Evaluating Combinatorial Effects of 13-cis-RA and Polyamine Synthesis Inhibition on Neuroblastoma Cell Fate

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Introduction

- Neuroblastoma (NB) is an aggressive pediatric cancer arising from sympathetic nervous system precursors.
- A hallmark of NB is arrested differentiation of neural crest-derived sympathoadrenal cells.
- Inducing differentiation is a promising therapeutic approach to counteract tumor progression.
- Retinoic acids: derived from vitamin A, used in maintenance therapy for NB. However, potential synergies with other agents to enhance differentiation and improve outcomes remain largely unexplored.
- Polyamines (putrescine, spermidine, spermine) are critical for NB cell proliferation.
- Depletion of PAs via difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase (ODC), disrupts oncogenic translation & reprograms translation to enhance neural differentiation and repress proliferation.
- The synergistic potential between transcriptional (RA-mediated) and translational (polyamine depletion -mediated) outcomes remains unknown.
- This study evaluates the effects of 13-cis-RA, polyamine depletion (via DFMO & arginine depletion), and combinatory treatments to assess interactions and/or synergistic effects on SH-SY5Y neuroblastoma cell differentiation and viability.

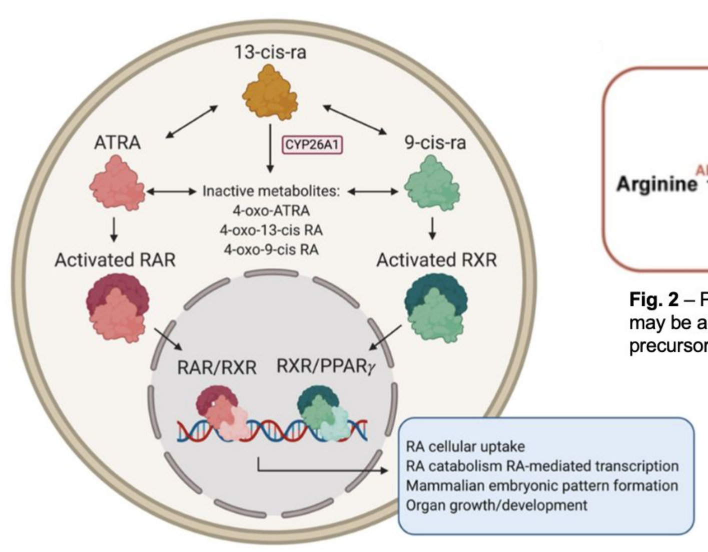

Fig. 1: RA binds nuclear receptors (RARs, RXRs) to form heterodimers that bind RA response elements (RAREs) in target gene promoters, inducing expression.

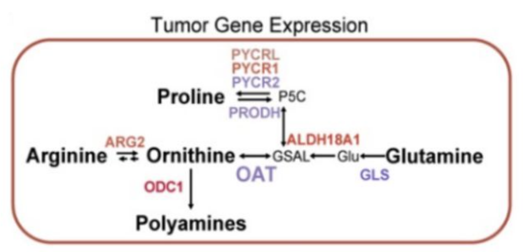

Fig. 2 – Polyamine biosynthesis pathway. Inhibition may be accomplished by directly depleting amino acid precursors (Pro, Arg) or by inhibiting key enzymes.

Methods

- SH-SY5Y neuroblastoma cells were cultured at 37°C, 5% CO_2, then treated with RA (2.5, 5, 10, 20 μM) and DFMO (0.5, 1.0, 2.5 mM) for 48 and 96 hr.
- Cells maintained in 50% arginine-depleted and cRPMI media to assess combinatorial treatment responses.
- Morphological analysis: phase-contrast microscopy used to assess cell elongation and neurite outgrowth.
- Differentiation quantified using a 0–3 scoring system based on neuronal differentiation hallmarks (see below).
- CellTiter-Glo Luminescent Assay (Promega) used to measure ATP levels as a proxy for metabolic activity.
  - Viability assessed at all treatment conditions.
  - All assays performed in triplicate.

