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Efficacy of Mcl-1 Inhibitors in Multiple Myeloma Cells Resistant to Bortezomib

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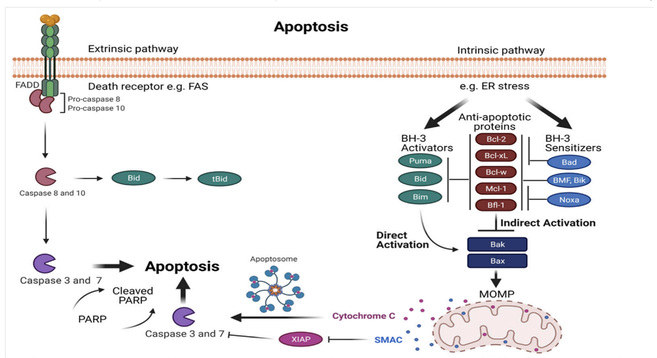
Abstract

Multiple myeloma (MM) is a type of cancer that affects plasma B cells. Patients with MM often experience frequent relapses and can develop resistance to drugs. As a medical researcher, it is important to understand the role of Mcl-1 in preventing intrinsic apoptosis and drug resistance. Mcl-1 belongs to the anti-apoptotic subgroup of Bcl-2 family proteins and plays a crucial role in these processes. Mcl-1 plays a crucial role in driving disease progression and contributing to drug resistance in MM. It has been observed that there is an increased expression of Mcl-1 in 52% of patients with MM during diagnosis, which further rises to 81% during relapse. Thus, researchers are investigating the potential of Mcl-1 inhibitors as a viable treatment option for patients with MM, particularly those who have not responded to previous therapies. Proteasome inhibitor Bortezomib (BTZ) is commonly prescribed as the initial treatment for MM, but unfortunately, patients eventually develop resistance to it. For this study, we created cells that are resistant to BTZ in order to explore the potential mechanisms behind the development of resistance. These cells have been treated with BTZ over a period of 6 months. Regrettably, there are currently no Mcl-1 inhibitors that have been approved by the FDA. However, there are several agents, such as S63845, AZ5991, and VU661013, that are currently undergoing clinical trials. Interestingly, Mcl-1 inhibitors demonstrated effectiveness against sensitive cells but showed a decrease in efficacy against BTZ resistant cells. Our research indicates that cells resistant to BTZ require a higher concentration of Mcl-1 inhibitors in order to undergo cell death. This suggests that these resistant cells may possess a compensatory mechanism that stabilizes the Mcl-1 protein and alters the effectiveness of Mcl-1 inhibitors in treatment. It is worth noting that the anti-apoptotic Bcl-2 protein exhibits heightened expression in resistant cells, even when inhibitors are present. This observation may provide valuable insights into a potential resistance mechanism and calls for further investigation into the compensatory mechanisms that play a crucial role in drug resistance.

Introduction

- The American Cancer Society estimates in the year 2024 that 12,540 multiple myeloma patients will die and almost 36 thousand new cases will be diagnosed within the United States. MM disease progression and acquired resistance to therapeutic agents is related to Mcl-1 overexpression (1, 2, 4).
- The Bcl-2 family subgroup of antiapoptotic proteins includes Mcl-1, Bcl-2, Bcl-xL, and Bcl-W. These proteins sequester Bak and Bax indirectly and inhibit BH-3 activators (Bcl-2 proteins part of the proapoptotic subgroup). Pro-survival proteins therefore prevent mitochondrial pore formation which would result in cell death (3).



Al-Odat, Omar, et al. "Mcl-1 Inhibition: Managing Malignancy in Multiple Myeloma." Frontiers in Pharmacology 12 (2021). Graphic made using Biorender.com.

- Mcl-1 has a short half-life due to degradation via ubiquitin-proteasome system. Extracellular signaling, such as IL-6, significantly increases Mcl-1 transcription (2,3).
- Various Phase I clinical trials have examined the dosing and benefits of Mcl-1 inhibitors. However, many have been discontinued prematurely due to negative cardiotoxic effects in patients.

Results

Development of BTZ resistance MM cell lines.

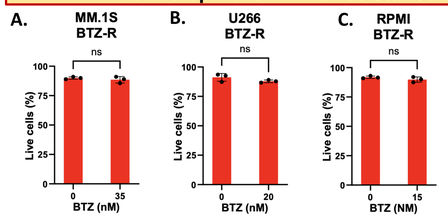
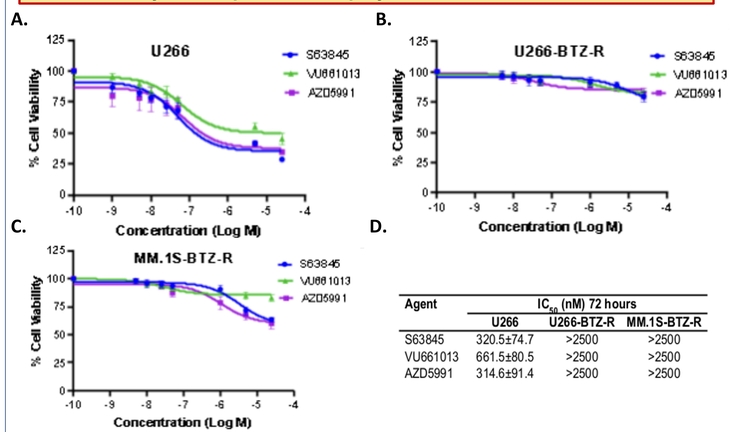


Fig 1. A-C, MM.1S, U266, RPMI BTZ-R cell lines treated with BTZ for 24hr and Annexin V staining was used to identify apoptotic cells with Muse® Cell Analyzer. Total amount of apoptotic cells identified and unpaired t test was performed using GraphPad prism software. ns: non-significant.

