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Katelyn King April 10, 2024

# Unlocking New Avenues in Multiple Myeloma Treatment: Targeting Anti-Apoptotic Genes to Enhance Drug Efficacy

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Abstract

Unlocking New Avenues in Multiple Myeloma Treatment: Targeting Anti-Apoptotic Genes to Enhance Drug Efficacy

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This study explored the role of Bcl-2 and Bcl-xL, anti-apoptotic genes known for promoting cancer cell survival, in the drug resistance of multiple myeloma by using CRISPR/Cas9 to knock them out in the KMS18 cell line. The efficacy of these knockouts was confirmed through Western blotting, and their impact on drug-induced apoptosis was assessed using Annexin-V and propidium iodide staining with flow cytometry, particularly in response to the proteasome inhibitor bortezomib. Results showed that knocking out these genes increased the susceptibility of multiple myeloma cells to apoptosis, highlighting their potential as therapeutic targets. Despite facing methodological challenges such as maintenance of cell lines and issues with co-immunoprecipitation reagents, the study offers valuable insights into overcoming drug resistance and improving treatment outcomes in multiple myeloma. Future research should investigate the combinatorial effects of these gene knockouts and develop in vivo models to further validate these findings, advancing personalized medicine in cancer treatment.

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# Introduction

## Characteristics of Cancer

Cancer cells exhibit a unique set of physiological alterations that enable their unchecked growth and spread, distinguishing them significantly from normal, healthy cells. Each of these alterations, or hallmarks, contributes to the development and progression of cancer in distinct ways.

The first hallmark is self-sufficiency in growth signals. In healthy cells, growth signals are needed to initiate the process of cellular proliferation. Unlike normal cells, cancer cells have the ability to produce their own growth signals, therefore altering the state of homeostasis within the system. Furthermore, this self-sufficiency allows them to proliferate without the constraints typically imposed by the surrounding cellular environment, leading to rapid and uncontrolled tumor growth (Cooper, 2000).

In addition to generating their own growth cues, cancer cells also display insensitivity to growth-inhibitory signals. Normally, cells receive cues from their environment that direct them to stop growing, helping to maintain tissue integrity and function. Cancer cells, however, can ignore these inhibitory signals, continuing to grow even when conditions are unfavorable or when they are in contact with other cells, further contributing to their malignant behavior (Cooper, 2000).

Another critical characteristic is the evasion of apoptosis or programmed cell death. Apoptosis serves as a natural barrier to cancer, eliminating cells that have sustained genetic

damage or have become dysfunctional. Cancer cells, through various genetic alterations, are able to avoid this mechanism, allowing them to survive and accumulate despite serious cellular stresses that would normally trigger their destruction (Cooper, 2000).

Another hallmark of cancer cells is limitless replicative potential, While normal cells can only divide a finite number of times before they become senescent and die, cancer cells can maintain or even lengthen their telomeres, the protective caps on chromosome ends, enabling them to divide indefinitely (Hanahan & Weinberg, 2011). This immortal behavior underlies the persistent growth of tumors and is a key target for potential cancer therapies.

In addition to the advanced replicative potential, sustained angiogenesis is another hallmark of cancer. Angiogenesis is the process of forming new blood vessels, which is necessary to supply the tumor with oxygenated blood to enable uncontrolled growth. Cancer cells can secrete factors that stimulate angiogenesis, ensuring that they have access to the oxygen and nutrients needed for their continued expansion (Hanahan & Weinberg, 2011). This ability not only supports tumor growth but also provides a pathway for cancer cells to enter the bloodstream and metastasize to distant sites.

Finally, the ability to invade tissue and metastasize distinguishes cancer cells from normal cells, which are typically confined to their original tissue (Hanahan & Weinberg, 2011). Cancer cells have the ability to penetrate the barriers that typically confine cells within their intended areas. They can invade adjacent tissues, and through the bloodstream or lymphatic system, travel to other parts of the body. This capability for invasion and metastasis is often the

reason why cancer is deadly, as it makes treatment more complicated and can affect numerous bodily functions.

Together, these six hallmarks of cancer form a framework that helps scientists understand the complex biology of cancer and guides the development of new therapeutic strategies aimed at interfering with these malignant characteristics.