Score	Cell Morphology	Neurites
0	Rounded, irregular	None visible, or minimal
1	Slightly elongated, stubby, polygonal	Minimal to very short (<1x cell body length)
2	Moderately elongated, slender or spindle-shaped	May or may not possess; if so, 1-1.5x cell body length
3	Highly elongated, extremely slender	Distinct neurites ≥2x cell body length, branching

Results

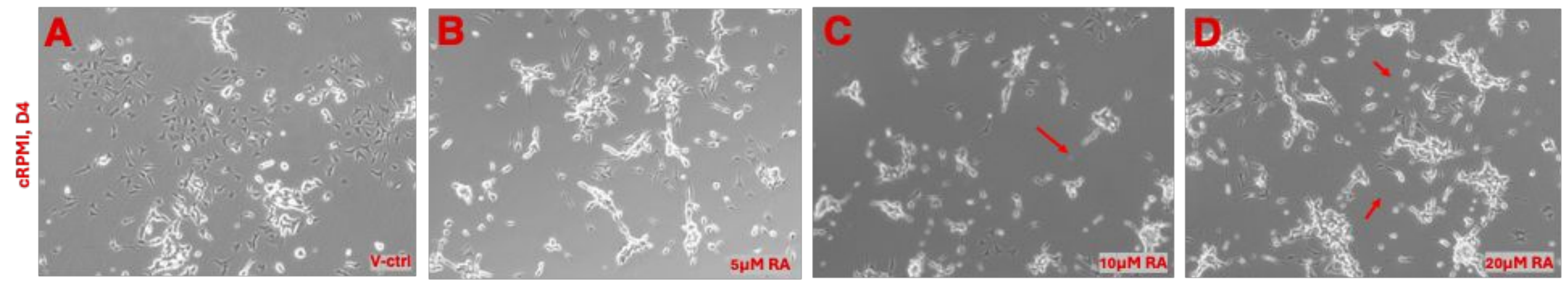

Fig. 3: 13-cis-RA successfully induces SH-SY5Y differentiation, as evidenced by lengthened neurite projections (red arrows), in complete RPMI conditions.

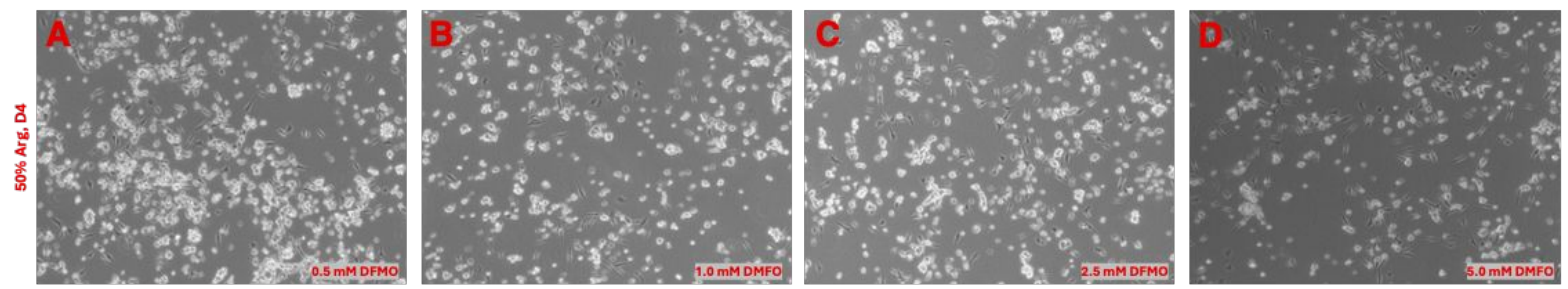

Fig. 4: Significant cell detachment (glowing edges) and rounding occur under conditions of polyamine perturbation (arginine depletion, DFMO administration).

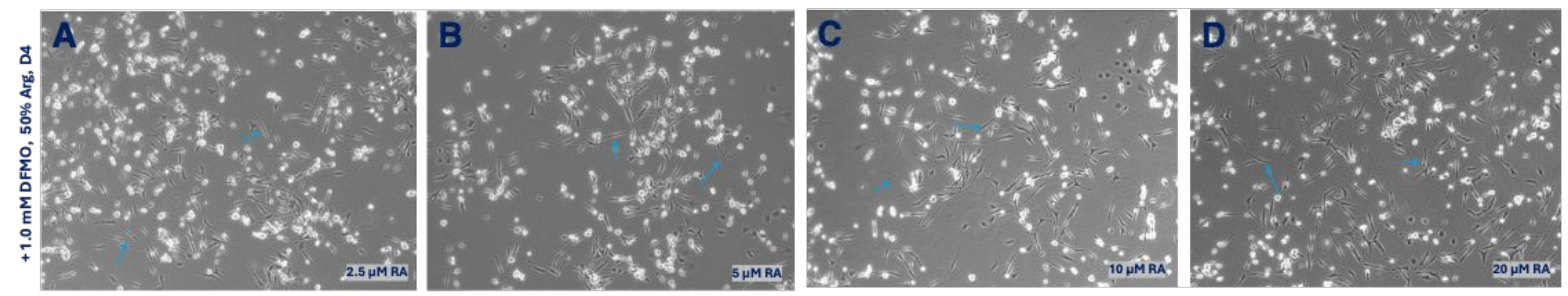

Fig. 5: Representative panel depicted; combination treatment of low-dose DFMO with 13-cis-RA somewhat restores a differentiation phenotype (blue arrows).

