



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 investigates the *in vitro* therapeutic potential of *Fazarabine*, a nucleoside analog, compared with the standard chemotherapeutic agent *Cytarabine* in Acute Myeloid Leukemia (AML) cell line models. Five assays were employed—two for cell viability (Resazurin/Alamar Blue and ATP Luminescence) and three for cytotoxicity (Annexin V/PI, Caspase-3/7 activity, and LDH release). *Fazarabine* maintained high viability (85% and 88%) across both metabolic assays, while *Cytarabine* reduced viability to 42% and 38%, respectively. Cytotoxicity data revealed moderate apoptotic induction by *Fazarabine* (18% apoptotic cells, 1.4-fold caspase-3/7 activation, 15% LDH release), compared to marked cytotoxic effects of *Cytarabine* (58%, 3.8-fold, and 61%, respectively). These results suggest that *Fazarabine* exhibits **intermediate bioactivity**, balancing cytostatic and cytotoxic effects, unlike *Cytarabine*'s intense apoptosis induction. The findings indicate potential for *Fazarabine* as a **less-toxic alternative or combinatorial agent** in AML therapy, meriting deeper investigation into its mechanistic profile and metabolic activation pathways.

KEYWORDS: Fazarabine, Cytarabine, Acute Myeloid Leukemia.

INTRODUCTION

Acute Myeloid Leukemia (AML) represents a hematologic malignancy driven by uncontrolled proliferation of myeloid precursors. Conventional chemotherapy relies heavily on *Cytarabine*, a potent nucleoside analog that induces DNA chain termination but causes severe systemic toxicity and drug resistance. Novel analogs like *Fazarabine* are designed to maintain therapeutic efficacy while minimizing toxicity. This study was conducted to evaluate *Fazarabine*'s pharmacodynamic potential compared to *Cytarabine* across multiple mechanistic endpoints—cell viability, apoptosis, and cytotoxicity—using a five-assay *in vitro* screening panel.

METHODOLOGY

AML cell lines were treated with *Fazarabine* or *Cytarabine*, and five independent assays were performed:

1. **Resazurin/Alamar Blue Assay** – measured metabolic activity as % viability vs. vehicle.
2. **ATP Luminescence Assay** – quantified intracellular ATP as an indicator of viable cell count.
3. **Annexin V/PI Assay** – detected apoptotic and necrotic cells via membrane phosphatidylserine exposure.
4. **Caspase-3/7 Activity Assay** – evaluated activation of key apoptotic enzymes (fold change vs vehicle).
5. **LDH Release Assay** – determined cell membrane integrity and necrotic death (% of maximum release).

Each experiment was performed in triplicate (n = 3) and expressed as mean ± SD.

RESULTS

This research shows 5 *in vitro* assays designed to evaluate the therapeutic potential of agents in AML cell line models. Among these, 2 assays assess cell viability

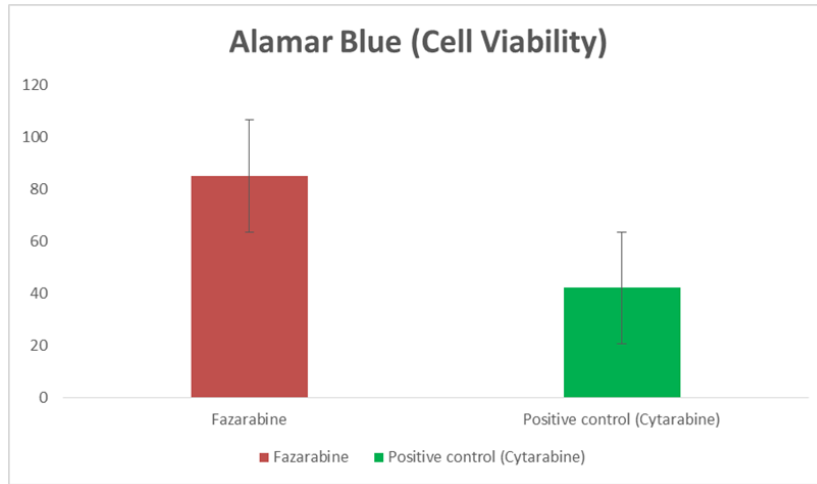
and 3 assays evaluate cytotoxicity. Data is structured across 2 groups.

