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Mcl-1 inhibition modulates ERK-mediated resistance in Multiple Myeloma

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Abstract

Novel multiple myeloma (MM) treatments have significantly improved over the previous several decades, primarily on account of targeting bone marrow microenvironment (BMM) pathways. However, drug resistance and patient relapse remain major clinical problems. The role of BMM in the upregulation of anti-apoptotic protein Mcl-1 is well documented. The Mcl-1 protein plays a critical role in the progression and acquired drug resistance in MM. The regulation of Mcl-1, a protein characterized by a short half-life, from transcription to degradation is crucial for understanding its role in cell survival. The GSK3β and Erk play important role in the stability of Mcl-1. Also, overexpression of phospho Erk is associated with the acquired resistance. In this study, we investigated Mcl-1 regulation, focusing on transcriptional and post-translational modifications and their impact on protein stability in Mcl-1 inhibitor (KS18) treated cells. The small molecule inhibitor KS18 induces Mcl-1$^{Ser159/Thr163}$ phosphorylation and ubiquitination resulting in a sharp decline in Mcl-1 protein levels. Furthermore, we assessed the effects of the KS18 in a combination with ERK inhibitors on cell viability and found that blocking the Mcl-1 stabilization mechanism improves the effectiveness and potency of KS18. Furthermore, we compared KS18 to different classes of chemotherapeutic agents, such as GSK3β/α inhibitor (LY209031), ERK inhibitor (SEH77272), MEK inhibitor (PD18435), and Akt inhibitor (AZD5363). Interestingly, we found KS18 more potent than other agents. Combined, our results propose a strong rationale for novel combination therapies using selective KS18 and ERK inhibitors, which have the potential to markedly improve the outcome of MM treatment. This may also address one of the major clinical problems drug resistance and enhance the use of existing drugs.

