Rescuing nonsense in cancer: recovering p53 tumor suppressor gene expression by translational readthrough inducing drugs (TRIDs)

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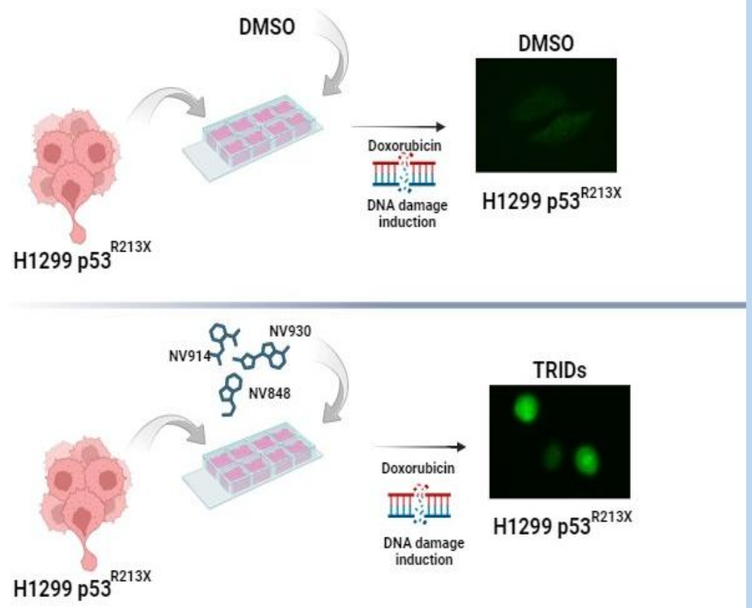
Background

Mutation-based treatments represent a burgeoning frontier in genetic medicine, wherein therapeutic interventions are tailored according to the specific mutation profile of a patient[1].

Recently, considerable attention has been directed towards addressing diseases stemming from premature termination codons (PTCs). A novel class of drugs, known as Translational readthrough-inducing drugs (TRIDs), has emerged with the capacity to facilitate the readthrough of PTCs, thereby reinstating the synthesis of full-length functional proteins[2].



Aim

Our study focuses on the potential of three newly synthesized TRIDs: NV848, NV914, and NV930, in promoting the production of functional p53 protein from a cDNA sequence carrying a PTC.

The investigation involved the treatment of a human cancer cell line (H1299-p53^{R213X}) engineered to harbor a PTC.

By Real-Time RT PCR and Western blot analysis, we explored the possibility of rescuing p53 protein expression and its functionality as transcription factor.

p53 expression in nonsense cell model system harboring a premature stop codon R213X

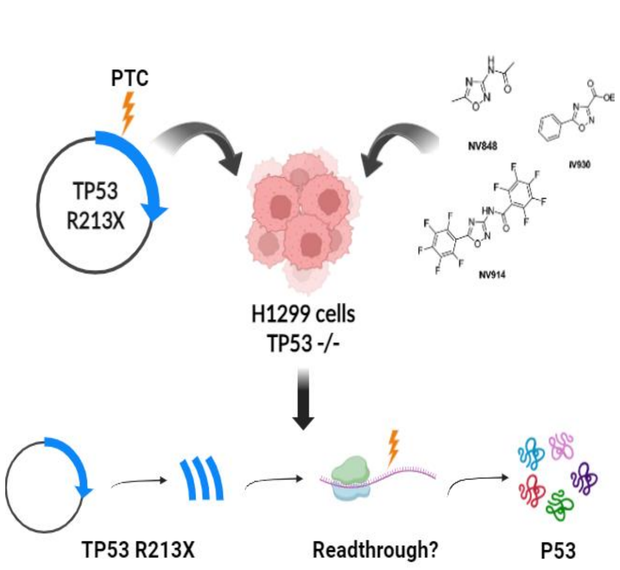


Figure 1: *Experimental workflow for the study of the p53 rescue in H1299 p53^{R213X} cells by NVs molecule treatment.*