Decreased cytotoxic potential displayed in BTZ Resistant cells in vitro.



Agent	IC_{50} (nM) 72 hours		
	U266	U266-BTZ-R	MM.1S-BTZ-R
S63845	320.5±74.7	>2500	>2500
VU661013	661.5±80.5	>2500	>2500
AZD5991	314.6±91.4	>2500	>2500

Fig 2. A, U266 cell line was treated separately with increasing doses of inhibitors S63845, AZ5991, and VU661013 (0-25μM) for 72h, and cell viability measured via MTT assay. ***B&C,*** BTZ resistant U266 and MM.1S cell lines generated and treated with increasing doses of inhibitors S63845, AZ5991, and VU661013 (0-2.5μM) for 72h, and cell viability measured via MTT assay. ***D,*** IC_{50} calculations were performed by GraphPad.

Antiapoptitic protein expression in BTZ resistant cells.

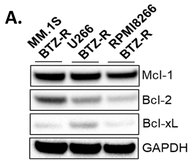


Fig 3. Pro-survival proteins Mcl-1, Bcl-2, and Bcl-xL expression in BTZ-R MM cell lines determined via western blot. BTZ-MM resistant cells including MM.1S-BTZ-R, U266-BTZ-R, and RPMI-BTZ-R were lyzed without treatment and immunoblotted with the aforementioned antibodies. GAPDH antibody was used as a loading control on the stripped membrane.

Mcl-1 overexpressed in BTZ-R cells, despite treatment with inhibitors.

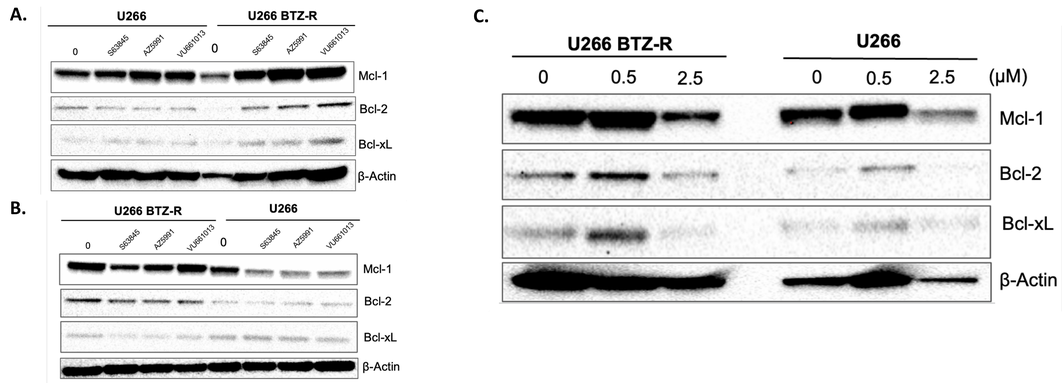
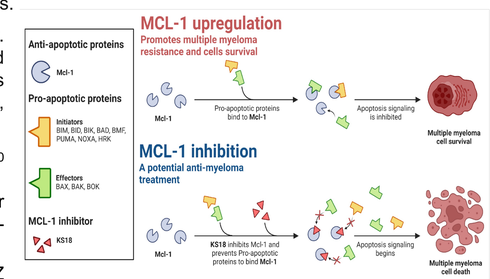


Fig 4. A&B, U266 and U266-BTZ-R cells were treated with S63845, AZ5991, and VU661013 at 0.5μM (A) and 2.5μM (B) for 24hrs, then western blot analysis used to investigate Mcl-1, Bcl-1, and Bcl-xL expression in each cell group. As a loading control, the stripped membrane was probed with β-Actin or GAPDH antibody, respectively. ***C,*** U266 and U266-BTZ-R cells treated with S63845 at 0 μM, 0.5μM and 2.5μM for 24hrs followed by western blot analysis of Mcl-1, Bcl-1, and Bcl-xL expression; β-Actin used as loading control.

Conclusion and Future Directions

- The BTZ-R MM cells exhibited a significant upregulation of Mcl-1. The Mcl-1 inhibitors S63845, AZ5991, and VU661013 showed no effectiveness in BTZ-resistant MM cell lines.
- Cells resistant to BTZ also showed the presence of Bcl-2. Furthermore, the administration of Mcl-1 inhibitors did not yield any impact on Bcl-2 expression. This implies that Bcl-2 does indeed contribute to resistance, alongside Mcl-1. Therefore, inhibiting Bcl-2 is necessary to prevent any potential resistance.
- In BTZ-resistant cells, Mcl-1 inhibitors demonstrated a higher IC_{50} compared to sensitive MM cell lines.
- Our next step is in vitro testing to determine the best inhibitor among the three. These inhibitors will also be tested in drug-resistant MM cells like Lenalidomide-R and Venetoclax-R.
- Further investigation is needed to understand how acquired BTZ resistance can bypass the actions of Mcl-1 inhibitors.



Graphic made using Biorender.com.

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Cooper Medical School of Rowan University