Introduction

- According to the American Cancer Society 2023 estimation, approximately 35 thousand new MM cases will be diagnosed, and 13 thousand death cases will occur from MM in the United States.
- The interaction of myeloma cells to bone marrow microenvironment (BMM) is the hallmark of MM. This interaction what makes MM is challenging to treat and all patients experience relapse.
- MM cells receive crucial signals from the BMM to overexpress anti-apoptotic Bcl-2 proteins, particularly Mcl-1, and ultimately evade apoptosis.
- Mcl-1 protein overexpression plays a crucial role in MM tumor initiation, progression, and drug resistance (1,2).
- Among MM patients, 52% had overexpressed Mcl-1 upon diagnosis, and 81% had done so after relapse.
- Mcl-1 overexpression is the major clinical problem for acquired resistance against various therapeutic agents (3-5).
- The regulation of Mcl-1, a protein characterized by a short half-life, from transcription to degradation is crucial for understanding its role in cell survival.
- ERK induction provides a compensatory mechanism by mediating Mcl-1 phosphorylation at T163 and upregulating of Mcl-1 (6).
- Despite the development of various Mcl-1 inhibitors to kill myeloma cells, no molecules have been approved for clinical use. Thus, more effective Mcl-1 inhibitors are needed to prolong the survival of MM patients.
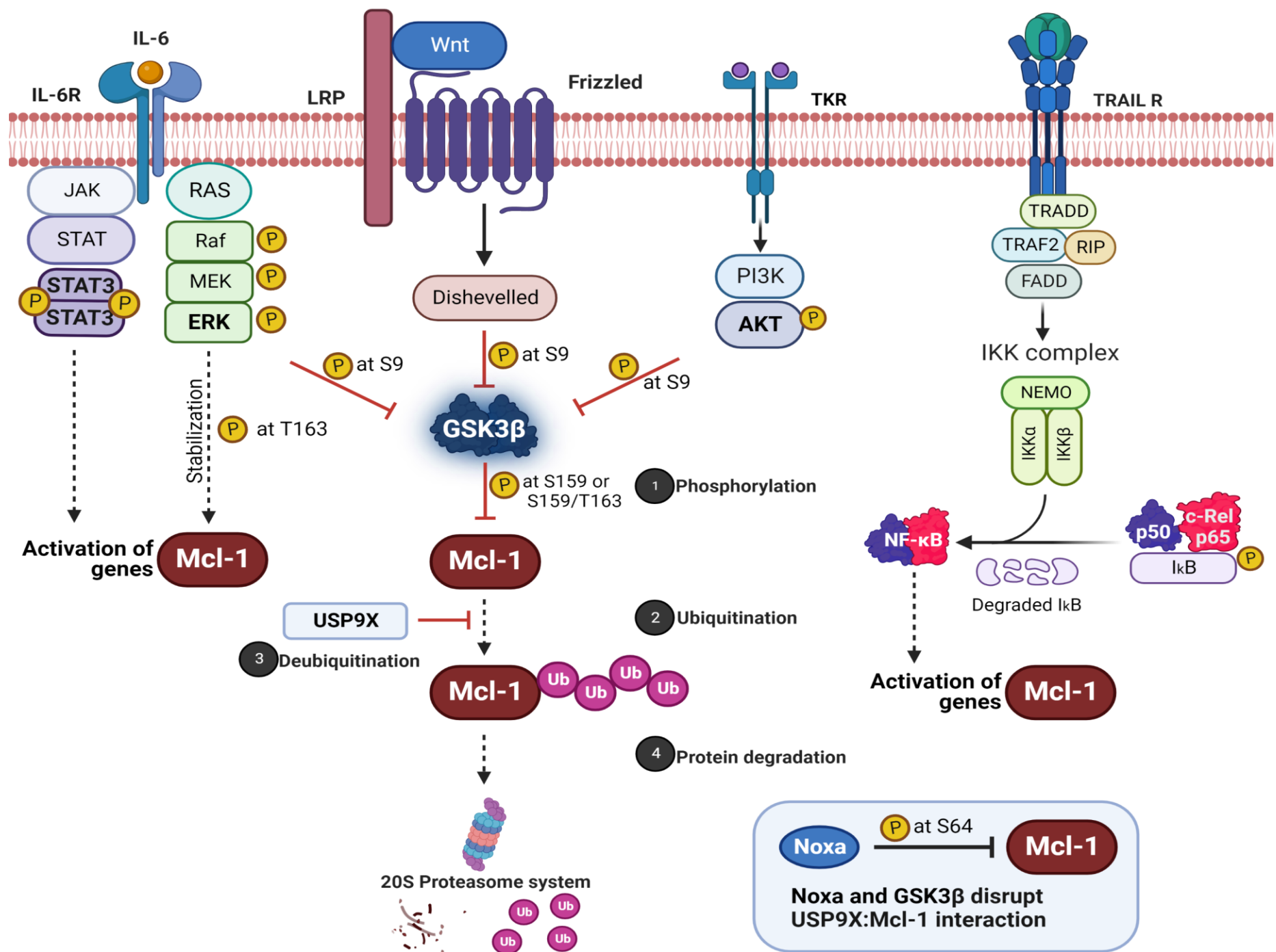
Results

Fig 1. The role of bone marrow microenvironment (BMM) in Mcl-1 regulation. BMM facilitates the long-term survival of MM. Stromal cells in BM regulate Mcl-1 anti-apoptotic protein by secreting various signaling molecules such as IL-6, which triggers JAK/STAT and Ras/MAPK pathways, leading to the upregulation of Mcl-1. GSK3β regulates Mcl-1 phosphorylation (S159/T163) and ubiquitin/proteasome–dependent protein degradation system (UPS).