**Multiple Myeloma**

Following the discussion on the general characteristics of cancer, it's important to delve into the specifics of multiple myeloma, a cancer that highlights the complexity and diversity of malignant diseases. Multiple myeloma is a type of blood cancer that originates in the plasma cells, a form of white blood cell crucial for the immune system's ability to fight infections. Unlike many cancers that form solid tumors, multiple myeloma primarily affects the bone marrow, the soft, spongy tissue inside bones where blood cells are produced. This section explores multiple myeloma's pathophysiology, its impact on the body, and the current approaches to its management.

Multiple myeloma is characterized by the proliferation of malignant plasma cells in the bone marrow, leading to several direct and indirect effects on patient health. These cancerous cells produce abnormal antibodies, known as M proteins, which can cause a variety of problems, including kidney damage and bone lesions. The excessive growth of these abnormal plasma cells also interferes with the production of healthy blood cells, leading to anemia, increased risk of infections, and impaired blood clotting (Fairfield et al., 2016).

**Epidemiology Multiple Myeloma**

Multiple myeloma is an increasingly prevalent cancer of the plasma cells, notable for its significant impact on populations worldwide. As of 2018, multiple myeloma accounted for nearly 0.9% of all cancer diagnoses globally, with an estimated 160,000 new cases. The incidence has been rising sharply; globally, the number of cases increased by 126% from 1990 to 2016, underscoring a growing public health issue. The disease primarily affects older adults, with the median age of diagnosis around 69 years in the United States, and it is slightly more common in men than in women. There has been a marked improvement in survival rates due to advancements in treatment, with the 5-year survival rate in the U.S. more than doubling over recent decades, reflecting a significant decrease in mortality despite the increased incidence (Padala et al., 2021).

A particularly concerning aspect of multiple myeloma epidemiology is its disproportionate effect on African Americans, who are more than twice as likely to be diagnosed with multiple myeloma compared to their Caucasian counterparts. This disparity is not just limited to a higher incidence but also manifests in a younger average age at diagnosis among African Americans, suggesting potential genetic, environmental, or socioeconomic factors contributing to the risk and progression of the disease in this group. The incidence rate among African American men stands at 16.5 per 100,000, and 12.0 per 100,000 among African American women, compared to 8.2 and 5.0, respectively, for Caucasians. Despite the higher risk and incidence rates, survival rates do not significantly differ by race, pointing towards a

complex interplay of factors that influence the disease's outcome beyond genetic predisposition (Padala et al., 2021).

Moreover, family history has emerged as a risk factor, indicating a hereditary component to multiple myeloma risk. Studies suggest that first-degree relatives of multiple myeloma patients have an increased risk of developing the disease themselves, with particularly strong associations observed among men and African Americans. This familial linkage highlights the need for further research into the genetic and molecular underpinnings of multiple myeloma, which could pave the way for targeted screening and intervention strategies (Padala et al., 2021).

The epidemiology of multiple myeloma indicates that it is a disease that is increasing globally, but with significant advancements in treatment, survival outcomes have improved. However, there is a noticeable difference in the incidence of multiple myeloma among African Americans compared to other racial groups. This, coupled with the implications of family history, highlights the need for ongoing research into the genetic, environmental, and social determinants of multiple myeloma. Understanding these factors is crucial in developing more effective prevention, diagnosis, and treatment strategies that can address the disparities and reduce the overall burden of multiple myeloma.

**Pathophysiology of Multiple Myeloma**

The pathophysiology of multiple myeloma involves complex interactions between malignant plasma cells and the bone marrow microenvironment, leading to distinctive clinical manifestations and challenges in treatment. Multiple myeloma is a malignant neoplasm of

plasma cells that accumulate in the bone marrow, leading to bone destruction and marrow failure. This cancer is characterized by the uncontrolled growth of mutated plasma cells within the bone marrow, predominantly affecting patients over the age of 65. Multiple myeloma's hallmarks include elevated calcium, renal failure, anemia, and bone lesions. These features are often remembered with the acronym CRAB. CRAB, alongside heterogeneous chromosomal abnormalities and numerous gene mutations, complicates therapeutic targeting (Fairfield et al., 2016).

A precursor of multiple myeloma is monoclonal gammopathy of undetermined significance (MGUS). MGUS is an asymptomatic premalignant plasma cell disorder that is characterized by the presence of serum M-protein, bone marrow clonal plasma cells less than 10%, and an absence of B-cell lymphoma or other disease known to produce an M-protein. Not all individuals with MGUS will develop multiple myeloma (Kaseb et al., 2024). The progression of multiple myeloma from MGUS to asymptomatic myeloma and then to symptomatic multiple myeloma is marked by increased bone marrow infiltration and the development of osteolytic lesions. This transition is fueled by an imbalance between osteoblastic (bone-building) and osteoclastic (bone-resorbing) activities, shifting towards net bone loss. Osteolysis releases growth factors from the bone matrix, which in turn promote multiple myeloma cell expansion within the bone marrow niche, establishing a vicious cycle of tumor growth and bone destruction (Fairfield et al., 2016).