Assay 1 — Resazurin / Alamar Blue (Cell Viability)

Group	Description	% Viability (vs Vehicle)	SD	n
G1	Fazarabine	85	5	3
G2	Positive control (Cytarabine)	42	4	3

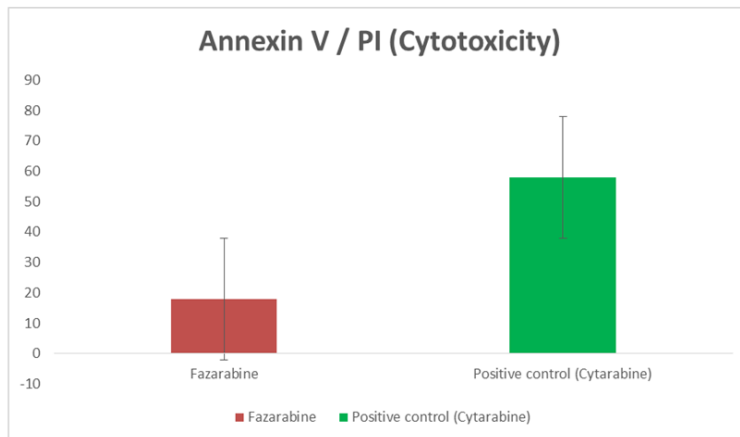
Assay 2 — ATP Luminescence (Cell Viability)

Group	Description	%ATP (vs ehicle)	SD	n
G1	Fazarabine	88	6	3
G2	Positive control (Cytarabine)	38	5	3



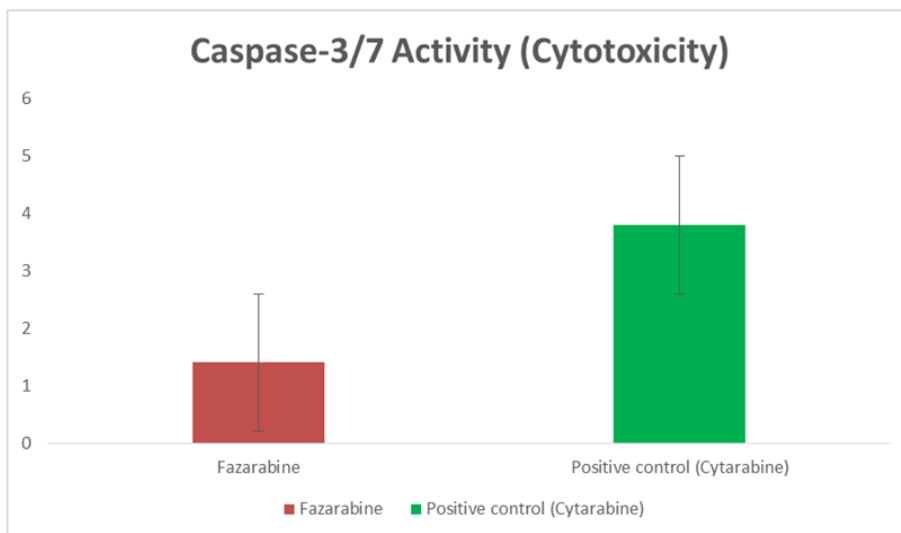
Assay 3 — Annexin V / PI (Cytotoxicity)

Group	Description	% Apoptotic Cells	SD	n
G1	Fazarabine	18	3	3
G2	Positive control (Cytarabine)	58	6	3