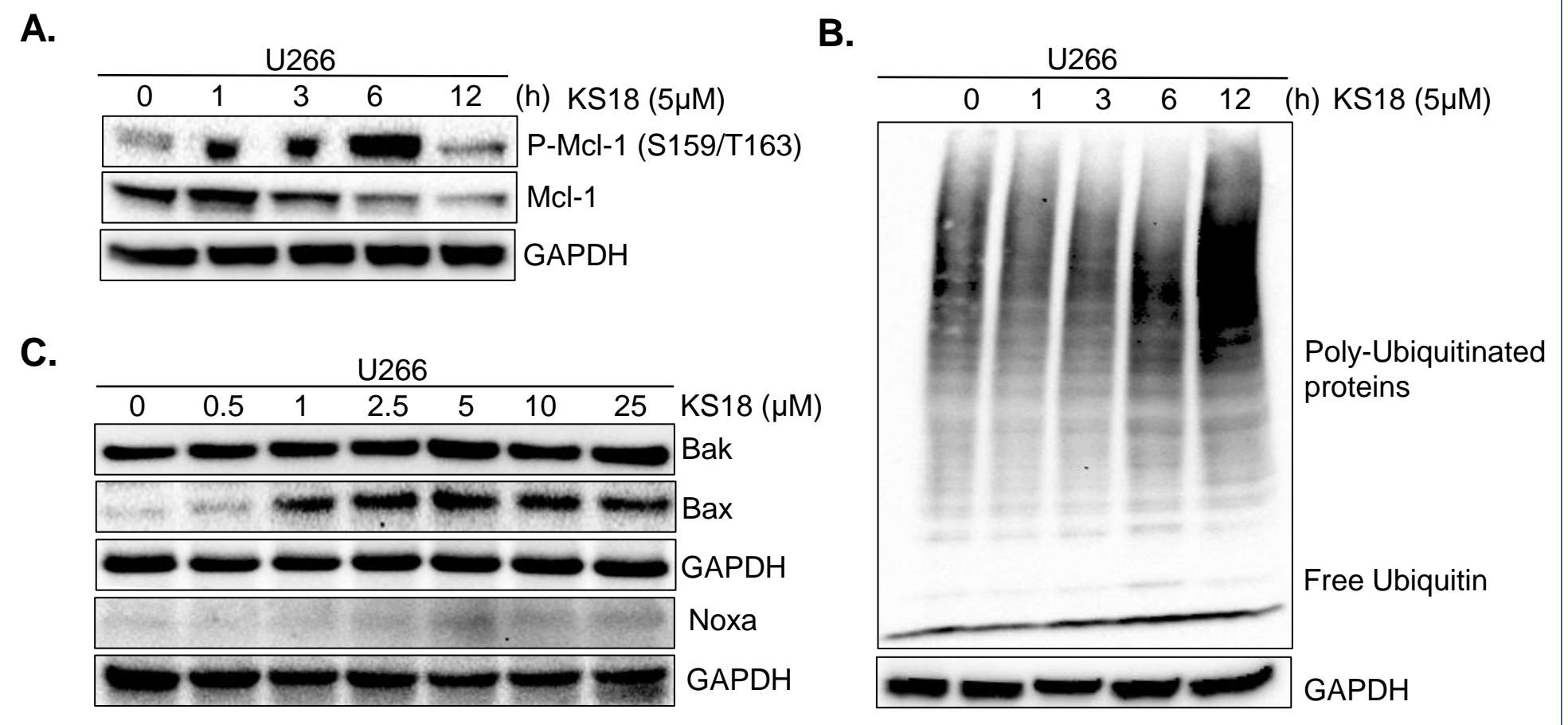### KS18 induces Mcl-1$^{Ser159/Thr163}$ phosphorylation and ubiquitination

Fig 2. KS18 inhibits Mcl-1 through UPS. **A&B,** U266 cells were treated with 5μM KS18 (0-12) hours then western blot was performed to investigate the effect of KS18 on Mcl-1 phosphorylation and ubiquitination. **C,** KS18 induces Bax in a dose-dependent manner. U266 cells were treated with KS18 (0-25) μM for 24h then western blot was performed to investigate Bak, Bax, and Noxa pro-apoptotic proteins. As a loading control, the stripped membrane was probed with GAPDH antibody.