The bone marrow microenvironment plays a pivotal role in the establishment and progression of multiple myeloma. Multiple myeloma's interaction with the bone marrow is

multifaceted, involving mesenchymal stem cells, osteoblasts, osteoclasts, and other niche cells like adipocytes and immune cells. These interactions contribute not only to the pathogenesis of multiple myeloma itself but also to the associated bone disease, highlighting the significance of the bone marrow niche in both the development and treatment of multiple myeloma (Fairfield et al., 2016).

Advances in understanding multiple myeloma genetics have highlighted the complexity of its cytogenetic changes, including mutations related to oncogenes and tumor suppressors. These genetic insights have facilitated the stratification of multiple myeloma into different risk categories based on specific genetic markers, aiding in prognosis and tailoring of therapeutic approaches. Despite this progress, the treatment of multiple myeloma remains challenging due to the disease's genetic heterogeneity and its interaction with the bone marrow microenvironment, underscoring the need for ongoing research into novel therapeutic targets and strategies.

**Management and Treatment of Multiple Myeloma**

The development of drug therapies for multiple myeloma has significantly improved patient survival rates. These therapies include immunomodulatory agents, proteasome inhibitors, monoclonal antibodies, and immunocellular therapies, which have extended life expectancy beyond a decade for many individuals.

The journey from diagnosis to management of multiple myeloma involves navigating symptoms that can mimic more common, less severe conditions, leading to potential delays in identifying the disease. A definitive diagnosis is achieved through the application of the

International Myeloma Working Group criteria, which necessitate the presence of clonal bone marrow plasma cells or a biopsy-proven plasmacytoma, alongside specific clinical features or biomarkers (Rajkumar et al., 2014).

Management strategies for multiple myeloma are tailored to individual patient profiles, taking into account factors such as eligibility for stem cell transplantation. For transplant-eligible patients, the approach typically includes induction chemotherapy, followed by stem cell collection and autologous stem cell transplantation (ASCT), and then maintenance therapy. In contrast, transplant-ineligible patients may receive a triplet drug regimen, with considerations for dose adjustments based on the patient's age and frailty (Devarakonda et al., 2021).

The management of relapsed or refractory multiple myeloma is particularly complex, requiring a careful balance between aggressive treatment to control the disease and the mitigation of side effects to maintain quality of life. This necessitates a holistic approach to care, involving not just the targeting of the myeloma cells themselves but also addressing the broader impacts of the disease and its treatment on the patient's health and well-being (Sonneveld, 2017).

Survivorship has become a key aspect of care for patients with multiple myeloma. The focus is not only on extending life, but also on maintaining a good quality of life. This involves managing both disease-related and treatment-related complications, such as bone disease, renal disease, infections, thrombosis, second malignancies, peripheral neuropathy, gastrointestinal issues, and cardiotoxicity. A multidisciplinary team approach, incorporating

primary care, specialty oncology, and supportive care services, is essential to address the diverse needs of multiple myeloma patients.

**Targeting Anti-Apoptotic Genes in Multiple Myeloma**

In recent years, the scientific community has turned its attention toward the sophisticated mechanisms through which multiple myeloma cells elude the cytotoxic effects of treatment. Central to this phenomenon is the role of anti-apoptotic genes, which have been shown to play a crucial part in the survival and drug resistance of cancer cells. These genes function by inhibiting the apoptotic pathways that normally lead to cell death, thereby granting cancer cells an unusual form of longevity and resilience against chemotherapy and targeted therapies. The overexpression or dysregulation of such genes can significantly reduce the sensitivity of multiple myeloma cells to anti-cancer agents, posing a substantial barrier to treatment success. Consequently, the modulation of anti-apoptotic gene activity presents a promising avenue of research and development, aiming to deactivate this protective mechanism and render the cancer cells more vulnerable to therapeutic interventions.

