Combination of TH-302 and Bortezomib Has Synergistic Activity in Multiple Myeloma

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Introduction

Multiple Myeloma (MM) is malignant plasma-cell disorder that accounts for ~10% of all hematologic malignancies. Recent advances in the treatment of MM have resulted in improved response rates and overall survival, however, nearly all patients eventually relapse.

TH-302 is a nitroimidazole-triggered bromo-isophosphoramidate mustard hypoxia-activated cytotoxic prodrug. Under hypoxic conditions it releases a cytotoxic DNA cross-linking bis-alkylator. TH-302 is currently in clinical trials for the treatment of solid tumors and hematologic malignancies.

As previously demonstrated by us and other groups, hypoxia is a critical microenvironment factor in multiple myeloma (MM). Treatment with the hypoxia-activated prodrug TH-302 has showed promising effectiveness in preclinical MM models (1).

Aims

The aim of this study was to investigate the combinatorial effects of TH-302 and bortezomib on MM.

Results

1. Hypoxia confers MM cell resistance to bortezomib.

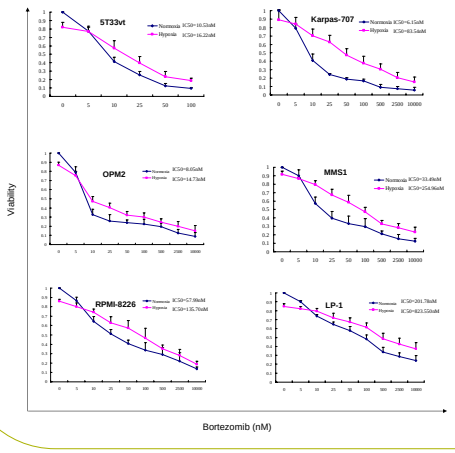

Figure 1. The increase of IC50 of bortezomib in hypoxia compared to normoxia. Bortezomib was less effective under hypoxic conditions. Normoxic (20%O_2) and hypoxic (1%O_2) conditions were established by culturing myeloma cells in a sealed chamber with fixed gas mixtures. After incubation for 16h, cell viability assays were performed using the CellTiter-Glo Luminescent Cell Viability Assay according to the protocol provided by Promega (Madison, WI, USA). The total ATP levels were measured as an index of the viable cell number.

2. Synergistic effects of TH-302 and bortezomib in hypoxia (*in vitro*) (5T33vt cells as an example) .

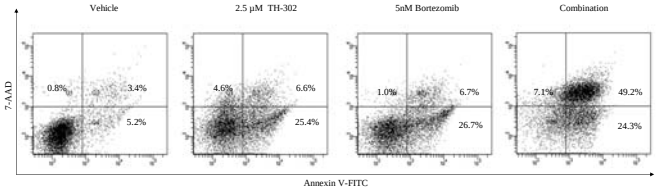

Figure 2. Combination treatment of TH-302 and bortezomib increases the level of apoptosis in 5T33vt cells in hypoxic condition (1% O_2). Apoptosis was measured by the flow cytometry analysis of MM cells stained with FITC-labeled Annexin V and 7-AAD after 16h with each indicted treatment. Percentage of cells is shown in the corner of each quadrant. Results are representative of three independent experiments.

Table 1 Combination index analysis of TH-302 combined with bortezomib at a non-constant ratio in 5T33vt cells

TH-302 μM	*Btz nM*	*Fa*	*CI*	*Description*
2.5	*5*	*0.382*	*0.638*	*Synergism*
2.5	*10*	*0.556*	*0.569*	*Synergism*
2.5	*20*	*0.605*	*0.891*	*Slight synergism*
5	*5*	*0.535*	*0.452*	*Synergism*
5	*10*	*0.603*	*0.550*	*Synergism*
5	*20*	*0.828*	*0.370*	*Synergism*
10	*5*	*0.576*	*0.538*	*Synergism*
10	*10*	*0.777*	*0.308*	*Synergism*
10	*20*	*0.826*	*0.404*	*Synergism*

Abbreviations: Fa, fraction affected as tested by the flow cytometry analysis of MM cells stained with FITC-labeled Annexin V and 7-AAD after 16h with each indicated treatment; CI, combination index. Analysis was performed using the CompuSyn software (ComboSyn, Inc.). Descriptions are based on CI values and the recommendations of ComboSyn: <0.1=very strong synergism, 0.1–0.3=strong synergism, 0.3–0.7=synergism, 0.7–0.85—moderate synergism, 0.85–0.9=slight synergism.

