The 2025 San Antonio Breast Cancer Symposium - December 9-12, 2025

The Role of Washout Efficiency in Minimizing Toxicity of Cytotoxic Anticancer Drugs: A Study on CMPD1

Poster Number D31

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Background

Microtubule-targeting agents (MTAs) such as Taxol have been central to the treatment of various cancers, including breast cancer. However, their clinical utility is often limited by significant off-target toxicity, including myelosuppression, neuropathy, and organ damage, due to their indiscriminate action on both malignant and healthy dividing cells. To improve the therapeutic index of such agents, a new generation of drugs must achieve cancer cell selectivity without compromising efficacy.

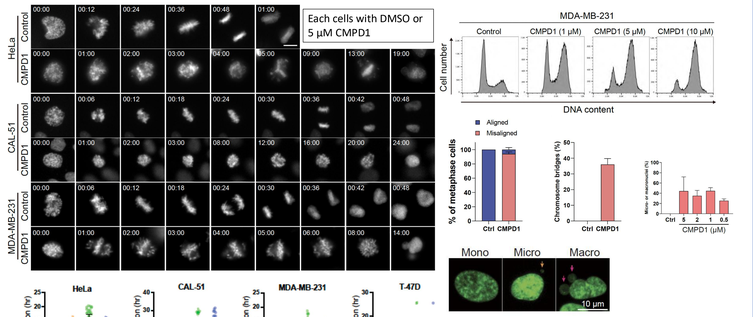
The p38-MK2 signaling pathway plays a key role in cell cycle progression and stress response and is frequently activated in cancer cells. Inhibiting this pathway has emerged as a promising approach to sensitize cancer cells to cytotoxic agents. CMPD1, a dual-function inhibitor of both MK2 and microtubule dynamics, demonstrates a unique property: it causes irreversible mitotic arrest selectively in cancer cells, while allowing normal cells to recover after drug withdrawal. This is largely due to its differential washout efficiency, wherein cancer cells exhibit prolonged drug retention and mitotic disruption, while normal cells rapidly regain normal division.

Methods

- **Cell Line Models**
  Breast cancer cell lines (MDA-MB-231, CAL-51, T-47D) and non-transformed human epithelial cells (MCF10A, RPE1) were cultured and treated with CMPD1 or control agents (DMSO, Taxol, vinblastine). Time-lapse live-cell imaging was performed to assess mitotic progression and duration.
- **Washout Assays**
  Cells were exposed to 2 μM CMPD1 for 12 hours, followed by drug washout. Recovery of mitotic progression was monitored to evaluate residual drug effects and washout efficiency in cancer vs normal cells.
- **Mitotic Error and Apoptosis Quantification**
  Chromosomal segregation errors (misalignment, lagging chromosomes, etc.) were quantified from live-cell imaging data. Apoptotic cell death post-mitosis was also recorded.
- **In Vivo Xenograft Models**
  Orthotopic breast tumor models were generated in immunodeficient mice via injection of MDA-MB-231 or CAL-51 cells. Mice were treated with CMPD1 or Taxol for up to three weeks. Tumor growth, body weight, and survival were monitored.
- **Toxicity Assessment**
  At study endpoints, liver and kidney function were assessed by serum biochemistry (AST, ALT, BUN, creatinine) and histopathological analysis. White blood cell counts were measured to evaluate bone marrow suppression.
- **RNA-Seq and Pathway Analysis**
  RNA-sequencing was performed on cancer (MDA-MB-231) and normal (RPE1) cells after CMPD1 exposure. Differentially expressed genes were analyzed using DESeq2 and GO enrichment analysis to identify pathways selectively affected in cancer cells.
- **Microtubule Dynamics Imaging**
  Total Internal Reflection Fluorescence Microscopy (TIRFM) was used to observe in vitro microtubule dynamics in response to CMPD1 and vinblastine, focusing on plus- vs minus-end depolymerization.
- **Migration and Invasion Assays**
  Wound healing and transwell invasion assays were used to evaluate the effect of CMPD1 on cancer cell motility and invasiveness in vitro and in xenografted tumor sections.

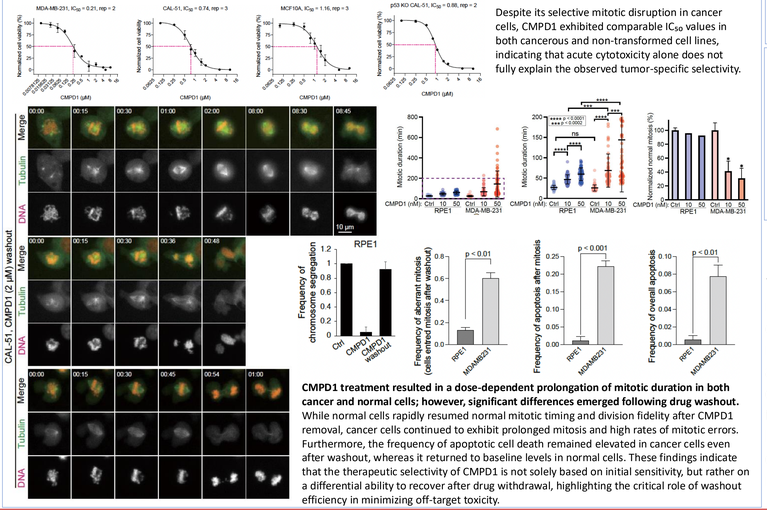
Results

1. CMPD1 induces severe mitotic arrest in different cell types



Representative images of H2B-EGFP RPE1 cells harboring normal nucleus, micronuclei, or macronuclei. Frequency of micro/macro or multi-nuclei.