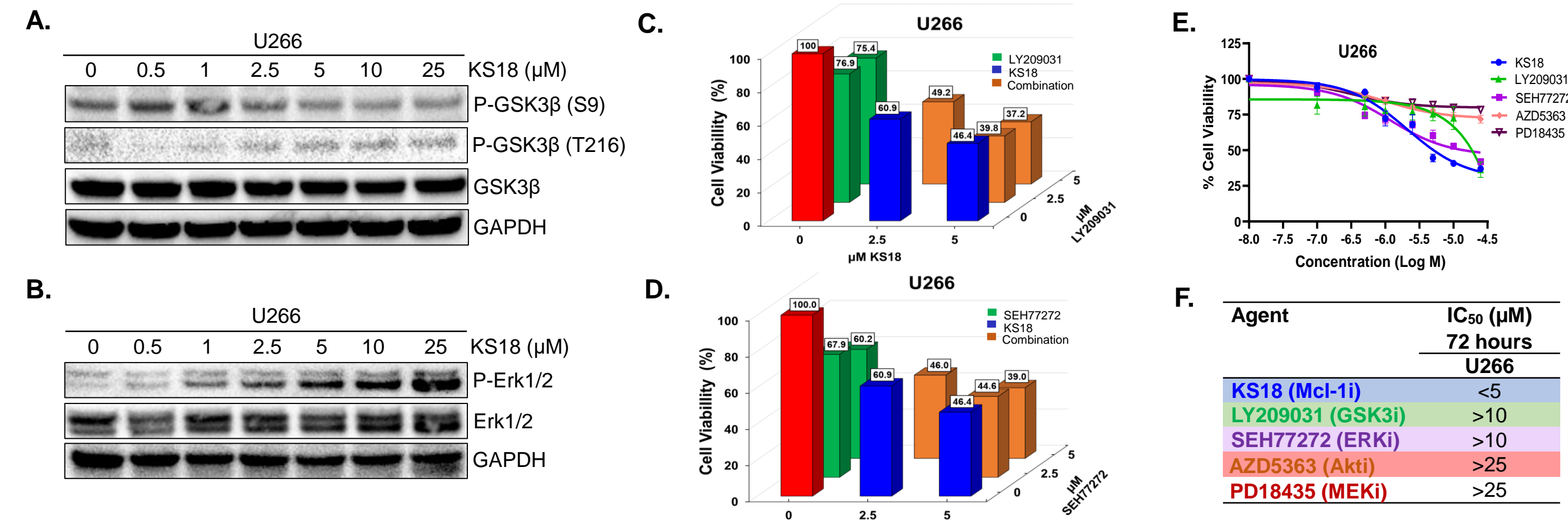## ERK and Mcl-1 inhibition synergistically reduce cell viability in MM cells

Fig 4. A&B, KS18 induces ERK activation in a dose-dependent manner. U266 cells were treated with KS18 (0-25) μM for 24h then western blot was performed to investigate P-GSK3β, GSK3β, P-ERK, and ERK. As a loading control, the stripped membrane was probed with GAPDH antibody. **C&D,** U266 cell line was treated with increasing doses of KS18 (0–25 μM) in combination with GSK3β/α inhibitor (LY209031) and ERK inhibitor (SEH77272) for 72h, and cell viability was measured by MTT assay. 3D Graphical representations were performed by Microsoft Excel. **E&F,** U266 cell line was treated with increasing doses of KS18, LY209031, SEH77272, PD18435, and AZD5363 (0–25 μM) for 72h, and cell viability was measured by MTT assay. Graphical representation and IC$_{50}$ calculation were performed by GraphPad.