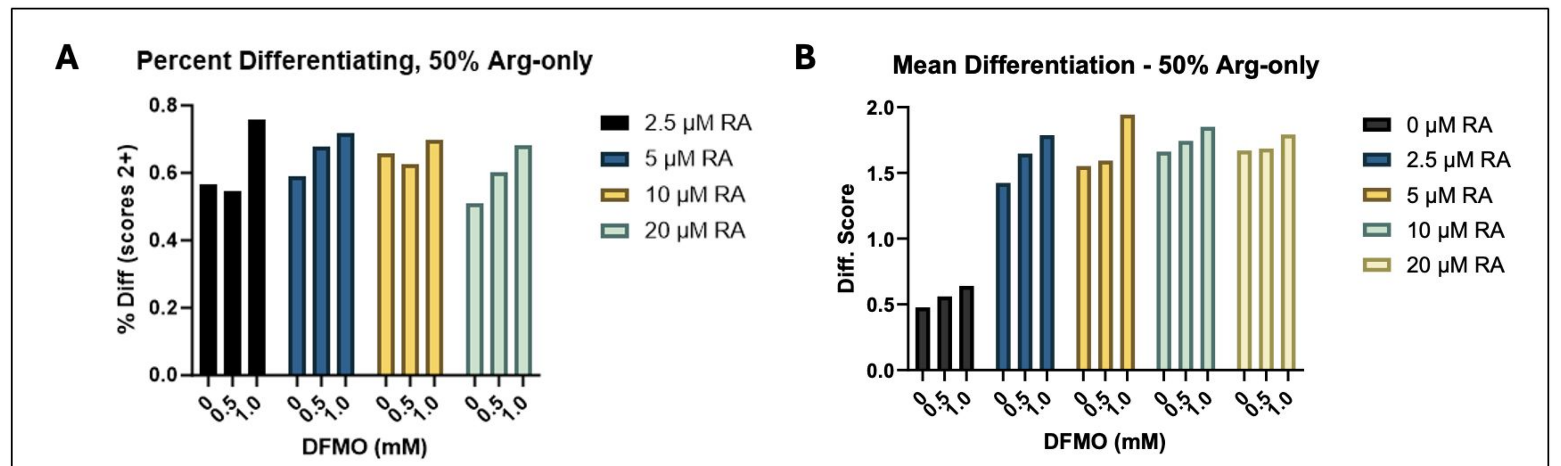

Fig. 6: Percentage of differentiating SH-SY5Y cells (designated by scores of 2+) and mean differentiation score across overall population, per condition of RA-only or in combination with low-dose DFMO. In adherent cells, increased [DFMO] generally leads to higher scores.

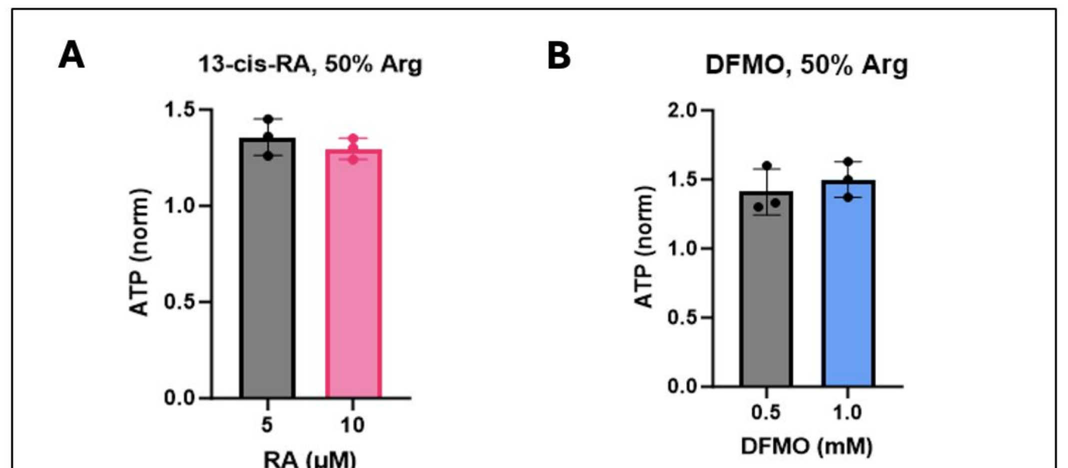

Fig. 7: SH-SY5Y cell viability across 13-cis-RA and DFMO treatment in Arg-depleted media. All [ATP] normalized to controls. Both drugs exert moderate effects on viability when administered alone.

