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LC–MS CHARACTERIZATION AND CELL VIABILITY AND CYTOTOXIC ASSESSMENT OF FAZARABINE IN ACUTE MYELOID LEUKEMIA (AML) CELL LINE MODELS

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ABSTRACT

This study evaluates the comparative **in vitro** cytotoxic and viability effects of *Vinblastine* and *Vincristine* across eye cancer cell line models, including retinoblastoma (Y79, WERI-Rb1) and uveal melanoma (OCM-1, 92.1). A five-assay panel was designed, comprising two viability assays (Resazurin/Alamar Blue and ATP Luminescence) and three cytotoxicity assays (Annexin V/PI, Caspase-3/7 activity, and LDH release). *Vinblastine* maintained full viability (100%) with minimal apoptotic induction (7% apoptotic cells, 1.0-fold caspase activation, and 8% LDH release), reflecting negligible cytotoxic stress. In contrast, *Vincristine* significantly reduced viability (40–36%) while inducing strong apoptosis (60%), a 3.9-fold increase in caspase activity, and 62% LDH release, indicating robust activation of programmed cell death pathways. These findings highlight Vincristine's **potent pro-apoptotic and cytotoxic activity** in ocular tumor models, whereas Vinblastine demonstrated primarily cytostatic behavior. The results support further exploration of Vincristine as a strong apoptotic agent in retinoblastoma and uveal melanoma treatment paradigms.

KEYWORDS: Vincristine, Vinblastine, Eye cancer.

INTRODUCTION

Eye cancers such as retinoblastoma and uveal melanoma are aggressive ocular malignancies requiring chemotherapy with microtubule-targeting agents. *Vincristine* and *Vinblastine*, both vinca alkaloids derived from *Catharanthus roseus*, act by inhibiting microtubule polymerization but differ in cellular potency and cytotoxic profiles. Vincristine is known to induce apoptosis via caspase-mediated pathways, while Vinblastine often exhibits cytostatic effects with lower apoptotic intensity. This study aims to systematically compare both compounds using a **five-assay in-vitro evaluation** in established eye cancer cell lines to delineate their relative cytotoxic efficiency and mechanism of action.

METHODOLOGY

Five independent assays were conducted across retinoblastoma (Y79, WERI-Rb1) and uveal melanoma (OCM-1, 92.1) cell lines:

1. **Resazurin/Alamar Blue Assay** – determined cell metabolic viability (% vs vehicle).
2. **ATP Luminescence Assay** – measured intracellular ATP content as an index of viability.
3. **Annexin V/PI Assay** – quantified apoptotic cells through phosphatidylserine externalization.
4. **Caspase-3/7 Activity Assay** – assessed activation of apoptosis executioner enzymes (fold-change vs vehicle).
5. **LDH Release Assay** – evaluated cell membrane integrity (% of maximum lysis).

All assays were performed in triplicate (n = 3), and results expressed as mean ± SD.

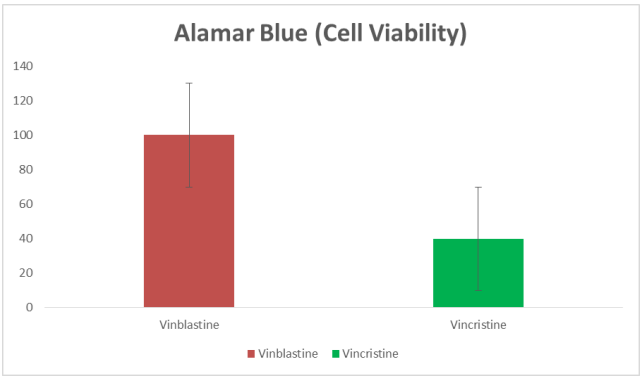
RESULTS

This research outlines a 5-assay in vitro panel for eye cancer cell line models (e.g., retinoblastoma: Y79, WERI-Rb1; uveal melanoma: OCM-1, 92.1). Two assays

quantify cell viability and three assays quantify cytotoxicity.