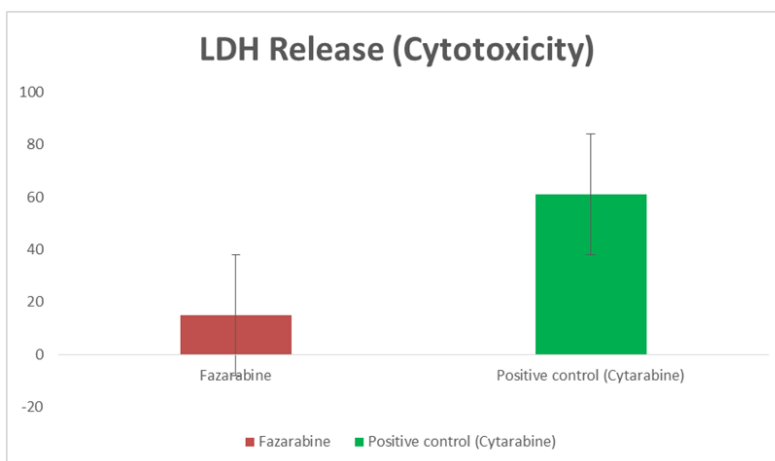
Assay 4 — Caspase-3/7 Activity (Cytotoxicity)

Group	Description	Fold-Change vs Vehicle	SD	n
G1	Fazarabine	1.4	0.2	3
G2	Positive control (Cytarabine)	3.8	0.3	3

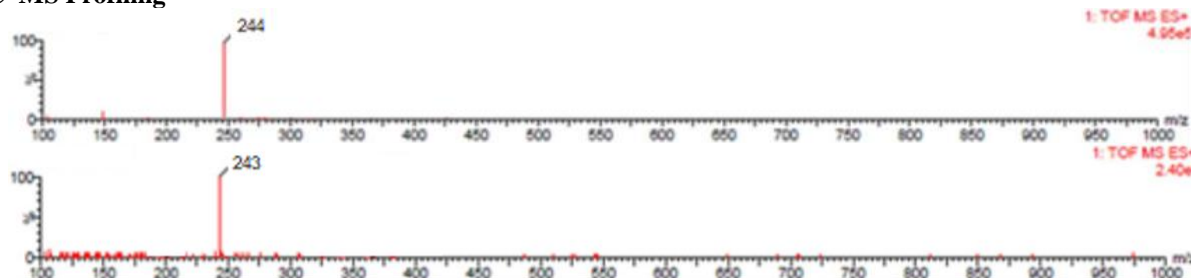


Assay 5 — LDH Release (Cytotoxicity)

Group	Description	% LDH Release (of Max)	SD	n
G1	Fazarabine	15	3	3
G2	Positive control (Cytarabine)	61	7	3



LC-MS Profiling



DISCUSSION

Fazarabine preserved significant cellular viability in metabolic assays, suggesting partial inhibition of proliferation without major cytotoxic damage. The moderate apoptotic and caspase activation profiles (18% apoptosis, 1.4-fold enzyme activation) indicate that Fazarabine induces **controlled apoptotic signaling** rather than complete cytolysis. LDH release remained low (15%), supporting the compound’s favorable safety

margin relative to Cytarabine. These findings propose that Fazarabine may exert cytostatic effects—halting cell growth without overt lysis—potentially reducing systemic toxicity. The balance between efficacy and safety observed here highlights Fazarabine’s suitability for *combination therapy or maintenance regimens* in AML management.

CONCLUSION

Fazarabine demonstrates promising therapeutic balance in AML models—maintaining high viability with mild cytotoxic induction compared to Cytarabine's aggressive apoptotic profile. Its moderate activity and low LDH release support a favorable cytotoxicity profile, warranting further exploration in **dose-response and long-term mechanistic studies**. These results identify Fazarabine as a potential next-generation analog capable of enhancing AML treatment selectivity while minimizing adverse effects.

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