Identification and Development of ALYREF Inhibitors Targeting MYCN-Driven Neuroblastoma

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Introduction: Neuroblastoma (NB) is a paediatric cancer that accounts for approximately 15% of all childhood cancer deaths. MYCN amplification is a key driver of high-risk neuroblastoma. Previously, we identified ALYREF as a direct protein-binding partner of MYCN, forming a positive feedback loop that enhances MYCN expression and drives NB tumorigenesis.[1] Despite its critical oncogenic role, no ALYREF specific inhibitors are currently available. Therefore, the aim of my project is to identify ALREF specific inhibitors and develop novel, effective combination therapies that integrate these inhibitors with chemotherapy for the treatment of high-risk NB patients. We have now identified a number of candidates ALYREF inhibitors by screening NIH drug/compound libraries.

Methods and Results: We have established doxycycline (Dox)-inducible SK-N-BE(2)-C cell line to knockdown ALYREF protein expression following 72 hours of Dox treatment .Using Dox- inducible ALYREF knockdown in SK-N-BE(2)-C cell line, we conducted a high-throughput drug screening of 4 sets of NIH drug/compound libraries, including approved oncology drugs (147 drugs), Mechanistic Set (811 compounds), Diversity Set (1584 compounds) and natural products set (390) using Alamar blue cell viability assay. The shALYREF/SK-N-BE (2)-C cells were treated with 5 µM compounds with or without Dox treatment for 72 hours. We have selected 98 small molecules heterocyclic compounds based on their cell viability difference, structural analysis and drug-like properties, that will potentially target ALYREF oncogenic signals. Two of the compounds from the candidate list, designated as ALY2 and raloxifene has exhibited strong cytotoxicity in MYCN-amplified neuroblastoma cell lines and demonstrated a favourable therapeutic window. Importantly, we showed that these two compounds reduced ALYREF, MYCN and USP3 protein levels, suggesting that they are potential inhibitors for ALYREF/MYCN/USP3 axis. These lead compounds will be further studied for their molecular mechanism, protein-drug binding and their therapeutic efficacy in NB cell lines and *in vivo* models.

Conclusion: Our discovery of small molecule compounds that suppress NB cell growth by reducing ALYREF and MYCN protein levels, will paves the way for development of the first-in-class novel ALYREF inhibitors. These findings have the potential to advance novel therapeutic strategies for MYCN-driven NB, with the ultimate goal of translating them into clinical applications for the treatment of high-risk NB.

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References:

[1] Nagy, Z.; *et. al. Nature Communications* **2021,** 12, 1881.