Therapeutic targeting of TP-53 mutated acute myeloid leukemia by reintroduction of wild type TP53 enhances chemosensitivity

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Introduction

- TP53, the most frequently mutated tumor suppressor gene, occurs in about 10% of de novo AML.
- These mutations predict poor prognosis, with a median survival of only 5–10 months and limit the success of current therapies.
- To address this, we explored whether overexpression of wild-type TP53 could offer therapeutic benefit in AML, particularly in combination with existing drugs.

Aim

- Overexpression of wild-type TP53 in TP53 mutated acute myeloid leukemia and evaluation of chemotherapeutic drug sensitivity.

Objectives

- ❑ Development of a doxycycline inducible TP53 over expressing vector.
- ❑ Lentiviral transduction of suitable AML cell lines with TP53-OE vector.
- ❑ Evaluate the drug sensitivity of AML cell lines with wild-type TP53 overexpression

Methods

❖ **Drug sensitivity screening and TP53 status evolution of AML cell lines.**

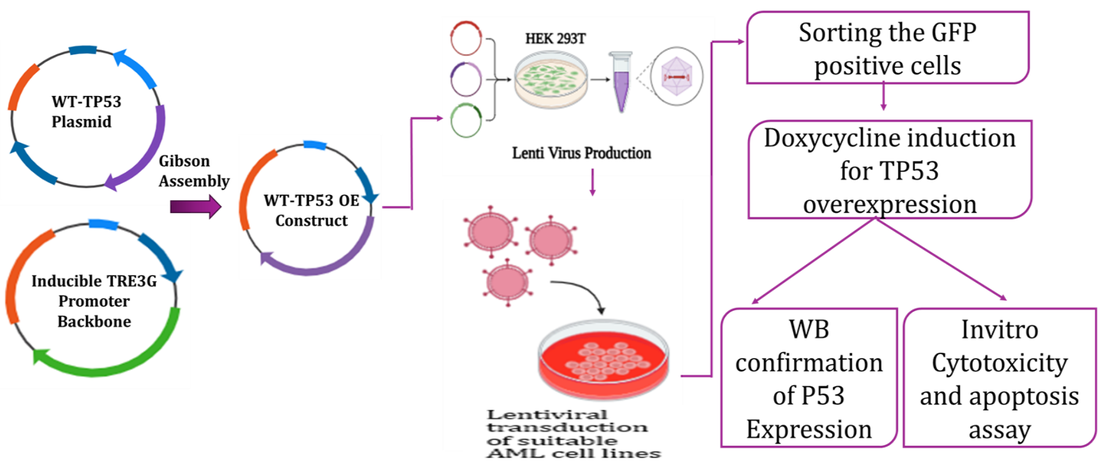
AML Cell lines (U937, THP-1, KASUMI-1, KG1, KG1A, MV-4 11, MOLM-13, HL-60, NB-4)

TP53 mutation analysis by Sanger Sequencing

P53 protein expression by Western Blot

In-vitro cytotoxicity assay- IC50 calculation

❖ **Development of wt-TP53 overexpressing Lentivirus & transduction of suitable AML cell lines.**



Result

In Vitro Sensitivity of AML Cell Lines to Venetoclax and Azacitidine

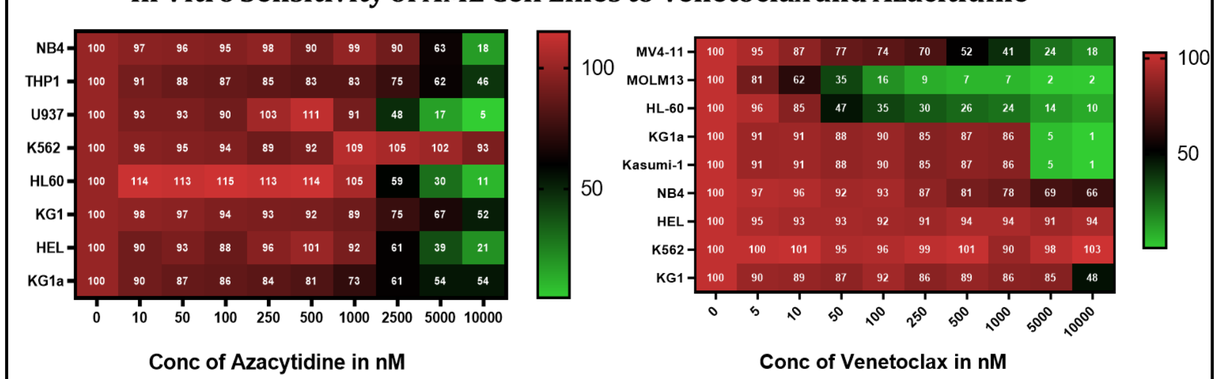


Fig - 1: In vitro cytotoxicity of AML cell lines treated with Azacitidine and Venetoclax

