Induces Mast Cell Apoptosis In Vitro and In Vivo: Potential for Therapeutics

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Mast cells and their mediators are implicated in the pathogenesis of many different diseases. One possible therapeutic intervention in mast cell-associated diseases can be to reduce the number of tissue mast cells by inducing mast cell apoptosis. In this study, we demonstrate that mast cells exhibit a high sensitivity to ABT-737, a BH3-only mimic molecule that induces apoptosis through high- affinity binding to the prosurvival proteins, Bcl-2, Bcl-XL, and Bcl-w. Primary mast cells as well as mast cell lines tested succumbed to apoptosis in response to the inhibitor at varying but seemingly low concentrations compared with other leukocytes investigated. L. p. injections of ABT-737 in mice resulted in a total abolishment of mast cells in the peritoneum. Confocal microscopy analysis of peritoneal cells revealed apoptotic bodies of mast cells being phagocytosed by macrophages. In addition, ex vivo treatment of human skin biopsies with ABT-737 demonstrated increased mast cell apoptosis. The data we present in this article show exceptional mast cell sensitivity to ABT-737, a selective inhibitor of antiapoptotic proteins, rendering a possible application for BH3-only mimetic compounds like ABT-737 in mast cell-associated diseases, such as mastocytosis, allergy, asthma, and other chronic inflammations. The Journal of Immunology, 2010, 185: 000–000.
cancer cell lines as well as in vivo xenografts of small-cell lung carcinoma (25), acute myeloid leukemia (27), and lymphoma (28).

In this study, we demonstrate mast cell apoptosis in response to ABT-737 both in vitro, ex vivo, and in vivo. Primary mast cells as well as mast cell lines tested succumbed to apoptosis in response to the inhibitor at varying but seemingly low concentrations compared with other cell types investigated. I.p. injections of ABT-737 into mice eliminated peritoneal mast cells and further evaluation of peritoneal-derived mast cells, macrophages, neutrophils, and B lymphocytes ex vivo revealed extensively higher mast cell sensitivity to ABT-737 than any other hematopoietic cells analyzed. Finally, ex vivo experiments performed on human skin biopsies demonstrated mast cell apoptosis on ABT-737 treatment. This study provides the first evidence that mast cells exhibit high sensitivity to BH3-mimetics and suggests that this could be a plausible way for treatment of mast cell-associated diseases.

Materials and Methods

Animals and cell cultures

Male BALB/c mice, 6–8 wk of age for in vivo and 10 wk of age for ex vivo experiments were used. For confocal microscopy we used male C57BL/6 KitWsh/KiWsh mice reconstituted i.p. with GFP-expressing marrow-derived mast cells (BMMCs, 5 × 10⁶) (derived from C57BL6-γ–actin–EGFP transgenic mice, The Jackson Laboratories, Bar Harbor, ME). Animal protocols were approved by the Local Ethical Committee for Animal Experimentation as stated by the Swedish Legislation. Mouse bone marrow-derived mast cells were obtained as previously described (29) in mice containing 15% WEHI-3 enriched conditioned RPMI 1640 (containing IL-3). The generation of bak−/−bax−/− double-deficient mice have been published previously (30). Fetal liver-derived mast cells from wild-type (wt) or bak−/−bax−/− C57BL/6 mice were differentiated into connective tissue-like mast cells (CTLMC) or mucosal-like mast cells (MLMCs) using previously described protocols (31). Murine mast cell lines C57 and MC9 (American Type Culture Collection, Manassas, VA) were cultured as described elsewhere (29). Human cord blood-derived mast cells (CBMCs) were differentiated from umbilical cord blood CD34+ progenitor cells for 4 wk in STEM-PRO-34 SFM medium (Life Technologies, Rockville, MD) supplemented with STEM-PRO-34 supplement (Life Technologies), 2 mM l-glutamine, 100 IU/ml penicillin, and 50 μg/ml streptomycin, 100 ng/ml SCF, 10 ng/ml IL-6 (both PeproTech, Rocky Hill, NJ). A total of 1 ng/ml PRO-34 supplements, 2 mM-glutamine, 100 IU/ml penicillin, and 50 μg/ml streptomycin, 100 ng/ml SCF. All mouse and human cells were cultured in 37°C in a humidified atmosphere with 5% CO₂.

