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Histone deacetylases 8 and 10 in neuroblastoma: co-expression as a biomarker for high-risk disease and simultaneous inhibition as a novel synergistic treatment approach

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Neuroblastoma is a heterogeneous malignancy of childhood, with a broad range of presentations and outcomes. This tumor entity is the most common extracranial solid tumor in children and with survival rates under 30% in the highest-risk patients, it accounts for 10-15% of cancer-related deaths in children. Thus, the improvement of outcomes for high-risk neuroblastoma patients is of great interest. Recent advances in targeted and immunotherapy have improved outcomes, however, there is still room for improvement, and patients who have endured a high load of chemotherapy to survive the tumor are at risk for therapy-related sequelae. Therefore, there is intensive focus on targeted therapy and an overall reduction of the classical chemotherapy burden on these patients.

Members of the histone deacetylase family have been shown to play important roles in multiple aspects of development and several disease states including neurodegenerative disease and cancer. Histone deacetylases are of great interest due to the ability for these enzymes to be inhibited with small molecules. In the case of certain histone deacetylases, such as HDAC8 and HDAC10 in neuroblastoma, the role in tumor pathology is of critical importance compared with non-transformed cells, where other processes compensate when these enzymes are inhibited. This is important for potential therapeutic use, since the avoidance of systemic toxicities is a high priority. In the case of HDAC8, this has already been explored in a mouse model, where the inhibition of HDAC8 in combination with retinoic acid was able to significantly slow tumor growth without systemic toxicities. For HDAC10, initial data from cell culture experiments points toward a tumor-specific importance of HDAC10, as cell death is not induced in non-transformed cells. However, this needs to be validated in mouse experiments. Combined inhibition of HDAC8 and HDAC10 leads to a decrease in cell growth and increased cell death compared with individual treatment of aggressive neuroblastoma cells. Addition of chemotherapy yields viable cell regression, potentially due to an increased dependence on HDAC8 and HDAC10 for stress response and DNA repair mechanisms. This combination has the potential to reduce chemotherapeutic burden as a lower concentration of chemotherapy is needed to achieve efficient cell death. In addition, it sensitizes otherwise resistant neuroblastoma cells to chemotherapy.

An initial indicator of tumor entities reliant upon HDAC8 and HDAC10 for survival was provided by a gene signature including the autophagy-related gene family member *ATG4D* and the heat shock protein 70 family member *HSPA4* together with *HDAC8* and *HDAC10*. The expression of this gene signature was explored in multiple tumor entities and found to have a tumor-specific effect, effectively separating neuroblastoma, group 3 medulloblastoma and ovarian cancer patients by overall survival. In neuroblastoma, high signature expression was correlated with features of aggressive disease. This retrospective signature provides an

initial indicator as to which tumor entities could be reliant upon HDAC8 and HDAC10 for survival.

Taken together, these results indicate that aggressive, chemotherapy resistant neuroblastoma cells are dependent on HDAC8 and HDAC10 for survival. When these HDACs are inhibited in the context of non-lethal chemotherapy, viable cell regression is induced in aggressive neuroblastoma cells. Initial experiments with non-neuroblastoma tumor entities indicate that other entities respond to this combination (medulloblastoma group 3, MED8A) whereas others are not dependent on HDAC8 and HDAC10 (embryonal rhabdomyosarcoma, RD). Thus, the effects of this combination treatment are tumor entity specific. Likewise, the ability of the hypothesis-driven gene signature to separate patients by overall survival is tumor entity specific. The development of a prospective biomarker would be useful for clinical implementation to identify patients who could benefit from treatment with this combination. So far, aggressive neuroblastoma cell lines show a potent response in cell culture experiments, which holds promise for a chemotherapy burden-reducing treatment for a patient population that is in need of effective, minimally toxic, targeted therapies.