P53 Protein Expression in AML Cell Lines

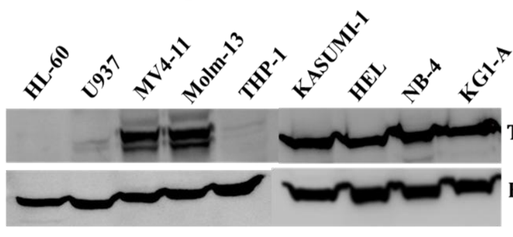


Fig - 2: Screening the P53 protein expression pattern in AML cell lines by WB. The right-side table showing the TP53 mutation status of AML cell lines.

TP53 Mutation Status in AML Cell Lines

AML Cell Line	TP53 Status	Mutation type	TP53 Function
MV-4-11	Wild-type	NA	Functional
MOLM-13	Wild-type	NA	Functional
U-937	Null Tp53	Splice	NA
HL-60	Null Tp53	Large deletion	NA
THP-1	Null TP53	Frame shift	NA
K-562	p.C135FS	Frame shift	Nonfunctional
KASUMI-1	p.R248Q	Missense	Nonfunctional
HEL	p.M133K	Missense	Nonfunctional
NB-4	p.R248Q	Missense	Nonfunctional
KG-1	Null Tp53	Splice	NA
KG-1A	p.V225I	Missense	Functional

Confirmation of TP53 Overexpression in AML Cell Lines by Western Blot

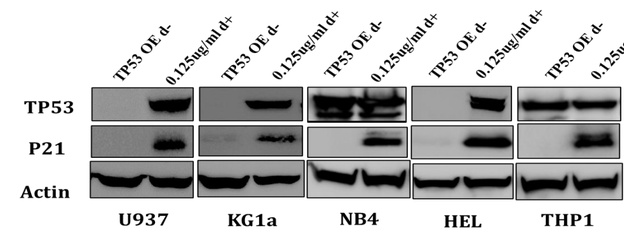
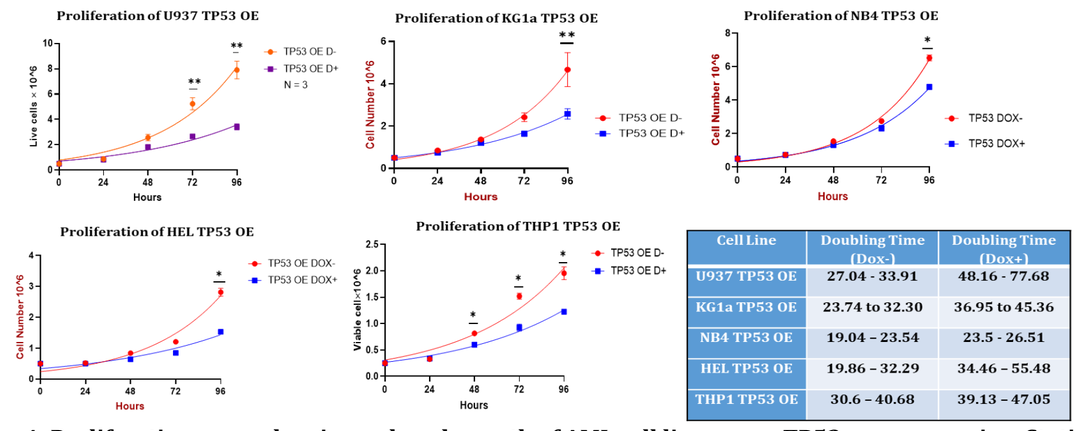


Fig - 3: Western blot validation of TP53 overexpression and its downstream target P21 (CDKN1A) expression by western blot.

Effect of TP53 Overexpression on Proliferation of AML Cell Lines



Cell Line	Doubling Time (Dox-)	Doubling Time (Dox+)
U937 TP53 OE	27.04 - 33.91	48.16 - 77.68
KG1a TP53 OE	23.74 to 32.30	36.95 to 45.36
NB4 TP53 OE	19.04 – 23.54	23.5 - 26.51
HEL TP53 OE	19.86 - 32.29	34.46 - 55.48
THP1 TP53 OE	30.6 – 40.68	39.13 - 47.05

Fig - 4: Proliferation assay showing reduced growth of AML cell lines upon TP53 overexpression. Statistical significance was determined using Mann–Whitney test, $p < 0.05$.