ABT-737 treatment and mast cell survival

Mast cells (7 × 10⁵ cells/ml) were cultured in medium for respective cell type. ABT-737 or a less active enantiomer (Abbott Laboratories, Abbot Park, IL) was added to cells at concentrations ranging from 0.01 μM to 50 μM as indicated in the respective figure. Cell viability was analyzed by flow cytometric analysis excluding cells stained with 5 μg/ml propidium iodide (BD Pharmingen, San Diego, CA) and 0.3 μg/ml FITC-conjugated annexin V (BD Pharmingen) using a FACSCalibur (Becton Dickinson) after 48 h of ABT-737 treatment for dose response experiment. For kinetic studies, cell viability after treatment with ABT-737 (0.4, 1, or 10 μM) was analyzed at time points indicated.

Confocal microscopy

Male C57BL/6 KitWsh/KiWsh mice were reconstituted i.p. with GFP-expressing BMMCs (5 × 10⁶). After 24 h, ABT-737 (40 mg/kg) was injected i.p. Mice were killed after another 4 h and peritoneal lavage was taken. Macrophages were visualized by biotin-linked CD11b Ab (BD Pharmingen), followed by streptavidin-linked Qdot 605 (Invitrogen, Carlsbad, CA). Cells were fixed in 2% paraformaldehyde before microscopic analysis.

Western blot analysis

Lysates were prepared from 2–3 × 10⁶ cells of peritoneal lavage-derived mast cells (PLMCs), C57, MC9, BMMC, LAD2, and CBMC. For analysis of caspase-3 cleavage, cell lysates were prepared after 8 h of incubation with ABT-737 (0.4 μM), zVAD-fmk (Chemicon International, Temecula, CA) was added 10 min before ABT-737. Cells were lysed in 2× SDS gel sample buffer (20 mM DTT, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromphenol blue), sonicated (2 × 7 s), and heated for 5 min at 95°C. A total of 10 μl protein was resolved on SDS-10% NuPAGE polyacrylamide gels (NOVEX, Carlsbad, CA). Proteins were electroblotted onto nitrocellulose membranes (Hybond ECL, Amersham Biosciences, Uppsala, Sweden). Proteins of interest were detected using HRP-conjugated Abs against Actin (1:1200; Erk1/2 1:4000 (Santa Cruz Biotechnologies, Santa Cruz, CA); rabbit anti-mouse Mcl-1 1:10 000 (Rockland, Gilbertsville, PA); rabbit anti-human Mcl-1 1:2 000 (StressGen Biotechnologies, Victoria, British Columbia, Canada); rabbit caspase-3 1:1000; rabbit Bak-2 1:1000 (Cell Signaling, Beverly, MA), followed by HRP-linked anti-rabbit IgG at a 1:2000 dilution (Cell Signaling). Western blots were visualized by enhanced chemiluminescence (LumiGLO, Cell Signaling) and exposed to Hybond ECL film (Amersham Biosciences).

FIGURE 1. Murine mast cells are sensitive to ABT-737. A, Dose response of murine mast cell lines C57 (filled triangle), MC9 (filled square), and BMMCs (open square) after the 48 h treatment with ABT-737. B, Time course study of 1 μM ABT-737 on mast cell lines C57 (filled triangle), MC9 (filled square), and BMMCs (open square). Relative viability was measured using PI and annexin V staining analyzed by flow cytometry, normalized against viability of untreated cells and presented as means (± SEM) of three to five independent experiments. C, PLMCs were cultured 30 d in SCF conditioned medium. Thereafter, the cells were treated with ABT-737 at concentrations from 0–10 μM (left panel). Cell viability was measured by flow cytometry and PI and annexin V exclusion after a 48 h treatment with ABT-737. For the kinetic study (right panel), PLMCs were treated with 0.4 μM ABT-737. Data are presented as mean (± SD) from three independent experiments. Student t test. *p < 0.05; ***p < 0.01; ****p < 0.001. PI, propidium iodide.
**In vivo administration of ABT-737**

Mice were injected i.p. at day 1, 3, and 5 with ABT-737 (0, 40 or 75 mg/kg). A stock solution of ABT-737 (0.5 g/ml in DMSO) was diluted in a mixture of 30% propylene glycol (Sigma-Aldrich, St. Louis, MO), 5% Tween 80, 65% D5W (5% dextrose in water), pH 4. The final concentration of DMSO was <2%. At day 8 (day 22 for Fig. 4D), mice were sacrificed and peritoneal lavage collected. The cell content was analyzed in a FACS Calibur using fluorescence-labeled anti-mouse CD11b, anti-mouse Ly6G, anti-mouse CD117, anti-mouse B220, and anti-mouse Trk receptor (BD Pharmingen).

