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Antitumor activities of selective HSP90α/β inhibitor, TAS-116, in combination with bortezomib in multiple myeloma

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Letter to the Editor

Heat shock protein 90 (HSP90) is a highly conserved molecular chaperone that interacts with various client proteins in eukaryotic cells1: Akt (PI3K/Akt pathway), IL-6R (JAK/STAT pathway), Bcr-Abl (RAS/ERK pathway), CDK4, 6, 9 (cell cycling), and IκB kinases (NF-κB pathway).2 The expression of HSP90 is upregulated (2- to 10-fold) in tumor cells compared with normal cells, reflecting multiple oncogenic pathways and maintenance of homeostasis within tumor cells.1, 2 Because HSP90 inhibition triggers downregulation/degradation of client proteins and triggers apoptosis, it is considered a promising target for novel targeted therapies. Indeed HSP90 inhibitors (e.g., geldanamycin analog 17-allylamo-17-demethoxy-geldanamycin (17-AAG), resorcinol derivatives, purine analogues) have shown early promising results in vitro and in vivo in solid tumors and some hematological malignancies, including multiple myeloma (MM).3, 4 However, some clinical studies have been discontinued due to adverse effects including ocular toxicity.3, 5 Therefore, development of a next-generation less-toxic HSP90 inhibitor remains an important therapeutic goal.

In the present study, we demonstrate in vitro and in vivo preclinical anti-MM activity of TAS-116, an oral selective HSP90α/β inhibitor, alone and in combination with BTZ. TAS-116 shows favorable oral bioavailability in rodent and non-rodent species, as well as
good metabolic stability. Importantly, TAS-116 demonstrates less ocular toxicity and greater anti-tumor activity in multiple xenograft models, compared to other HSP90 inhibitors at their MTD in rats. Our data therefore provide the preclinical framework for clinical evaluation of TAS-116, alone and with BTZ, to improve patient outcome in MM.

First we examined the growth inhibitory effect of TAS-116, a novel oral selective HSP90α/β inhibitor (Supplementary Figure S1A), in MM cell lines (Supplementary Figure S1B). TAS-116 significantly inhibited growth of these MM cell lines and patient MM cells (Supplementary Figure S1C), without affecting normal donor PBMNCs (Supplementary Figure S1D). Interestingly, we confirmed that TAS-116 was also active in N-Ras mutated cell lines (the proliferation/viability of NALM-6 is affected only at higher concentrations of 17-AAG) (Supplementary Figure S2A and S2B). We next examined the effect of TAS-116 on HSP90 client protein degradation. Significant degradation of HSP90 client proteins was triggered by TAS-116 in a dose-dependent manner in MM.1S cells (Supplementary Figure S1E).

We and others have shown that N-Ras mutation and HSP27 confers significant resistance to chemotherapies. Moreover, treatment with other HSP90 inhibitors induces resistance mechanisms due to the upregulation of other HSP proteins such as HSP27. We therefore next examined whether TAS-116 can overcome 17-AAG-resistance associated with N-Ras mutation and upregulation of HSP27. Importantly, more significant degradation of phospho-C-Raf and phospho-MEK1/2, HSP90 client proteins and key RAS/RAF/MEK pathway regulators, was triggered by TAS-116 than 17-AAG in INa6 and NCI-H929 MM cells (Supplementary Figure S2D, 2E). In addition, HSP27 upregulation induced by TAS-116 was lower than by 17-AAG at equipotent doses (Supplementary Figure S2F). Taken together, these results indicate that TAS-116 induces cytotoxicity selectively and potently in MM cell lines and patient MM cells, even in NALM-6 cells, without toxicity in normal PBMCs; potently targets HSP90 client proteins including C-Raf and MEK1/2; as well as inhibits upregulation of HSP27 and overcomes 17-AAG resistance mechanisms in MM cells.

We further confirmed that TAS-116 induces apoptosis in MM cells (Supplementary Figure S3A–F and Supplementary Information); inhibits Akt and ERK pathway, and overcomes the growth stimulatory effects triggered by cytokines and the bone marrow microenvironment (Supplementary Figure S4A–C, S5A–E, and Supplementary Information); and induces synergistic cytotoxicity with BTZ in vitro (Supplementary Figure S6A–D, Supplementary Table S1,2, and Supplementary Information).

