Targeting Ref-1 in Cancer: Progression of Advanced Generation Ref-1 Inhibitors Through the Drug Discovery Pipeline

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APE1/Ref-1 (Apurinic endonuclease1 / Redox factor-1, referred to as Ref-1) is a multifunctional protein with both DNA repair and redox activities. The redox activity of Ref-1 activates transcription factors such as HIF1a, STAT3, and NFkB that are involved in cancer cell survival and proliferation. Targeting Ref-1 with specific inhibitors represents a promising therapeutic strategy across multiple cancer types. This study focuses on screening and characterizing advanced-generation Ref-1 inhibitors to identify potential candidates for further preclinical development. As part of this drug discovery pipeline, we evaluated four advanced generation compounds and compared them to second-generation compound, APX2009. We selected malignant peripheral nerve sheath tumor (MPNST) cells for evaluation because these tumors often exhibit elevated oxidative stress and redox signaling, making them more dependent on Ref-1 activity. Using MPNST cells, I measured inhibition of downstream Ref-1 target, NFκB and cytotoxicity after treatment. NFκB activity was measured via a luciferase-based reporter assay and two viability assays were compared: Alamar Blue and Methylene Blue. Luciferase assays further confirmed on target inhibition of Ref-1–regulated NFκB activity. Both cell viability assays demonstrated dose-dependent reduction in cellular viability, confirming compound efficacy. Among the compounds tested, WGK2105 exhibited high potency with an IC_{50} of ~2 μM. WGK2110 and WGK2111 demonstrated similar potency and Ref-1 inhibition when compared to APX2009, while WGK2092 required significantly higher concentrations and will not be prioritized for further development. These findings support the potential for discovery of more potent Ref-1 inhibitors and identify multiple promising candidates for preclinical advancement. Future efforts will assess metabolic stability in mouse and human liver microsomes to determine *in vivo* suitability. *In vivo* efficacy studies and full pharmacokinetic profiling (ADME) will follow for top candidates. Comparative analysis of active versus inactive compounds will inform structure-activity relationship (SAR) development, guiding next-generation inhibitor design. These efforts are key steps toward Investigational New Drug (IND) applications and clinical trial readiness.