**Ex vivo assay**

Peritoneal cells from male BALB/c mice were used. To increase the number of PLMCs, peritoneal lavage cells were cultured for 30 d in OptiMEM (Life Technologies) media supplemented with 10% FBS, 2 mM l-glutamine, 100 IU/ml penicillin, 50 μg/ml streptomycin, and 4% SCF-conditioned medium (supernatant from CHO-K1 cells, kindly provided by Odile Malbec, Institute Pasteur, Paris, France) (33). The purity of mast cells was determined to be >95% by toluidine blue staining. Macrophages were obtained from peritoneal lavage from untreated mice. Influx of granulocytes was induced by one i.p. injection of vehicle buffer at low pH (pH 3) and analyzed after 24 h. The final concentration of DMSO of granulocytes was induced by one i.p. injection of vehicle buffer at low pH (pH 3) and analyzed after 24 h. Final concentration of DMSO.

Skin organ culture

After ethical reviewing at Kuopio University Hospital and obtaining a consent from patients a sample of the left-over skin tissue was collected anonymously according to Finnish tissue law. Skin specimens were obtained in connection to mastectomies for breast cancer or of breast reduction surgery from a total of four female donors after obtained consent. After removal, the tissue specimen was released from the s.c. fat with a knife, several adjacent 4-mm punch biopsies were taken, and residual blood was washed away with PBS. The procedure for cultivation of skin specimens has been described previously (34). Briefly, the skin specimens from four subjects were cultured at air-liquid interface in the medium (10% FCS and DMEM) in the presence of 0, 1, 10, or 25 μM ABT-737. After 3 d in culture skin, specimens were collected, frozen, and processed for histochemical cryosections.

**Detection of mast cells in skin sections**

To identify apoptosis in mast cells the immunohistochemical staining of chymase and TACS/TdT in situ apoptosis detection kit was used in sequential order according to the procedures of the manufacturer (R&D Systems, Minneapolis, MN). The mast cell was judged to be apoptotic when the red color for cytoplasmic chymase and bluish color for nuclear apoptosis were seen in close contact and the morphologic criteria for apoptosis were fulfilled (34). The mast cells were counted in a total area of 1 mm² just beneath the epidermis and the apoptosis index (%) was defined as the number of apoptotic mast cells in relation to all chymase-positive cells. The enzyme-histochemical staining method for identifying the activity of chymase and the immunohistochemical staining method for identifying chymase protein have both been described previously (34). The mast cells were counted in a total area of 1 mm² just beneath the epidermis and the chymase activity/protein ratio (%) was defined as the number of chymase activity containing cells in relation to all chymase-immunopositive cells.

**Results**

**ABT-737 treatment eliminates mast cells in vitro**

The compound ABT-737 mimics the binding properties of the proapoptotic BH3-only protein Bad with high affinity to the prosurvival proteins, Bcl-2, Bcl-XL, and Bcl-w, but not to Mcl-1 and Bfl-1/A1 (16, 25). Depending on their expression of prosurvival proteins,
different cell types may vary in their sensitivity to ABT-737. Mouse embryonic fibroblasts are relatively insensitive (16), whereas platelets die through apoptosis already at low concentrations of ABT-737 (25, 35). As the effect of ABT-737 on mast cells has not previously been investigated, we analyzed primary murine mast cells as well as mast cell lines. All mast cell sources analyzed showed reduction in cell viability at 48 h of ABT-737 treatment in a concentration dependent manner with concentrations ranging from 0.4 μM to 50 μM (Fig. 1). The murine mast cell line C57 was highly sensitive to ABT-737 with <30% cell viability at 0.4 μM ABT-737, compared with the MC9 cell line and primary BM MCs with cell viability of ~60% at 0.4 μM ABT-737 (Fig. 1A). Kinetic studies demonstrate a decrease of cell viability within the time period of 72 h for all mast cells analyzed (Fig. 1B). The concentration of DMSO in the dilution buffer was <1% and did not affect mast cell viability significantly (± 0.8%).

To further evaluate the sensitivity of different mast cell populations to ABT-737, we next treated PLMCs ex vivo. PLMCs were more sensitive than any other mast cell type tested. At concentrations of 10 nM ABT-737 the cell viability was reduced by 40%. Less than 10% cell viability was detected 48 h after treatment with 0.4 μM ABT-737 and at 72 h all PLMCs were dead (Fig. 1C).