Building upon this understanding, this research aims to delve deep into the mechanisms by which dysregulation of apoptosis contributes to the persistence of multiple myeloma cells against conventional and novel treatments. By focusing on the selective knockdown of key anti-apoptotic genes, this project endeavors to dismantle the molecular safeguards that protect cancer cells, thereby enhancing their susceptibility to apoptosis. This strategy is based on the idea that by understanding how anti-apoptotic genes and drug resistance pathways interact, we can develop targeted approaches to sensitize multiple myeloma cells. Through rigorous

experimentation and analysis, this investigation seeks to identify and validate novel therapeutic targets within the apoptotic machinery, with the ultimate goal of integrating these findings into the development of innovative, more effective treatments.

## Methods

First, the sgRNA targeting anti-apoptotic genes Bcl-2 or Bcl-xL were complexed with a Cas9 enzyme. Then, the complex was introduced into KMS18 cells, a multiple myeloma cell line, using the non-viral transfection technology, Amaxa nucleofector. This generated two cell lines: KMS18 cells with Bcl-2 knocked out (Bcl-2 KO) and KMS18 cells with Bcl-xL knocked out (Bcl-xL KO). Then, the knockouts were validated using Western blotting to ensure that there was no expression of the selected genes. Then, parental KMS18 and the knockout cell lines were exposed to anti-cancer drugs commonly used in multiple myeloma treatment. These drugs include proteasome inhibitors such as bortezomib and DNA-damaging reagents like etoposide. After exposing the cells to the selected drug for 24 hours, the cells were collected and stained with Annexin-V and propidium iodide (PI). The cell solution was then given to the BD FACSymphony™ A1 Cell Analyzer Flow Cytometer to detect the translocation of phospholipid phosphatidylserine (PS) from the inner side of the plasma membrane to the outer layer. Using single-cell analysis software FlowJo, cells that were PI negative and Annexin-V negative were considered viable, healthy cells, while those that were PI negative and Annexin-V positive were considered apoptotic cells. The percentage of viable cells for each cell line was calculated and compared.

## Results

Based on the percentage of viable cells, there appears to be increased drug sensitivity in the Bcl-2 KO and Bcl-xL KO cells. At a concentration of 3nm of bortezomib, about 90% of the parental cells, 60% of the parental Bcl-2 KO, and 40% of the Bcl-xL KO were viable. While attempting to run a co-immunoprecipitation test with Bim and Bcl-xL, there were technical issues with the reagent.

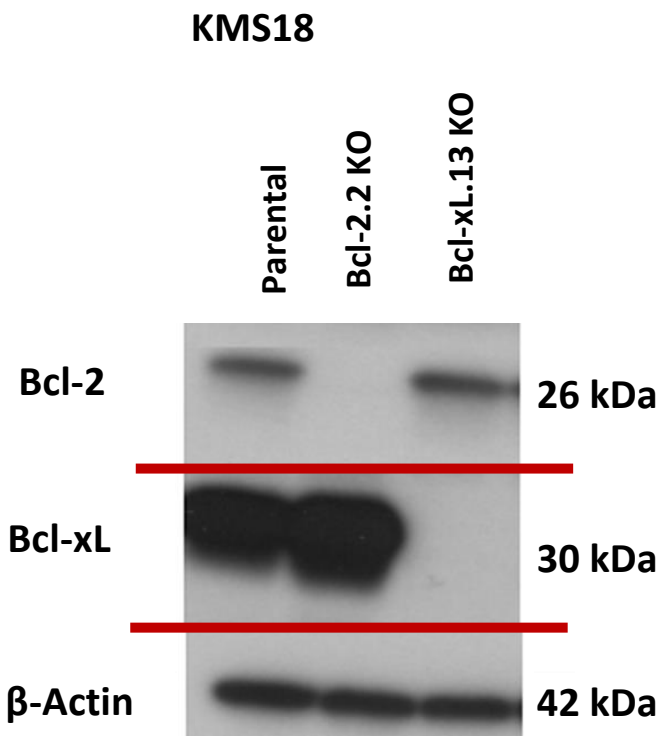


**Figure 1. Western Blot Verification of Bcl-2 KO and Bcl-xL KO in KMS18 cell line.**

CRISPR/Cas9 technology was utilized to knockout Bcl-2 and Bcl-xL in KMS18 cells. The knockout of these genes was confirmed by western blotting. β-Actin was used as a loading control.