Agent	IC$_{50}$ (μM) 72 hours U266
KS18 (Mcl-1i)	<5
LY209031 (GSK3i)	>10
SEH77272 (ERKi)	>10
AZD5363 (Akti)	>25
PD18435 (MEKi)	>25

Conclusion and Future Directions

- KS18 induces Mcl-1$^{Ser159/Thr163}$ phosphorylation and ubiquitin/proteasome–dependent protein degradation system (UPS)
- KS18 induces MM cell death in a mitochondrial outer membrane permeabilization (MOMP) pathway by selectively targeting Mcl-1.
- No disruption of Mcl-1: Noxa interaction followed by KS18 treatment.
- ERK induction by KS18 provides a compensatory mechanism and triggers Mcl-1^{Thr163} phosphorylation, which contributes to Mcl-1 stability.
- KS18 improves the therapeutic outcome and enhances the efficacies of ERK inhibitors.
- KS18 is a sufficient cell viability redactor with IC$_{50}$ less than 5μM.
- Our future studies will uncover the mechanism of action and provide the foundation for *in vivo* assessment of KS18.
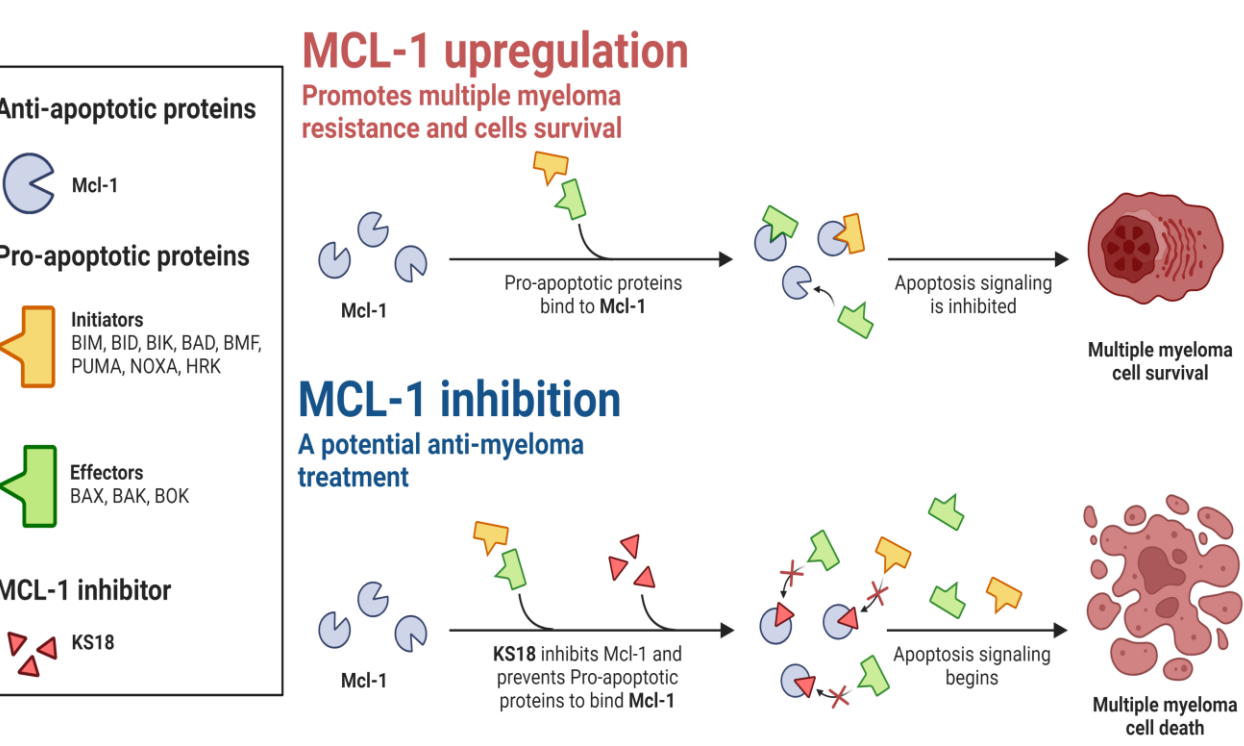
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Acknowledgment

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