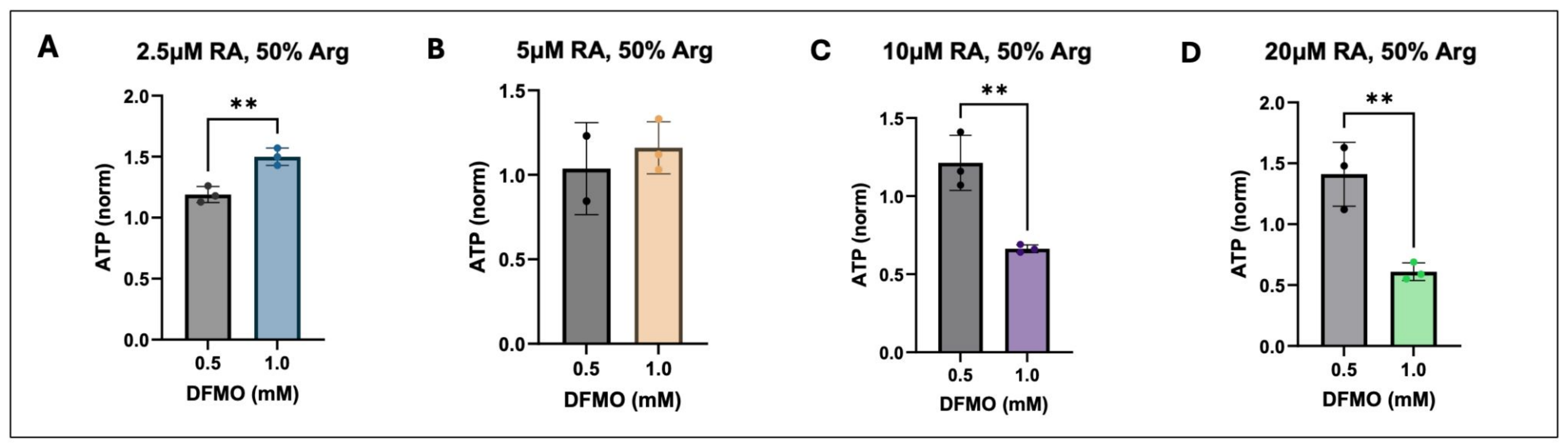

Fig. 8: Normalized [ATP] in cells under varying 13-cis-RA and DFMO conditions in 50% Arg media. In low-dose RA (2.5-5.0 μM), increasing [DFMO] results in improved viability, while the opposite is observed for 10-20 μM RA.

Discussion

RA and DFMO May Cooperate to Promote Differentiation

- 13-cis-RA successfully induced differentiation, evidenced by elongated cell bodies and neurite outgrowths.
- RA and low-dose DFMO (0.5–1.0 mM) significantly enhanced differentiation compared to RA or DFMO alone.
- High-dose DFMO (2.5–5.0 mM) led to cytotoxic effects, including increased cell rounding and detachment.
- Findings suggest RA and DFMO may act synergistically, but dose optimization is critical to balance differentiation and cytotoxicity.

Dose-Dependent Effects on Differentiation and Viability

- Cells treated with RA + low-dose DFMO exhibited:
  - Increasing differentiation scores in a generally DFMO dose-dependent manner.
  - Higher ATP levels when combined with low-dose 13-cis-RA (2.5–5.0 μM).
- In contrast, high-dose RA (10–20 μM) + low-dose DFMO led to dramatic drops in viability.

Interplay Between Differentiation and Viability

- Differentiation scores positively correlated with viability in many conditions.
- Higher viability may enable better differentiation by allowing cells to adhere and extend neurites.
- Conversely, differentiation signaling may also reduce cytotoxicity, improving cell survival despite DFMO-induced polyamine depletion.
- Further interrogation may define the mechanistic link between differentiation and cell survival.

Conclusions

- Current findings suggest that the combination of 13-cis-RA and low-dose DFMO presents a potentially promising therapeutic approach for NB.
- The observed synergy underscores the importance of targeting both differentiation and metabolic pathways.
- Amino acid depletion further sensitized cells to treatment, indicating a potential vulnerability.
- Future directions include validating findings *in vivo* and investigating the molecular mechanisms underlying observed interactions.
- Temporal shifts in order of compound administration may also be considered.
- By understanding the pathways involved, further optimization of combination therapies could maximize differentiation while minimizing toxicity, ultimately improving treatment outcomes for NB patients.

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