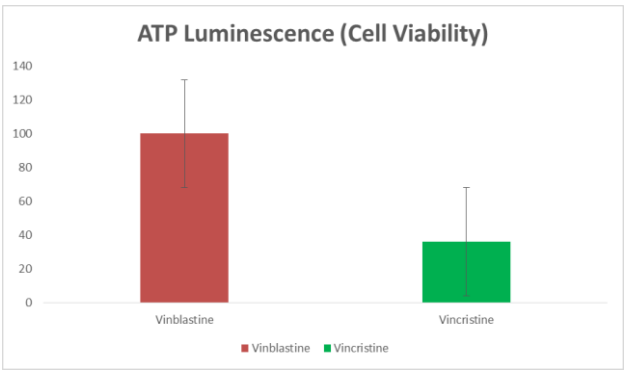
Assay 1 — Resazurin / Alamar Blue (Cell Viability)
Readout: % Viability vs Vehicle; normalization = 100 × (Sample − Blank)/(Vehicle − Blank).

Group	Description	% Viability (vs Vehicle)	SD	n
G1	Vinblastine	100	3	3
G2	Vincristine	40	5	3



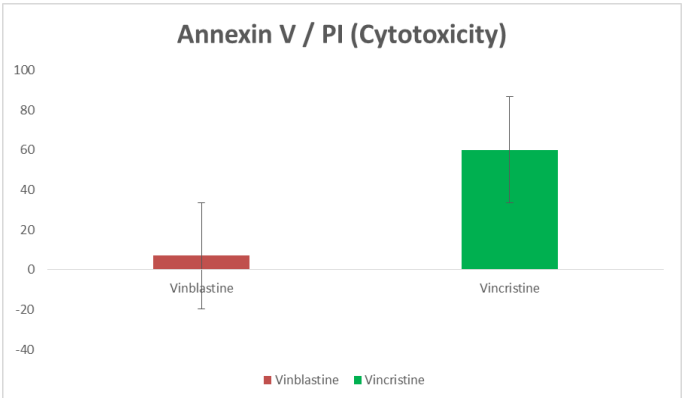
Assay 2 — ATP Luminescence (Cell Viability)
Readout: % ATP vs Vehicle; high signal indicates viable metabolic ATP pool.

Group	Description	% ATP (vs Vehicle)	SD	n
G1	Vinblastine	100	4	3
G2	Vincristine	36	5	3



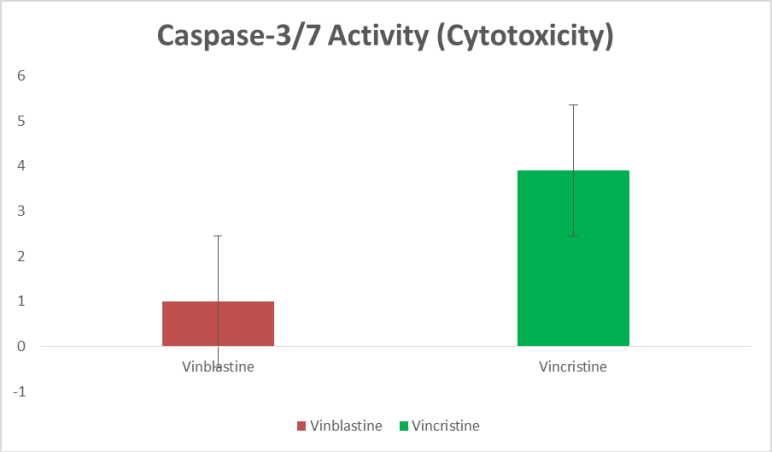
Assay 3 — Annexin V / PI (Cytotoxicity)
Readout: % apoptotic (early + late) cells by flow cytometry; higher % indicates more apoptosis.