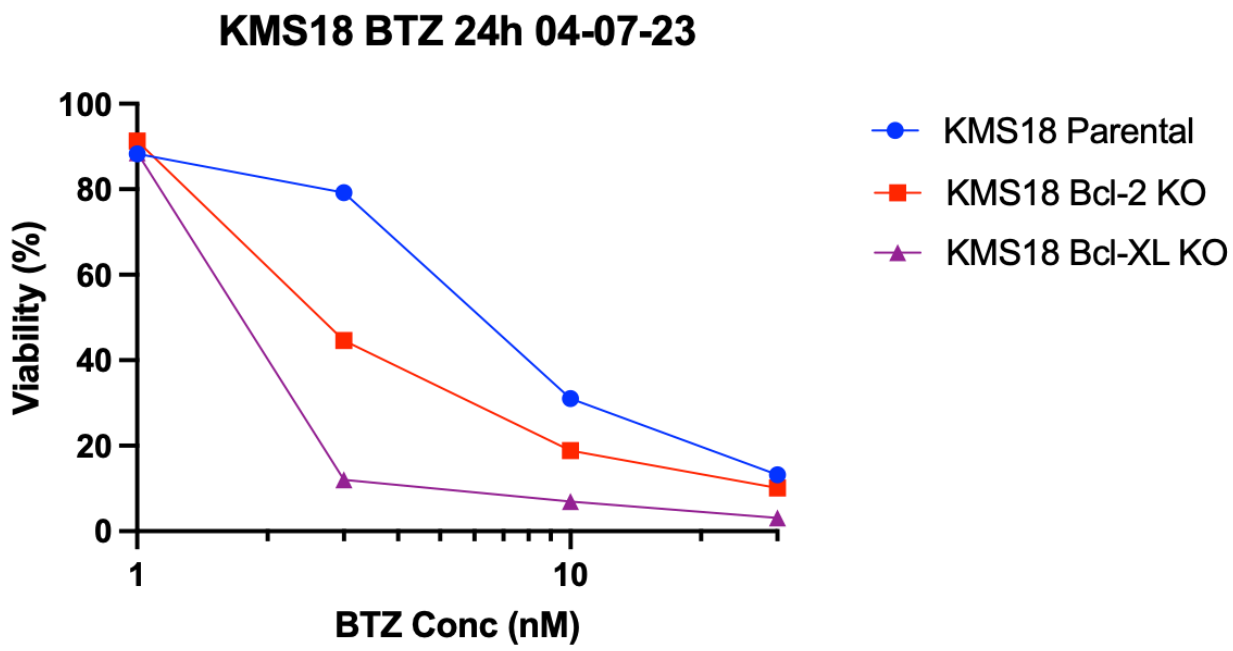


**Figure 2. First Trial of Dose-Curve Bortezomib with KMS18 Parental, Bcl-2 KO, and Bcl-xL KO cells.** KMS18 parental (blue), Bcl-2 KO (red), and Bcl-xL KO (purple) were exposed to 1, 3, 10, 15 nM of bortezomib. The viability of each cell line was measured with each cell line having decreasing cell viability as the dose of bortezomib increased. The Bcl-xL cells were the least viable cell line with increasing bortezomib.