We and others have previously shown that HSP90 inhibitors such as 17-AAG inhibit NF-κB signaling and induce terminal unfolded protein response (UPR). Whereas, BTZ induces both terminal UPR and canonical NF-κB pathway activation. We therefore hypothesized that TAS-116 could enhance the terminal UPR and inhibit canonical NF-κB pathway induced by BTZ, thereby augmenting BTZ-induced cytotoxicity. Although BTZ triggers activation of IκB kinase (IKKβ) and Akt, TAS-116 significantly downregulated IκKα/β in a time-dependent manner (Supplementary Figure S7A). Importantly, we observed that enhanced phosphorylation of Akt and key canonical NF-κB pathway regulators (p65, IκBα, and IκKα/β) triggered by BTZ in MM cell lines were significantly inhibited by
TAS-116. Since Akt associates with IKK to induce IKK activation leading to NF-κB activation, these results indicate that TAS-116 significantly inhibits bortezomib-induced canonical NF-κB pathway.

We next evaluated the effect of this combination on UPR. TAS-116 markedly upregulated p-IRE1α, p-eIF2α, and CHOP, a transcription factor leading to apoptosis due to endoplasmic reticulum (ER) stress, at early time points (within 4 hours) (Supplementary Figure S7B). Importantly, TAS-116 in combination with BTZ enhanced phosphorylation of IRE1α and eIF2α in MM cell lines, indicating that BTZ-induced UPR was enhanced by TAS-116 (Supplementary Figure S6H, S7C). Moreover, cleavage of PARP was significantly enhanced by TAS-116 in combination with BTZ in both MM cell lines, associated with increased CHOP (Supplementary Figure S6I, S7D). These results suggest that TAS-116 augments BTZ-induced ER stress, followed by the terminal UPR and apoptosis.

We next examined the in vivo efficacy of TAS-116 in combination with BTZ using a murine xenograft model of human MM. Mice treated with TAS-116 (10 mg/kg and 15 mg/kg), BTZ, or TAS-116 plus BTZ showed significantly enhanced growth inhibition versus the vehicle control group (P = .004 and P = .0012, P = .003, and P < .0001, respectively; Figure 1A). Representative images of tumor growth inhibition by TAS-116 (10 mg/kg) are demonstrated in Figure 1B. The delay in tumor growth was greater in the combination-treated group compared with either monotherapy cohort (P = .0014 in TAS-116 vs the combination and P = .0001 in BTZ vs the combination; Figure 1A). Median overall survival of treated animals (TAS-116 10 mg/kg = 33 days, 15 mg/kg = 37 days, BTZ = 36 days, and the combination = 56.5 days) was significantly longer than vehicle control (29 days; P = .0064, P < .0001, P = .0009, and P < .0001, respectively; Figure 1C). The OS was significantly prolonged in the combination group compared with either monotherapy cohort (P = .004 in TAS-116 vs the combination, and P = .0004 in BTZ vs the combination; Figure 1C). These treatments were well tolerated, and no significant body weight loss was observed (Figure 1D). Importantly, immunohistochemical analysis of harvested human MM confirmed a significant increase in cleaved caspase-3- and TUNEL-positive cells in TAS-116 15 mg/kg-treated mice (Figure 1E). These results indicate that TAS-116 triggers enhanced in vivo anti-MM activities, both alone and in combination with BTZ, with a favorable safety profile.

Finally, we investigated the ocular toxicity profile of TAS-116, since ocular toxicity is one of the most notable toxicities limiting the clinical application of other HSP 90 inhibitors. Because others have shown that geldanamycin and its analogue 17-AAG inhibit proliferation of normal human retinal pigment epithelial ARPE-19 cells essential for the support of photoreceptors by inducing cell cycle arrest and apoptosis,15 we first examined the growth inhibitory effect of TAS-116 and 17-AAG in ARPE-19 cell line (Figure 2A). Importantly, TAS-116 was less toxic to ARPE-19 cells than 17-AAG. In addition, we assessed the cytotoxicity of TAS-116 or HSP90 inhibitor PF-04928473 (SNX-2112) in combination with BTZ in ARPE-19 cells. Surprisingly, low-dose BTZ significantly ameliorated the cytotoxicity induced by TAS-116, compared with PF-04928473 (SNX-2112) (Figure 2B). We next investigated the ocular toxicity profile of TAS-116 using an in vivo murine xenograft model. Importantly, TAS-116 15 mg/kg--treatment (maximum
tolerated dose) did not induce ocular toxicity in mice, in contrast to PF-04929113 (SNX-5422) 40 mg/kg, which was associated with increased photoreceptor cell death in all retinal layers (Figure 2C). Taken together, these results indicate that TAS-116 demonstrates a safer ocular toxicity profile than PF-04929113 (SNX-5422).