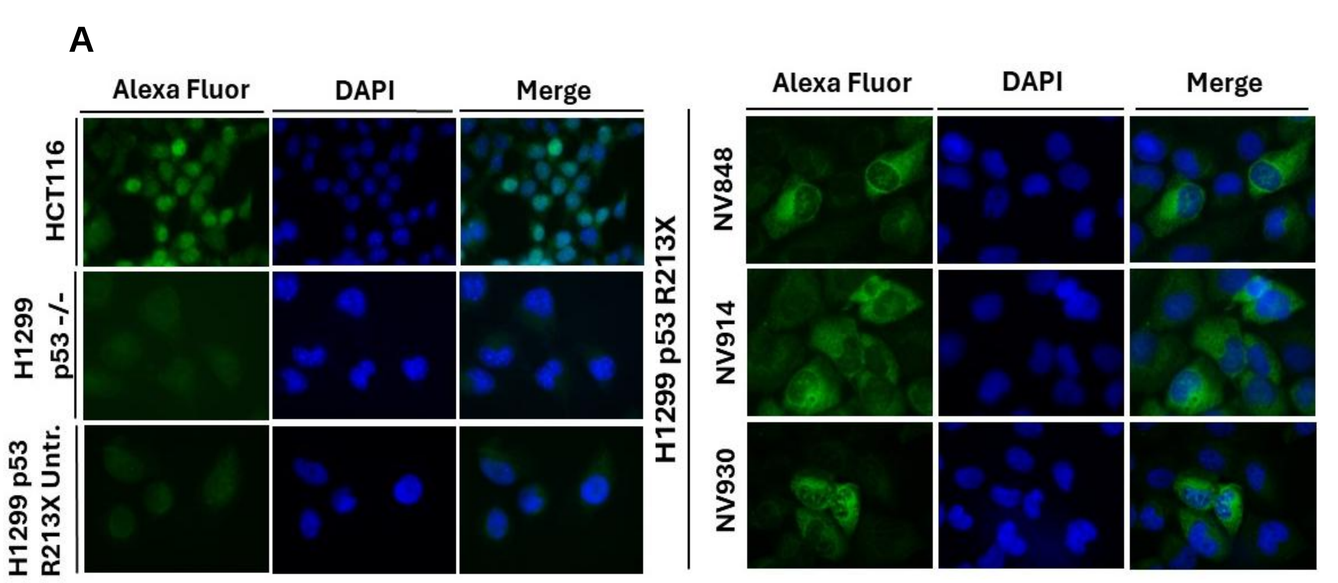
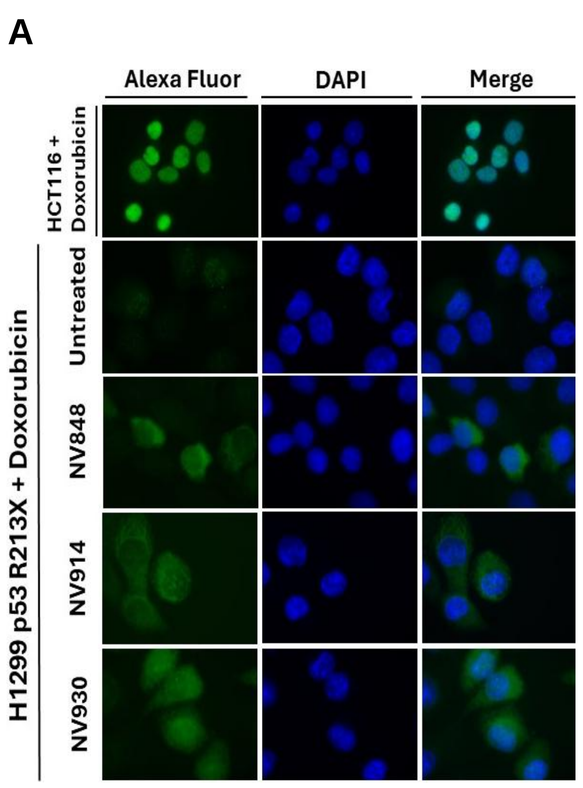


Figure 2: A) *Immunofluorescence analysis after NV848, NV914 and NV930 molecules treatment (12uM) in p53 ^{R213X} cells;* ***B)*** *p53 fluorescence quantification (p-value < 0.0001 was calculated by one-way ANOVA test statistical analysis).*

P53 induction and functionality in H1299^{R213X} cells after doxorubicin DNA damage