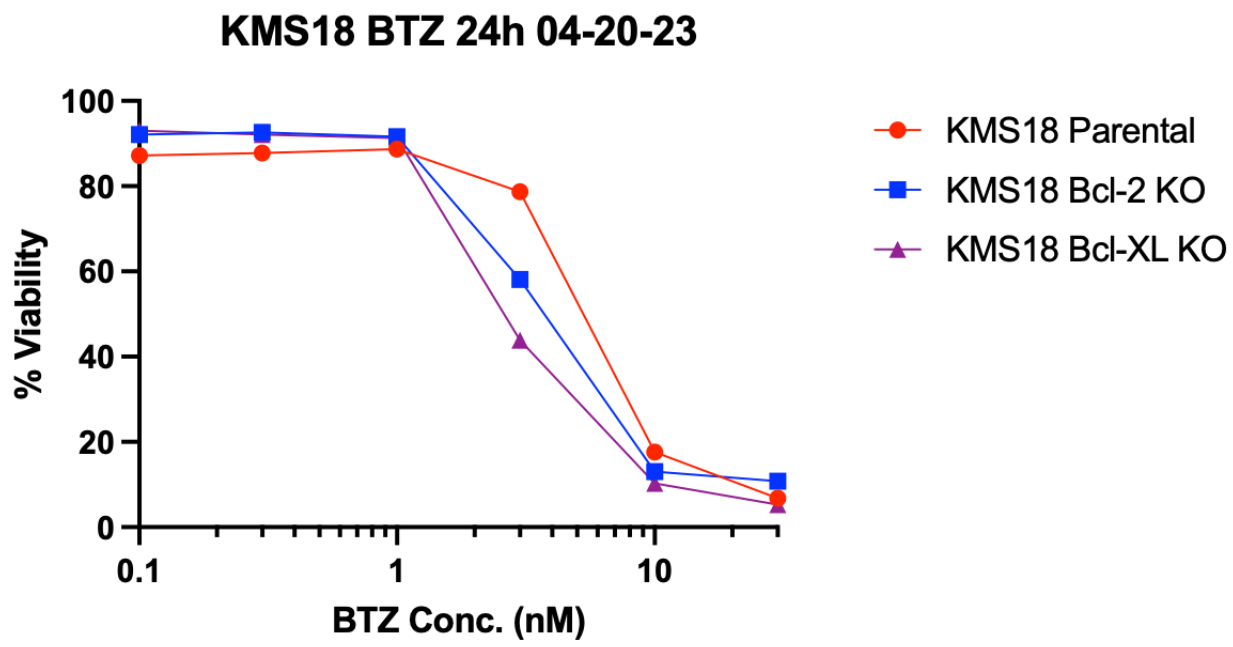


**Figure 3. Second Trial of Dose-Curve Bortezomib with KMS18 Parental, Bcl-2 KO, and Bcl-xL KO cells.** When treated with bortezomib at concentrations of 1, 3, 10, and 15 nM, the viability of KMS18 parental (blue), Bcl-2 knockout (KO) (red), and Bcl-xL KO (purple) cell lines was assessed. As the dosage of bortezomib escalated, a decline in cell viability was observed across all cell lines, with the Bcl-xL knockout cells demonstrating the most significant reduction in viability in response to increasing levels of bortezomib.