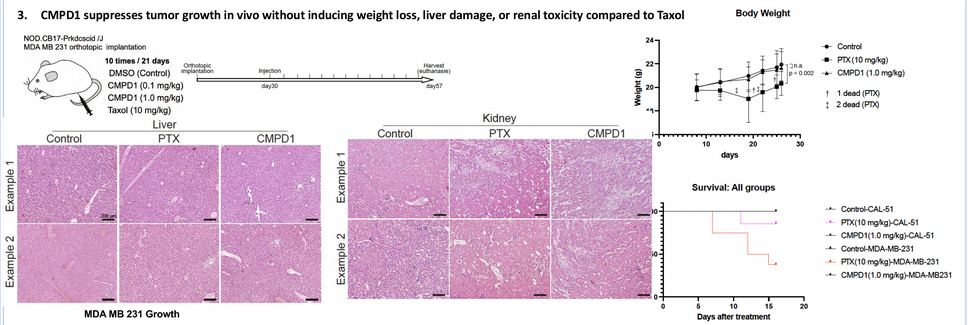
2.CMPD1 selectively induces mitotic defects in cancer cells but spares normal cells through differential washout recovery

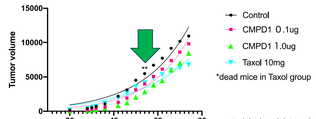


Despite its selective mitotic disruption in cancer cells, CMPD1 exhibited comparable IC_{50} values in both cancerous and non-transformed cell lines, indicating that acute cytotoxicity alone does not fully explain the observed tumor-specific selectivity.

CMPD1 treatment resulted in a dose-dependent prolongation of mitotic duration in both cancer and normal cells; however, significant differences emerged following drug washout. While normal cells rapidly resumed normal mitotic timing and division fidelity after CMPD1 removal, cancer cells continued to exhibit prolonged mitosis and high rates of mitotic errors. Furthermore, the frequency of apoptotic cell death remained elevated in cancer cells even after washout, whereas it returned to baseline levels in normal cells. These findings indicate that the therapeutic selectivity of CMPD1 is not solely based on initial sensitivity, but rather on a differential ability to recover after drug withdrawal, highlighting the critical role of washout efficiency in minimizing off-target toxicity.

3. CMPD1 suppresses tumor growth in vivo without inducing weight loss, liver damage, or renal toxicity compared to Taxol

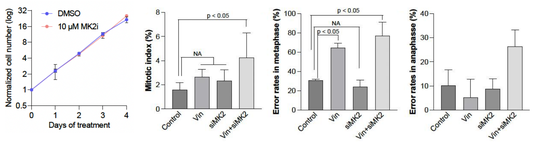




Sample	UN(mg/dL)	CRE(mg/dL)	AST(IU/L)	ALT(IU/L)	r-GTP(IU/L)	WBC
Ctrl	26.55	0.195	5617.5	3405.5	<6	124.8
CMPD1	27.4	0.275	7685	4300	<6	156
PTX	33.7	0.235	16749	10937.5	<3	35.2

In the in vivo xenograft experiments using MDA MB 231, CMPD1 exhibited comparable antitumor efficacy to Taxol despite being administered at only 1/100th the dose. While Taxol-treated mice began to show significant body weight loss around day 20 of treatment, mice treated with CMPD1 maintained stable body weight and appeared visibly healthier throughout the study. Hematological analysis revealed that Taxol caused marked reductions in blood cell counts, whereas no such reductions were observed in the CMPD1 group. Additionally, the Taxol group showed elevated transaminase levels and histological evidence of liver damage, while the CMPD1-treated mice displayed neither biochemical nor structural signs of hepatic injury. Importantly, deaths occurred in the Taxol-treated group during the course of treatment, but no mortality was observed in the CMPD1 group. These findings underscore CMPD1's superior safety profile and its potential as a low-dose, low-toxicity alternative to conventional MTAs like Taxol.

3. MK2 Inhibition Enhances Mitotic Stress and Antitumor Efficacy in Combination with Microtubule Inhibitors



While MK2 inhibition alone does not significantly alter mitotic progression in cancer cells, its combination with microtubule inhibitors such as vinblastine substantially amplifies mitotic stress. This enhanced stress leads to a higher frequency of abnormal mitotic events, including chromosome missegregation and mitotic slippage, thereby increasing apoptotic cell death in dividing cancer cells. These findings suggest that the MK2 pathway acts as a critical buffer against mitotic stress, and its inhibition renders cancer cells more vulnerable to the effects of microtubule-targeting agents. Therefore, the dual inhibition of MK2 and microtubule function represents a promising strategy to potentiate antitumor efficacy while maintaining a favorable safety profile.

Conclusions

This study identifies CMPD1 as a dual inhibitor of the p38-MK2 pathway and microtubule dynamics that selectively disrupts mitosis in cancer cells while sparing normal cells. This selectivity is attributed not to differences in acute cytotoxicity, but to differential washout efficiency: cancer cells fail to recover from transient mitotic arrest, whereas normal cells quickly resume normal mitosis after drug removal. In vivo, CMPD1 demonstrated antitumor efficacy comparable to Taxol despite being administered at only 1/100th the dose, and was associated with no body weight loss, no hematological toxicity, no liver or renal damage, and no treatment-related deaths—unlike Taxol. Furthermore, while MK2 inhibition alone had minimal impact on mitotic timing, its combination with microtubule inhibitors markedly increased mitotic stress and abnormal mitoses in cancer cells, highlighting a synergistic therapeutic potential. Together, these findings position CMPD1 as a highly promising therapeutic candidate with both enhanced safety and efficacy, and underscore the importance of washout efficiency and stress-adaptation mechanisms as key parameters in cytotoxic drug design.

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