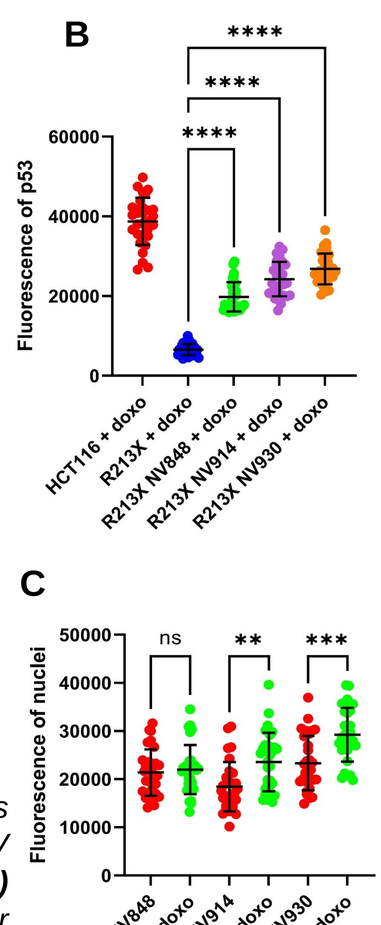
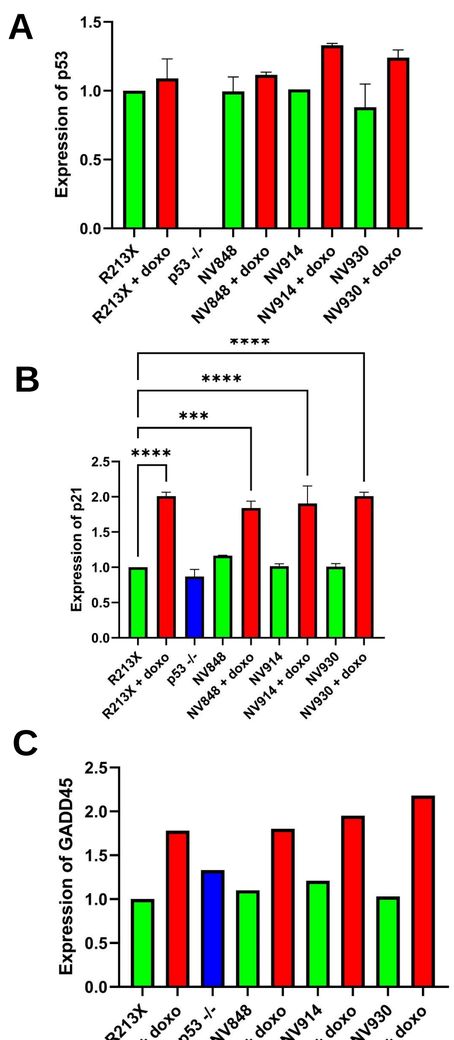


Figure 3: A) *Immunofluorescence analysis of H1299^{R213X} cells were treated with NV molecules and doxorubicin (0,2ug/ml);* ***B-C)*** *p53 fluorescence quantification after treatment with NV molecules and doxorubicin.*



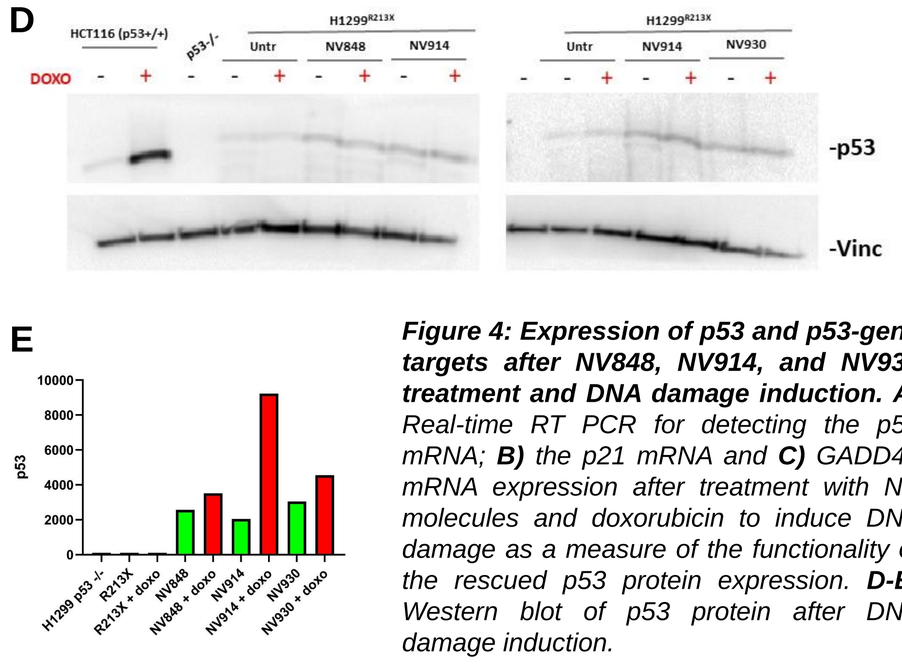


Figure 4: Expression of p53 and p53-gene targets after NV848, NV914, and NV930 treatment and DNA damage induction. A) *Real-time RT PCR for detecting the p53 mRNA;* ***B)*** *the p21 mRNA and* ***C)*** *GADD45 mRNA expression after treatment with NV molecules and doxorubicin to induce DNA damage as a measure of the functionality of the rescued p53 protein expression.* ***D-E)*** *Western blot of p53 protein after DNA damage induction.*

Conclusions

Our results show that NV molecules rescue p53 protein expression and that its localization is nuclear after DNA damage induction. The protein exhibits correct functionality, as observed following DNA damage stimuli induced by doxorubicin treatment.