In summary, we show the anti-MM activities of a novel HSP90α/β-selective inhibitor TAS-116 in MM. TAS-116 triggered cytotoxicity via apoptosis, associated with downregulation of phosphorylation of client proteins including Akt and ERK. TAS-116 overcame the anti-apoptotic effect triggered by IL-6, IGF-1, or bone marrow stromal cells. TAS-116 also induced cytotoxicity even in acute B cell leukemia cell line NALM6, as well as N-Ras mutated NCI-H929 and INA6 MM cell lines, and overcame 17-AAG resistance mechanisms. TAS-116 also enhanced bortezomib (BTZ)-induced MM cytotoxicity, due to inhibition of BTZ-triggered canonical NF-κB activation and enhancing endoplasmic reticulum stress. We confirmed that TAS-116, alone and in combination with BTZ, was well tolerated; triggered significant tumor growth inhibition; and prolonged host survival in a murine xenograft model of human MM. Importantly, TAS-116 showed lower ocular toxicity, a known toxicity of HSP90 inhibitors, than PF-04929113 (SNX-5422). Taken together, our studies show that TAS-116 blocks MM cell growth both in vitro and in vivo, and is well tolerated, providing the framework for its clinical evaluation to improve patient outcome in MM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. TAS-116 inhibits human MM cell growth and enhances bortezomib-induced cytotoxicity in vivo

(A–G) SCID mice were injected subcutaneously with $5 \times 10^6$ MM.1S cells and treated with 10 mg/kg oral TAS-116 5 days a week (n = 10; green line); 15 mg/kg oral TAS-116 5 days a week (n = 10; blue line); 0.5 mg/kg subcutaneous BTZ twice a week (n = 8; purple line); or 0.5 mg/kg subcutaneous BTZ twice a week and 10 mg/kg oral TAS-116 5 days a week (n = 10; red line) for 28 days. A vehicle control group received oral vehicle only and subcutaneous saline (n = 9; black line).

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(A) Tumor volume was calculated from caliper measurements every other day, and data represent mean ± SD.
(B) Representative whole-body images from a mouse treated for 29 days with control vehicle (bottom panel) or for 31 days with TAS-116 (10 mg/kg; top panel).
(C) Survival was evaluated from the first day of treatment using Kaplan-Meier curves.
(D) Change of body weight was expressed from the first day of treatment. Data represent mean ± SD.
(E) Tumors harvested from TAS-116- (15 mg/kg) and vehicle control- treated mice after 3 days of treatment were subjected to immunohistochemical analysis for cleaved caspase-3 and TUNEL staining.
Figure 2. TAS-116 is less toxic to human retinal pigment epithelial cells than other HSP90 inhibitors, and does not trigger ocular toxicity in mice

(A) Human retinal pigment epithelial ARPE-19 cell lines and NCI-H929 MM cells were cultured with TAS-116 or 17-AAG (0–5 μM) for 48 hours. Cell viability was assessed by CellTiter-Glo® assay of triplicate cultures, expressed as percentage of untreated control. Data represent mean ± SD.

(B) ARPE-19 cells were cultured for 48 hours with BTZ (0–2 nM) in combination with TAS-116 (0 μM: gray, 0.125 μM: gold, 0.25 μM: light orange, 0.5 μM: orange) or PF-04928473 (SNX-2112) (0 μM: gray, 0.125 μM: light green, 0.25 μM: sea green, 0.5 μM:...
green). Cell proliferation was assessed by CellTiter-Glo® assay of triplicate cultures, expressed as percentage of untreated control. Data represent mean ± SD. (* $P < .01$; ** $P < .001$)

(C) TAS-116 (15 mg/kg; 5 days a week), PF-04929113 (SNX-5422) (40 mg/kg; 3 times per week), or vehicle were administered orally in SCID mice for two weeks. Retinal morphology and photoreceptor cell death were evaluated by TUNEL staining. ONL indicates outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.