3. Caspase cascade was triggered by bortezomib, TH-302 alone and combination.

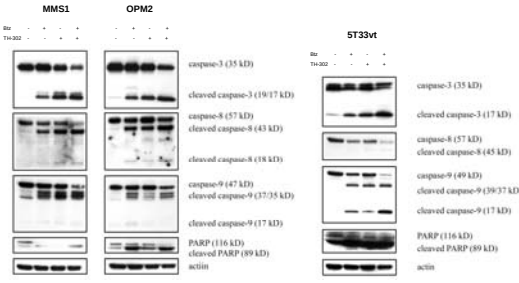

Figure 3. Western blot analysis of the caspase activation involved in bortezomib, TH-302 alone and the combination in hypoxia. The following doses of bortezomib and TH-302 were used in different MM cell lines: MMS1 (25nM Btz and 10μM TH-302), OPM2 (10nM Btz and 5μM TH-302) and 5T33vt cells (5nM Btz and 5μM TH-302). After incubation with drugs for 16h, the cells were harvested for Western blot analysis.

4. Combination effects of TH-302 with bortezomib in 5T33vv MM mouse model.

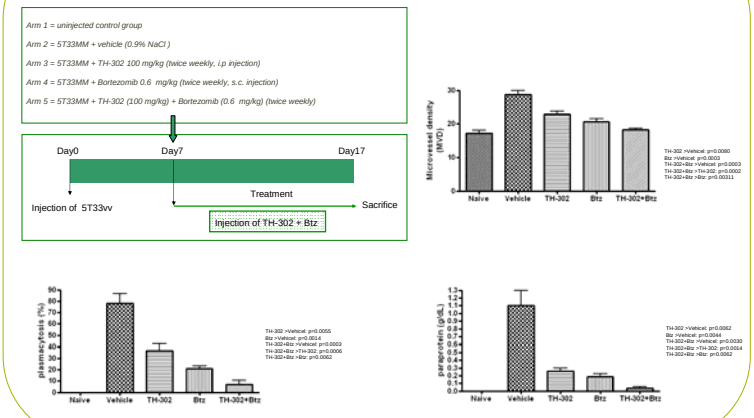

Table 2. The direction of change of BCL-2 family protein expression following bortezomib or TH-302 treatment in MM cells in hypoxia

Bcl-2 family protein	Pro- or antiapoptotic	Class	change (by Bortezomib)	change (by TH-302)
Bcl-2	antiapoptotic	multidomain	downregulation or no change	***downregulation***
Bcl-xL	antiapoptotic	multidomain	no change	***downregulation***
Mcl-1	antiapoptotic	multidomain	***upregulation***	***downregulation***
			cleavage	***cleavage***
Bax	proapoptotic	multidomain	no change	no change
Bad	proapoptotic	BH3	***cleavage***	***cleavage***
BID	proapoptotic	BH3	***cleavage***	***cleavage***
BIK	proapoptotic	BH3	***upregulation***	no change
BIM	proapoptotic	BH3	***upregulation***	no change
PUMA	proapoptotic	BH3	***upregulation***	***upregulation***
NOXA	proapoptotic	BH3	***upregulation***	***upregulation***

* Pro-apoptotic: in red; anti-apoptotic: in green.
* The cleaved pro-apoptotic Bcl-2 family proteins are stronger apoptosis inducers.
* The results were measured by Western blot in MM cell line *in vitro*.

5. Two mechanisms involved in reduction of Mcl-1 by TH-302: via ATF4 and via HIF1a/2a.

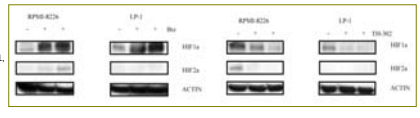

Figure 5A. The opposite effect of bortezomib and TH-302 on HIF1a/2a accumulation in MM in hypoxia. TH-302 decreases the accumulation of HIF1a/2a, which is the upstream regulator of Mcl-1, The following doses of bortezomib were used: RPMI-8226 (0, 5, 25nM), OPM2 (0, 2, 10nM). The doses of TH-302 were used in both RPMI-8226 and LP-1 cells: 0, 5, 10μM. After incubation with drugs for 16h, the cells were harvested for Western blot analysis.

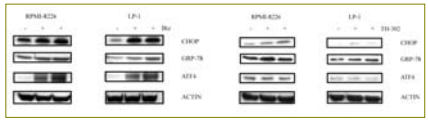

Figure 5B. The opposite effect of bortezomib and TH-302 on ATF4 (activating transcription factor 4; one of the key regulators of the Unfolded Protein Response) expression in MM in hypoxia. Bortezomib increases the expression, however, TH-302 decreases the expression of ATF4, which we recently identified as a new upstream regulator of Mcl-1. The following doses of bortezomib were used: RPMI-8226 (0, 5, 25nM), OPM2 (0, 2, 10nM). The doses of TH-302 were used in both RPMI-8226 and LP-1 cells: 0, 5, 10μM. After incubation with drugs for 16h, the cells were harvested for Western blot analysis.

CONCLUSIONS

1. Combination of TH-302 and bortezomib has synergistic cytotoxicity in multiple myeloma.
2. The synergistic effect is related to the changes of Bcl-2 family members.
3. The study provides the basis for clinical evaluation of the combination of TH302 and bortezomib for multiple myeloma patients.

Reference: (1) Hu J. et al. Blood. 2010;116(9):1524.

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