Cell Cycle Analysis of AML Cell Lines with and without TP53 Overexpression

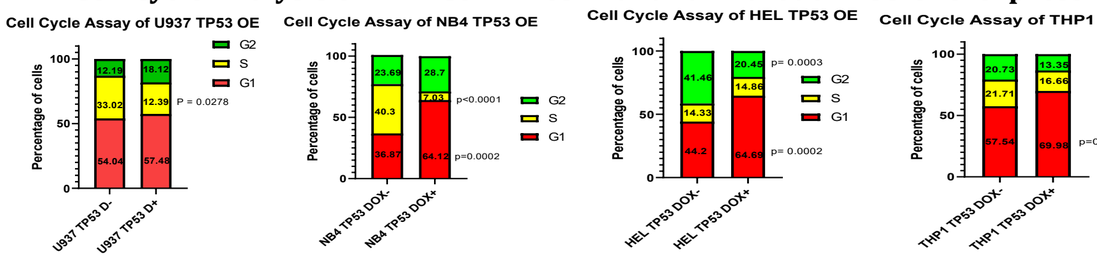
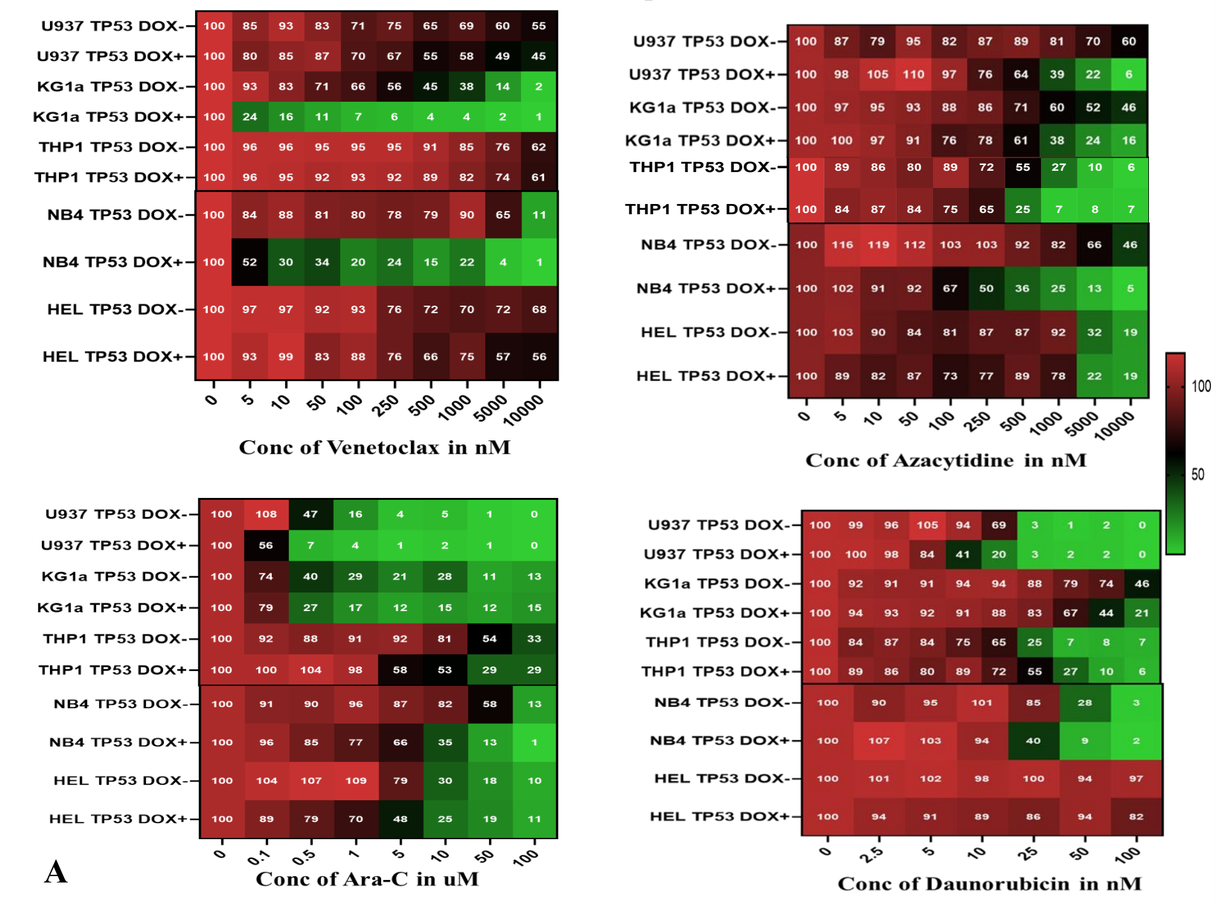