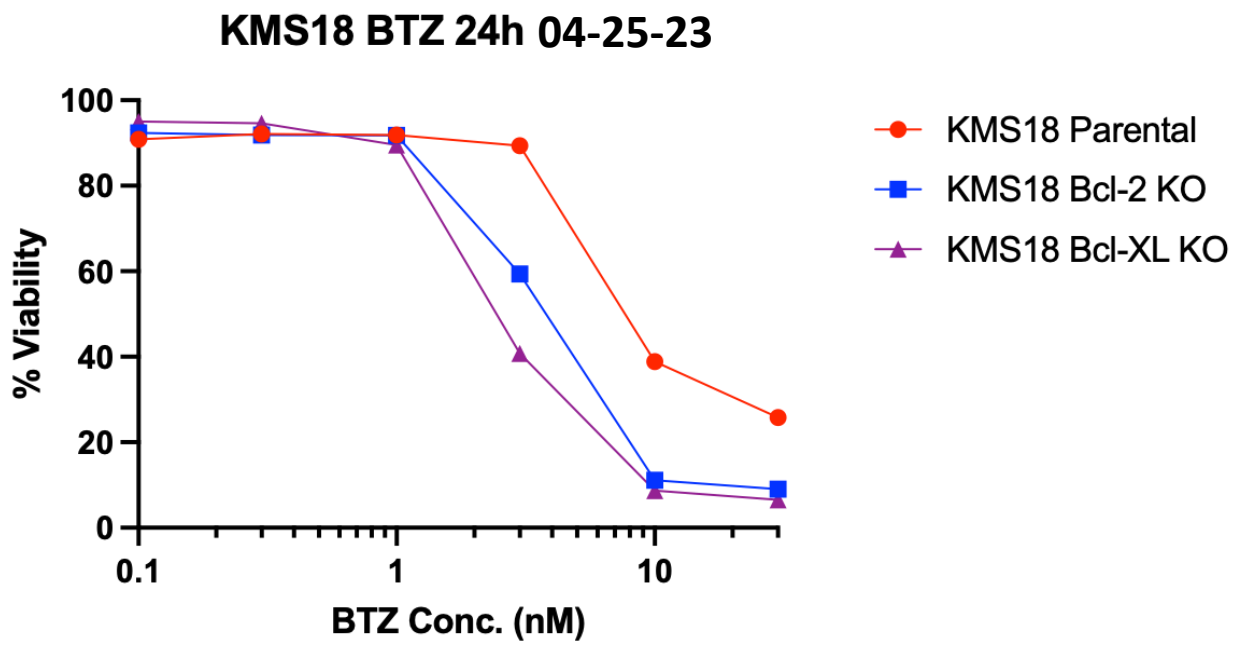


**Figure 4. Third Trial of Dose-Curve Bortezomib with KMS18 Parental, Bcl-2 KO, and Bcl-xL KO cells.** Exposure to bortezomib doses of 1, 3, 10, and 15 nM led to a dose-dependent decrease in cell viability among KMS18 parental (blue), Bcl-2 KO (red), and Bcl-xL KO (purple) cell lines. Notably, the Bcl-xL knockout cells exhibited the greatest decrease in viability, indicating heightened sensitivity to escalating doses of bortezomib compared to the other cell lines.

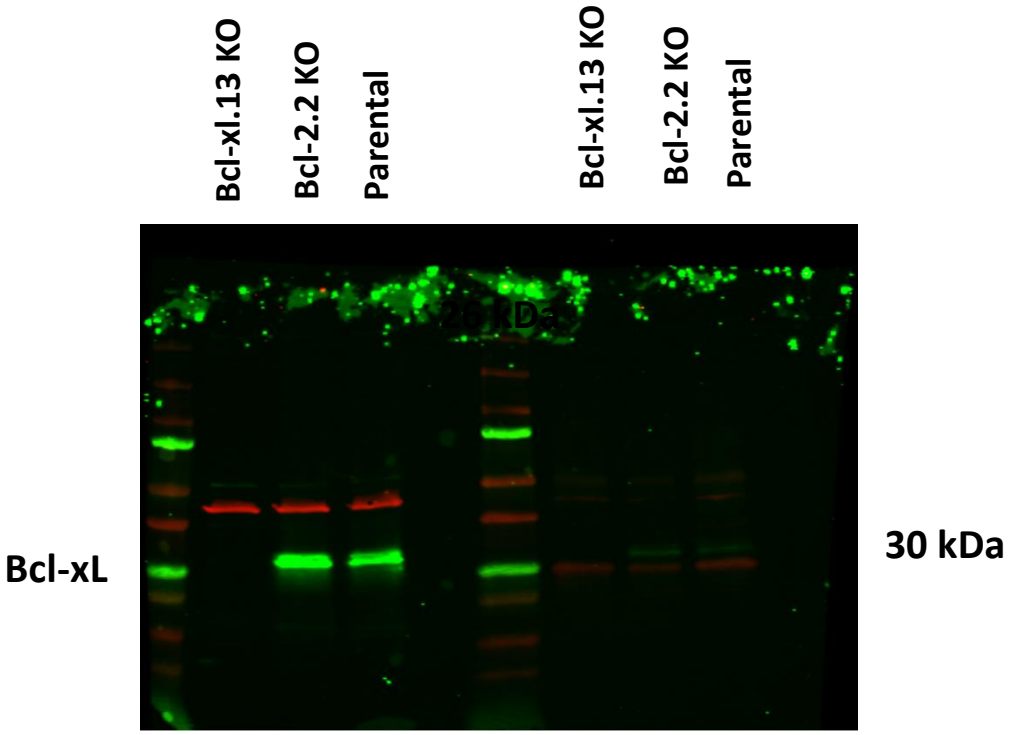


**Figure 5. Bim co-IP Actin and Bcl-xL.** Visualization of the Bim-Bcl-xL protein-protein interactions using co-immunoprecipitation. Beta-actin was used as a loading control.

## Discussion

The current study highlights the pivotal role of Bcl-2 and Bcl-xL in the drug resistance mechanisms of multiple myeloma cells. By utilizing CRISPR/Cas9-mediated knockouts, we demonstrated that the deletion of these anti-apoptotic genes renders multiple myeloma cells more susceptible to drug-induced apoptosis. This observation aligns with the broader understanding of cancer pathophysiology, particularly the hallmark of evading apoptosis, which is a critical driver of cancer progression and resistance to therapy (Hanahan & Weinberg, 2011). Our findings are not only important for understanding the intricate survival mechanisms of

multiple myeloma cells but also open avenues for targeted therapeutic strategies aimed at sensitizing these cells to existing treatments.

The role of Bcl-2 and Bcl-xL in cancer biology extends beyond multiple myeloma, as these proteins are key players in the regulation of apoptosis across various cancer types. Their overexpression has been correlated with increased resistance to chemotherapy and poor prognosis, underscoring the universal importance of targeting these pathways (Adams & Cory, 2018). Therefore, our study contributes to a growing body of evidence supporting the therapeutic potential of inhibiting anti-apoptotic genes in cancer treatment.