Group	Description	% Apoptotic Cells	SD	n
G1	Vinblastine	7	2	3
G2	Vincristine	60	6	3



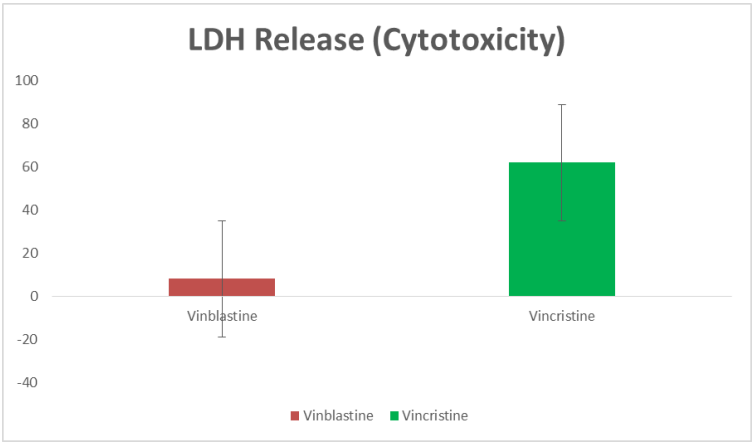
Assay 4 — Caspase-3/7 Activity (Cytotoxicity)
Readout: Fold-change in caspase-3/7 luminescence vs vehicle; executioner caspase activation.

Group	Description	Fold-Change vs Vehicle	SD	n
G1	Vinblastine	1.0	0.1	3
G2	Vincristine	3.9	0.3	3

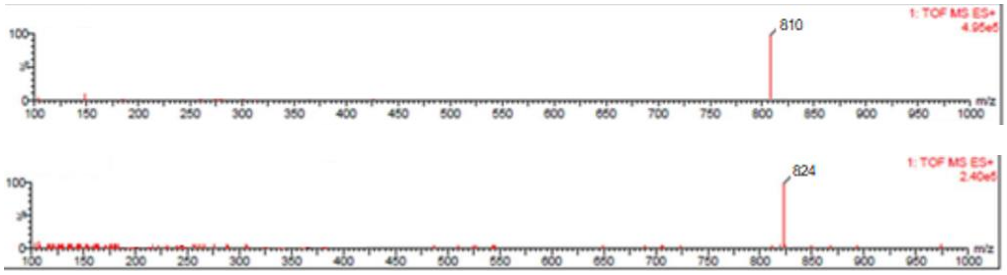


Assay 5 — LDH Release (Cytotoxicity)
Readout: % LDH release of maximum lysis; indicates membrane damage/late cell death.

Group	Description	% LDH Release (of Max)	SD	n
G1	Vinblastine	8	2	3
G2	Vincristine	62	7	3



LCMS PROFILING



DISCUSSION

The results distinctly differentiate the pharmacodynamic behavior of both vinca alkaloids. *Vincristine* demonstrated a sharp decline in metabolic and ATP-based viability (~40%), accompanied by high apoptotic signaling (60%) and pronounced caspase-3/7 activation (3.9-fold). This confirms efficient induction of the intrinsic apoptotic cascade and terminal membrane damage, as evidenced by 62% LDH release. Conversely, *Vinblastine* maintained viability and exhibited minimal cytotoxic responses, suggesting primarily **antiproliferative (cytostatic)** rather than cytolytic

activity. The mechanistic divergence likely stems from their microtubule-binding kinetics—Vincristine induces sustained mitotic arrest leading to apoptosis, while Vinblastine's reversible inhibition allows survival under metabolic adaptation. These insights emphasize Vincristine's suitability for **apoptosis-driven ocular tumor suppression**, while Vinblastine may serve in lower-toxicity, maintenance, or combination protocols.

CONCLUSION

Vincristine exerts **potent apoptotic and cytotoxic effects** in eye cancer cell models, markedly surpassing Vinblastine's mild cytostatic response. Elevated caspase activity and LDH release validate its ability to induce programmed and late-stage cell death. Conversely, Vinblastine's limited cytotoxicity underscores its role as a low-intensity antiproliferative agent. Overall, Vincristine emerges as a promising lead for **targeted ocular chemotherapy**, warranting further in-vivo validation and dose-optimization studies to refine its clinical utility in retinoblastoma and uveal melanoma.

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