Fig - 5: Proliferation assay showing reduced growth of AML cell lines upon TP53 overexpression. Statistical significance was determined using Unpaired t Test, $p < 0.05$.

Invitro Cytotoxicity Profiling of TP53-Overexpressing AML Cells with Standard Therapeutics



Apoptosis Assay of AML Cell Lines with and without TP53 Overexpression

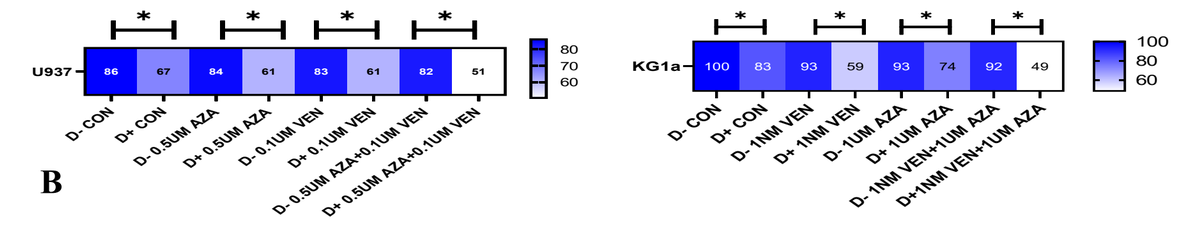


Fig - 6: A. MTT assay showing sensitivity ofTP53 overexpressing AML cell lines (Dox– vs Dox+) treated with AZA, VEN, Ara-C, and Daunorubicin. (N=4) B. Annexin V/7-AAD staining showing percentage of apoptotic cell death in TP53 overexpressing AML cell lines Statistical significance was determined using Mann–Whitney test, $p < 0.05$.

Validation of Apoptosis by Western Blot in p53-Overexpressing U937 Cells

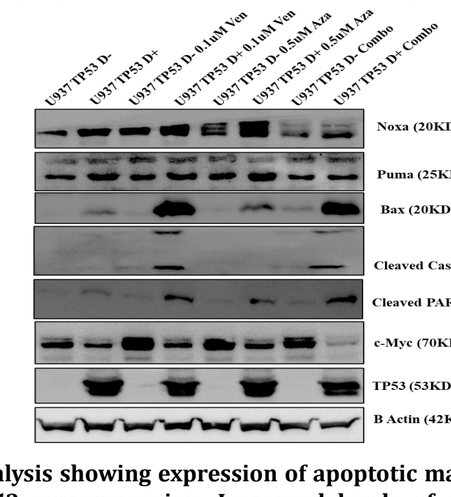


Fig- 7: Western blot analysis showing expression of apoptotic markers in U937 TP53 OE cells following doxycycline-induced p53 overexpression. Increased levels of cleaved PARP, cleaved caspase-3, Bax, PUMA, and NOXA were detected compared to Dox- control (β-actin used as loading control)

Summary

- ❑ **Doxycycline-induced p53 overexpression in AML cells resulted in reduced proliferation and increased drug-induced cytotoxicity.**
- ❑ **Cell cycle analysis revealed G1 arrest upon p53 overexpression.**
- ❑ **Apoptosis was confirmed by Annexin V/7-AAD staining and validated by Western blot showing upregulation of cleaved PARP, cleaved caspase-3, Bax, PUMA, and NOXA and downregulation of c-MYC.**
- ❑ **These findings demonstrate that overexpression of P53 enhances apoptotic response and chemotherapeutics sensitivity in AML cells.**

Future Direction

- **In vivo validation** – Evaluate the effect of p53 overexpression in AML xenograft mouse models to confirm therapeutic potential.
- **Gene therapy approach** – Explore AAV/lipid nanoparticle-mediated TP53 delivery as a translational strategy for restoring p53 function in TP53-deficient or mutant AML

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