Glutamine metabolic inhibition synergizes with L-asparaginase in MYCN-amplified neuroblastoma

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Abstract

Neuroblastoma is the most common extracranial solid tumor in children. Though it accounts for about 10% of pediatric cancer, it is disproportionately responsible for 15% of pediatric cancer deaths. MYCN is amplified in 20% of neuroblastomas and correlates with adverse outcome. MYCN is known to drive tumor cell reliance on glutamine as a cellular nitrogen donor. 6-diazo-5-oxo-L-norleucine (DON) is a well-characterized glutamine analogue that inhibits glutaminase metabolism by irreversibly inactivating multiple glutamine-utilizing enzymes. DON was well tolerated in a previous phase I clinical trial in pediatric patients, but it has never been systematically studied in neuroblastoma patients. We show that MYCN amplification confers sensitivity to DON therapy in vitro in models of neuroblastoma, and that DON administered by intraperitoneal injection twice weekly significantly reduces flank tumor volume in mouse models of MYCN-amplified neuroblastomas (mean tumor volume 1715 mm³ vs. 207 mm³ in control animals, p-value = 0.00017 by t-test). We have also developed an orally bioavailable DON prodrug, JHU083, and we found that this drug administered orally three times weekly was similarly able to suppress neuroblastoma tumor growth in vivo (mean tumor volume 1113 mm³ vs. 217 mm³ in control animals, p-value = 0.000008 by t-test). Stable isotope resolved metabolomics (SIMS) experiments tracing glutamine and glutamate formation of ¹⁵N and ¹⁵N vicinal glutaminase mass spectrometry, DON presents antagonistic synergy with antitumor asparaginase agents, confirming that DON reduces cell proliferation in MYCN-amplified neuroblastoma cells compared to controls (p-value = 0.0011). We hypothesized that treatment with DON-a-sagnopeptide would enhance DON efficacy. Indeed, DON combined with L-asparaginase synergistically inhibits growth of MYCN-amplified neuroblastoma cells (CI < 0.3 by the Chou-Talalay method, indicating strong synergy; p-value = 0.00011). We conclude that DON, despite its potential benefit in combination therapy with DON and L-asparaginase synergistically inhibits the growth of MYCN-amplified neuroblastoma. These studies provide the preclinical justification for potential clinical trials for the use of DON or DON prodrug in combination with L-asparaginase as new therapeutic options for patients with MYCN-amplified neuroblastoma.

Results

DON and JHU083 treatment both significantly reduce MYCN-amplified neuroblastoma flank xenograft growth

DON treatment primarily prevents asparagine synthetase in vitro in MYCN-amplified neuroblastoma cell lines

JHU083 and asparaginase combination therapy in vivo more effectively reduces MYCN-amplified flank xenograft growth than treatment with either agent alone

Conclusions

- DON (or DON prodrugs) combined with asparaginase could be a potent metabolic therapy in certain MYCN-driven neuroblastoma cell lines.
- The combination of JHU083 and asparaginase decreases growth and proliferation in vitro.
- The combination of DON and asparaginase in vivo reduces flank tumor growth even more effectively than either medication alone.
- We predict that this combination therapy will reduce growth of flank tumors generated from other MYCN-amplified neuroblastoma cell lines.
- We plan experiments in vivo stable isotope resolved metabolomics to confirm that treatment with DON and prodrug primarily prevents asparagine synthetase in MYCN-amplified neuroblastoma xenografts.

Future Directions

- Based on results from this study, we expect that a combination of DON and L-asparaginase might be a promising therapeutic option for patients with MYCN-amplified neuroblastoma.
- We anticipate that future clinical trials will include the combination of DON and asparaginase in patients with MYCN-amplified neuroblastoma.
- We also plan to investigate the potential of DON and L-asparaginase combination therapy in other MYCN-driven pediatric solid tumors.

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- We plan experiments in vivo stable isotope resolved metabolomics to confirm that treatment with DON and prodrug primarily prevents asparagine synthetase in MYCN-amplified neuroblastoma xenografts.

Future Directions

- Based on results from this study, we expect that a combination of DON and L-asparaginase might be a promising therapeutic option for patients with MYCN-amplified neuroblastoma.
- We anticipate that future clinical trials will include the combination of DON and asparaginase in patients with MYCN-amplified neuroblastoma.
- We also plan to investigate the potential of DON and L-asparaginase combination therapy in other MYCN-driven pediatric solid tumors.