**ABT-737 treatment induces apoptosis in mast cells**

The two effector proteins, Bak and Bax, are essential for cells to undergo mitochondria-dependent apoptotic cell death, because double deficiency render resistance to all intrinsic cell death pathway stimuli tested (36, 37). The nonspecific toxicity of ABT-737 is very low compared with other inhibitors of apoptotic cell death, because it only induces apoptosis through Bak and Bax upstream of the mitochondria (16). To verify that the decrease in mast cell viability in response to ABT-737 treatment was due to apoptosis, murine CTLMCs and ML MCs deficient for both Bak and Bax were totally resistant to ABT-737 treatment at concentrations that killed wt mast cells (Fig. 2A). Activation of caspases after ABT-737 treatment was also observed in murine PLMCs and the broad spectrum caspase inhibitor zVAD-fmk was shown to reduce the levels of cleaved caspase-3 after treatment (Fig. 2B).

Moreover, treating the murine mast cell line C57 with the less active enantiomer of ABT-737 induced a minor decrease in viability supporting the specific apoptotic effect of ABT-737 (Fig. 2C), as previously described (25). To analyze if apoptotic mast cells were engulfed by macrophages in vivo we reconstituted mast cell-deficient C57BL/6 Kit^Wsh/Kit^Wsb mice i.p. with GFP-expressing BM MCs. ABT-737 was thereafter injected i.p. and after 4 h we detected green fluorescent apoptotic bodies of mast cells inside phagocytizing macrophages in the peritoneal lavage using confocal microscopy (Fig. 2D), suggesting that apoptotic mast cells are removed by phagocytes.

**Mast cell expression of Bcl-2 family proteins dictates the sensitivity to ABT-737**

The inhibitor ABT-737 has shown single-agent activity to several cancer cell lines (16, 27, 38) although it has proven less effective to induce apoptosis in cancer cells expressing high levels of Mcl-1 (16, 39, 40). Moreover, cell sensitivity to ABT-737 has also been shown to be dependent on expression levels of Bcl-2. More sensitive ALL and small-cell lung carcinoma cell lines express higher levels of Bcl-2 (38, 39). We observed differences in IC_{50} and sensitivity to ABT-737 comparing different mast cell types (i.e., PLMCs, C57, BM MCs, and MC9s) and subsequently did a densiometric analysis of the protein levels of Mcl-1 and Bcl-2 by Western blot (Fig. 3). We hypothesized that mast cells are less sensitive to ABT-737 treatment, thereby having higher IC_{50}, would express Mcl-1 abundantly albeit small amounts of Bcl-2. Our results show that the expression of the prosurvival protein Mcl-1 correlates with increasing IC_{50} and resistance to ABT-737, and we also observed an inverse correlation between sensitivity to ABT-737 and expression of Bcl-2. Accordingly, the mast cell line MC9, expressing the highest protein level of Mcl-1 and the lowest level of Bcl-2, was also found to be the most resistant to ABT-737 treatment with a calculated IC_{50} of 25 μM, whereas the PLMCs with high Bcl-2 level and low Mcl-1 level had a calculated IC_{50} of <0.1 μM.

**ABT-737 eliminates mast cells in vivo**

Our in vitro data demonstrate that mast cells are sensitive to ABT-737 and die through apoptosis. We also saw differences in sensitivity among various mast cell types where PLMCs were the most sensitive cells analyzed. Next, we analyzed the effects of ABT-737 in vivo on mast cells and other cell populations in the peritoneum of mice after ABT-737 injections. BALB/c mice were injected i.p. at day 1, 3, and 5 with ABT-737. At day 8, peritoneal lavage was prepared and cell populations of mast cells, lymphocytes, macrophages, and neutrophils in the peritoneum were analyzed by flow cytometry. A major reduction in mast cell numbers was detected at 40 mg/kg ABT-737 (Fig. 4A). The lack of mast cells in the peritoneum was confirmed by toluidine blue staining of peritoneal lavage cytospins (data not shown). T lymphocytes seemed unaffected by ABT-737 injections (Fig. 4B), whereas a decrease in B lymphocytes (Fig. 4C) was observed that also has been reported by others (25). The induction of apoptosis of peritoneal cells, by injections of ABT-737 at pH4, caused an influx of macrophages and neutrophils into the peritoneum (Fig. 4D, 4E). At 75 mg/kg ABT-737, the influx of neutrophils increased, as well as the apoptotic effect on B lymphocytes. Taken into account, the influx of granulocytes and macrophages in peritoneum after ABT-737 treatment and the decrease of, for example,
lymphocytes, the total cell number in peritoneal lavage was still left largely unaffected and a major reduction of the total numbers of mast cells was observed (Fig. 4).