However, our research encountered several limitations that must be acknowledged. The inadvertent switching of cell lines during maintenance underscores the challenges of working with in vitro models and highlights the need for rigorous quality controls in experimental protocols. Furthermore, difficulties with co-IP reagents point to the complexities of studying protein-protein interactions, a fundamental aspect of deciphering the functional consequences of gene knockouts. These limitations, while affecting our study's progression, also reflect common hurdles in the field, emphasizing the importance of methodological advancements and validation strategies.

Looking forward, our preliminary results pave the way for further investigation into the role of anti-apoptotic genes in multiple myeloma and other cancers. Future studies could explore the combinatorial knockdown of Bcl-2 and Bcl-xL, as well as other Bcl-2 family members, to evaluate synergistic effects on drug sensitivity. The knockout effect on cells' ability to effectively proliferate could also be studied using clonogenicity assays. Additionally, the

development of more sophisticated models, including patient-derived xenografts and organoids, could provide deeper insights into the in vivo relevance of these findings.

The translational potential of targeting Bcl-2 and Bcl-xL in multiple myeloma is significant. Given the complex interplay between multiple myeloma cells and the bone marrow microenvironment, strategies that sensitize cancer cells to apoptosis could enhance the efficacy of existing therapies. This approach aligns with the current trend toward personalized medicine, where understanding a patient's specific genetic and molecular profile guides treatment choices.

Our study presents additional proof that anti-apoptotic genes, particularly Bcl-2 and Bcl-xL, are vital in drug resistance among patients with multiple myeloma. Despite encountering some limitations, our discoveries highlight the possibility of targeting these pathways to overcome therapy resistance. In the future, it is essential to delve deeper into these mechanisms, establish more dependable experimental models, and transform this knowledge into clinical approaches that can improve patient outcomes in multiple myeloma and potentially other cancer types.

## References


Adams, J. M., & Cory, S. (2018). The BCL-2 arbiters of apoptosis and their growing role as cancer targets. *Cell Death Differ*, *25*(1), 27-36. https://doi.org/10.1038/cdd.2017.161

Cooper, G. M. (2000). *The Cell: A Molecular Approach* (2nd ed.). Sinauer Associates. https://www.ncbi.nlm.nih.gov/books/NBK9839/

Devarakonda, S., Efebera, Y., & Sharma, N. (2021). Role of Stem Cell Transplantation in Multiple Myeloma. *Cancers (Basel)*, *13*(4). https://doi.org/10.3390/cancers13040863

Fairfield, H., Falank, C., Avery, L., & Reagan, M. R. (2016). Multiple myeloma in the marrow: pathogenesis and treatments. *Ann N Y Acad Sci*, *1364*(1), 32-51. https://doi.org/10.1111/nyas.13038

Hanahan, D., & Weinberg, Robert A. (2011). Hallmarks of Cancer: The Next Generation. *Cell*, *144*(5), 646-674. https://doi.org/10.1016/j.cell.2011.02.013

Kaseb, H., Annamaraju, P., & Babiker, H. M. (2024). Monoclonal Gammopathy of Undetermined Significance. In *StatPearls*. StatPearls Publishing.

Padala, S. A., Barsouk, A., Barsouk, A., Rawla, P., Vakiti, A., Kolhe, R., Kota, V., & Ajebo, G. H. (2021). Epidemiology, Staging, and Management of Multiple Myeloma. *Med Sci (Basel)*, *9*(1). https://doi.org/10.3390/medsci9010003

Rajkumar, S. V., Dimopoulos, M. A., Palumbo, A., Blade, J., Merlini, G., Mateos, M. V., Kumar, S., Hillengass, J., Kastritis, E., Richardson, P., Landgren, O., Paiva, B., Dispenzieri, A., Weiss, B., LeLeu, X., Zweegman, S., Lonial, S., Rosinol, L., Zamagni, E., . . . Miguel, J. F. (2014). International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*, *15*(12), e538-548. https://doi.org/10.1016/s1470-2045(14)70442-5

Sonneveld, P. (2017). Management of multiple myeloma in the relapsed/refractory patient. *Hematology Am Soc Hematol Educ Program*, *2017*(1), 508-517. https://doi.org/10.1182/asheducation-2017.1.508