

Dual targeting of nonsense mutations and G-Quadruplex DNA structures: a novel strategy in cancer treatment

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Nonsense mutations cause a variety of inherited genetic disorders, including cancer. This type of mutation generates premature termination codons (PTCs) that cause premature termination of mRNA translation, inhibiting the synthesis of the full-length protein. The consequent loss of essential cellular functions underlies the severe phenotypes of so-called genetic nonsense-related diseases (NRDs), such as cystic fibrosis (CF), Duchenne muscular dystrophy (DMD), Fabry disease, and certain types of cancer (Vitale et al., 2025). Nonsense suppression therapy mediated by translational readthrough-inducing drugs (TRIDs) is a promising approach to restore the synthesis of full-length and potentially functional proteins. G418, also known as Geneticin and the newer oxadiazole-core TRIDs NV848, NV914, and NV930, showed promising levels of translational readthrough activity in nonsense-related in vitro systems (Pibiri et al., 2020; Perriera et al., 2023). In tumor suppressor genes, such as TP53, the loss-of-function caused by nonsense mutations can critically impair the mechanisms controlling proliferation, DNA repair or apoptosis, thus favoring tumor progression. This gene is mutated in a large fraction of human tumors, and about 11% of TP53 mutations are nonsense mutations, with the most common being R213X (Strandgren et al., 2025). In this context, TRIDs represent a valuable resource to restore full-length p53 expression. Another emerging approach in cancer therapy involves the use of Schiff base metal complexes that selectively bind and stabilize DNA G-quadruplex (G4) structures, non-canonical DNA structures that are enriched in oncogene promoter regions, stabilization of which can interfere with their transcription, ultimately impairing tumor cell proliferation. In this project, we investigated the use of translational readthrough-inducing drugs (TRIDs), alone and in combination with the DNA G-quadruplex stabilizing agent RB1 (Terenzi et al., 2014), as a dual-targeted anticancer strategy. p53 R213X cells were treated with these compounds to assess changes in cell proliferation and death rate at various time points through vital cell counts, and DNA damage induction with doxorubicin was also employed to stimulate p53-dependent cellular responses. In parallel, immunofluorescence analyses were performed to assess the restoration of p53 protein expression and its intracellular localization following combined treatment. Furthermore, cell cycle progression was analyzed by flow cytometry after treatment with NV compounds, G418, or RB1+NV combinations at different time points to evaluate G0/G1 checkpoint activation and cell cycle arrest. The data obtained showed that combined treatments exerted stronger antiproliferative effects than single compounds and induced increased accumulation of cells in the G0/G1 phase, suggesting enhanced restoration of p53-dependent cell cycle control and

supporting the potential therapeutic value of combining nonsense mutation readthrough with G-quadruplex stabilization in TP53-related cancer treatment.

References

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