Effect of ABT-737 on human mast cells in vitro and ex vivo

Our results so far suggested that murine mast cells are sensitive to ABT-737-induced apoptosis. Because there are many similarities, but also differences, between human and murine mast cells, we next turned our focus to human mast cells. Just like murine mast cells, the human mast cells were sensitive to treatment of ABT-737 in vitro. At lower doses of ABT-737 (0.4–1 \( \mu \)M), human CBMCs as well as the human mast cell line LAD2 showed a cell viability of 70% and 50%, respectively, after 48 h treatment (Fig. 5A). Also, the kinetic studies at 10 \( \mu \)M ABT-737 demonstrate a constant decrease of cell viability over a time period of 72 h (Fig. 5B). Because we also in this study observed differences in IC50 and sensitivity to ABT-737 comparing LAD2 and CBMCs, we subsequently investigated the protein levels of Mcl-1 and Bcl-2 by Western blot (Fig. 5C). CBMCs, expressing moderate levels of both Mcl-1 and Bcl-2 (with a Mcl-1/Bcl-2 ratio of 1.4), was also found to be the most resistant to ABT-737 treatment with a calculated IC50 of 10 \( \mu \)M, whereas LAD2 cells with high Bcl-2 level and low Mcl-1 level (with a Mcl-1/Bcl-2 ratio of 0.7) had a calculated IC50 of 1 \( \mu \)M.

Finally, to examine if tissue mast cells exhibit similar sensitivity to ABT-737 treatment, we performed ex vivo experiments using human organ cultures. Skin biopsies were cultured at air-liquid interface in medium in the presence of 1, 10, or 25 \( \mu \)M ABT-737 for 3 d and apoptotic mast cells were measured. We found a significant increase in mast cell apoptosis in the biopsies treated with ABT-737 compared with the vehicle control (Fig. 5D). In addition, we also measured the presence of mast cells with chymase enzymatic activity and/or chymase immunoreactivity as a measurement of intact active mast cells (34). We found that on treatment of ABT-737, there was a decrease in the number of mast cells with chymase activity, but persistence of cells with chymase immunoreactivity (Fig. 5E), suggesting a chymase inactivation by serum protease inhibitors (34), which correlates with increased mast cell apoptosis. Thus, our data suggest that mast cells are sensitive to ABT-737 treatment and that BH3-mimetics might be a plausible way to induce mast cell apoptosis for treatment of mast cell associated diseases.

Discussion

Induction of cell apoptosis is emerging as one possible strategy for breaking an ongoing inflammatory response and to enhance the resolution of inflammation (12, 41). The proapoptotic BH3-mimetic
molecule ABT-737 is one possible candidate to be applied for such purposes (23, 25). In this study, we found that mast cells are especially sensitive to ABT-737, both after in vitro and in vivo exposure, and that human skin mast cells undergo apoptosis after treatment ex vivo. Hence, the novel experimental data in this study suggest that BH3-mimetics are potentially useful for inducing mast cell apoptosis as treatment of mast cell-associated diseases.

We examined mast cells of different origin, both mouse and human, and primary mast cells as well as cell lines. Although all different mast cell populations tested undergo apoptosis after treatment with ABT-737, both after in vitro and in vivo exposure, and that human skin mast cells undergo apoptosis after treatment ex vivo. Hence, the novel experimental data in this study suggest that BH3-mimetics are potentially useful for inducing mast cell apoptosis as treatment of mast cell-associated diseases.

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ABT-737 has been demonstrated to be well-tolerated when given to mice and has shown to have single-agent efficacy against certain tumors (25). Reduction in platelets (thrombocytopenia) has previously been described, but shown to be restored within 3 d after cessation of treatment (25, 35, 42). In this study, we found that in vivo administration of ABT-737 i.p. totally eliminated the mast cell population and also reduced the number of B lymphocytes, but not significantly T lymphocytes. Similarly, a reduction in blood lymphocytes was recently reported by Bardwell et al. (43). However, when the sensitivity of the different cell types was compared ex vivo, we found a much stronger apoptotic effect of ABT-737 on mast cells compared with lymphocytes, granulocytes, or macrophages. Peritoneal mast cells, treated with ABT-737 ex vivo, are also much more responsive than any other mast cell line or primary mast cell tested. This could be a consequence of peritoneal mast cells having differentiated into fully mature mast cells before they are being extracted from the peritoneum, thus probably representing a more mature mast cell population than the others tested.

