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Investigating ALK inhibitors alone or in combination as therapeutic options for ALK-positive neuroblastoma

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ABSTRACT

Neuroblastoma (NBL) is the third most common pediatric cancer after leukemia and cranial tumours and accounts for around 15% of death in pediatric malignancy. NBL develops due to the poorly differentiated progenitor cells of the sympathetic nervous system. Even after extensive chemo and immune therapeutic options, high-risk and relapse NBLs are still hard to treat and needs better and efficient therapeutic treatment. The most common genetic abnormalities in NBL are chromosome 1p and 11q deletion, 2p and 17q gain, MYCN amplification and mutation in anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor, part of the insulin receptor (IR) family, which has been involved in several solid and liquid cancers. ALK mutations are found in about 10% of NBL cases but in the relapsed patient population, the ALK-positive percentage increases significantly. Crizotinib was the first ALK tyrosine kinase inhibitor (TKI), which was approved clinically to treat ALK-positive lung cancer. In NBL, crizotinib has had a less striking effect, which urges to discover more efficient and potent ALK TKIs for NBL treatment. The overall aim of this thesis was to investigate ALK inhibitors alone or in combination as therapeutic options for ALK-positive NBL patients.

Alectinib is a second-generation ALK inhibitor and showed a dramatic effect in crizotinib resistant ALK-positive lung cancer patients. In our first project study, we interrogated the alectinib effect in preclinical settings of NBL. In vitro kinase assays and cell-based experiments examining ALK mutations show that alectinib is an effective inhibitor of gain-of-function ALK mutants in NBL models. Administration of alectinib showed efficient tumour effect in mouse xenograft model of NBL, in comparison to crizotinib

In the second study project, we interrogated the inhibitory effect of dihydroorotate dehydrogenase (DHODH) in NBL preclinical settings. Pyrimidine nucleotides play a vital role in tumour progression and these pyrimidines can be synthesized through either salvage or the de novo pathway. Tumour cells fulfil their need for nucleotides through the de novo pathway. Dihydroorotate dehydrogenase (DHODH) is an important player of de novo pyrimidine synthesis and by inhibiting DHODH, tumour cells proliferation is inhibited. Low levels of DHODH in NBL tumours is linked to good clinical outcome. BAY2402234, a novel DHODH inhibitor has shown striking inhibition in acute myeloid leukemia (AML) and we investigated BAY2402234 in NBL settings. In BAY2402234 treated NBL cells and in transgenic mouse models, inhibition of cell proliferation and significant reduction of tumour growth were observed. Biochemical analysis showed that BAY2402234 treatment inhibited MYCN expression and increased p53 and cleaved PARP protein levels. Synergy was observed in ALK-positive NBL cells upon the combination of BAY2402234 and lorlatinib treatment.

This thesis study shows the significance of ALK in NBL. To summarize, alectinib is an efficient inhibitor of ALK kinase activity in ALK addicted NBL and BAY2402234 inhibits NBL cell proliferation in vitro and in vivo. The combination treatment of BAY2402234 and lorlatinib showed synergy and as a promising future therapeutic option for the NBL patients, this should be considered alone or in combination.

Keywords: NBL, Anaplastic lymphoma kinase, MYCN, DHODH, alectinib, crizotinib, BAY2402234, lorlatinib

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I. **Muhammad Wasi Alam**., Borenäs, M., Lind, D. E., Cervantes-Madrid, D., Umapathy, G., Palmer, R. H., & Hallberg, B. (2019). Alectinib, an anaplastic lymphoma kinase inhibitor, abolishes ALK activity and growth in ALK-positive neuroblastoma cells. Frontiers in oncology, 9, 579. https://doi.org/10.3389/fonc.2019.00579

II. **Muhammad Wasi Alam**., Ganesh Umapathy., Yeshwant Kurhe., Dan E. Lind., Sonia Lain., Palmer, R. H., & Hallberg, B. BAY2402234, a dihydroorotate dehydrogenase (DHODH) inhibitor, abrogates proliferation and induces apoptosis in neuroblastoma cells. Manuscript 2021

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