The binding specificity of ABT-737 to Bcl-2, Bcl-XL, and Bcl-w, leaves the expression levels of the nonbinding proteins Mcl-1 and Bfl-1/A1 vital for how sensitive the different cell lines and cell types are. Reducing Mcl-1 either by RNAi, or the cyclin-
dependent kinase inhibitor roscovitine (44) or overexpressing the BH3-only protein Noxa (16) highly enhances the apoptotic effect of ABT-737 in tumor cell lines. However, the expression levels of Bcl-2 also seem to have an influence on the sensitivity. Analysis of four ALL cell lines has shown a correlation between Bcl-2 protein expression and response to ABT-737, where the most sensitive cell line expressed the most Bcl-2 (38). Our data on mast cells follow this correlation. In addition to expressing the highest protein level of Mcl-1, the ABT-737 insensitive MC9 cell line also expresses low levels of Bcl-2, whereas the most ABT-737 sensitive mouse mast cell line C57 expresses Bcl-2 abundantly. This demonstrates how dependent the cells are on the relative protein expression of Mcl-1 but also other antiapoptotic proteins such as Bcl-2 for maintaining homeostasis. Mast cells have been demonstrated to express and/or be dependent on Bcl-2 and Bcl-XL (17–19). Furthermore, targeting Bcl-2 in mast cells in vivo, renders the mast cells to go into apoptosis (45). These studies suggest that Bcl-2 and/or Bcl-XL are plausible targets for induction of mast cell apoptosis in vivo. However, mast cells might also express Mcl-1, especially mast cells with Kit D814V mutation found in mastocytosis (46). Thus, the expression of prosurvival Bcl-2-members that determines the sensitivity to BH3-mimetics has to be carefully investigated before any clinical studies are initiated. Furthermore, the mast cells used in this study do not carry the mastocytosis-associated c-kit mutation D816V (corresponding mutation in mice is D814V). The sensitivity of mast cells with mutated c-kit to ABT-737 is currently under investigation.

MC\textsubscript{TC} type of mast cells is numerous in the skin where they are mainly located below the epidermis. Mast cell activation and release of their inflammatory mediators have been associated with several chronic inflammatory skin diseases, such as atopic dermatitis and psoriasis (4, 6, 47). Because the skin is an easily accessible organ, we decided to measure the effect of ABT-737 ex vivo on skin mast cells using an air-liquid interface culture previously described by us (34). As with murine mast cells, we found that human skin mast cells are sensitive to ABT-737–induced apoptosis. The decrease in chymase-enzymatic activity further support the decline in mast cell numbers in treated skin because chymase is presumably inactivated when exposed to serum protease inhibitors after cell death (34). Thus, to evaluate the effect of a BH3 mimetic, like ABT-737, on cutaneous mast cells in skin disorders, a cream formulation of ABT-737 could well be tested on skin lesions as well as on healthy skin.

Bcl-2 family members as pharmacological targets have previously mainly been discussed in the context of malignancies (48). However, induction of inflammatory cell-apoptosis as therapy in inflammatory diseases like rheumatoid arthritis has been discussed as a possibility (49). Recently it was described by Bardwell et al. (43) that ABT-737 inhibits inflammation in animal models of arthritis and lupus. Although the authors did not investigate or discussed mast cells in their study, it has previously been described that mast cells contribute markedly to these diseases (7). Thus, one could speculate that part of the effect obtained was through induction of mast cell apoptosis. We therefore suggest that induction of mast cell apoptosis could be applied to mast cell associated diseases, such as chronic inflammations or mastocytosis. The second generation of BH3 mimetic compound ABT-263, developed by Abbott Laboratories, with improved oral availability and the same binding properties as ABT-737 (50), is now in clinical trials for the treatment of patients suffering from, for example, lymphoma. For the future, it would be reasonable to investigate the effect of ABT-263 on mast cell numbers in these patients. Such studies would provide important information about the translation of our in vitro and in vivo results into the patients.

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Disclosures
The authors have no financial conflicts of interest.

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