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# Functional analysis of in-frame variants in *TP53* gene identified in adult leukemias: A study of the *TP53* Network of European Research Initiative on CLL


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## INTRODUCTION

- Testing for *TP53* variants is included in diagnostic and treatment guidelines in several leukemias → the need for rapid and reliable laboratory analysis, interpretation, and reporting.
- While most somatic *TP53* variants can be interpreted using data from large-scale functional studies, data on in-frame deletions and insertions remain limited [1,2], and classification often relies on expert judgment.

## OBJECTIVES

- To collect in-frame deletions and insertions in TP53 gene identified in leukemic samples during routine molecular diagnostics.
- To analyze the impact of these variants on protein function using functional assay in yeast complemented with human cell line assay.

## CONCLUSIONS

- In-frame TP53 variants located within the core region of the DNA-binding domain resulted in a loss of transactivation function
- Variants downstream of codon 287 displayed heterogeneous phenotypes depending on testing system and localization.
- Our findings support the interpretation that in-frame TP53 variants within the core DBD region are likely pathogenic when identified in tumor samples.
- In contrast, interpretation of in-frame variants located outside this region requires integration of multiple data sources, including functional studies, published literature, and curated variant databases.


## ACKNOWLEDGMENTS

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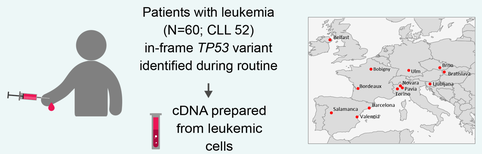
## METHODS and RESULTS

### Sample set

SAMPLES WITH *IN-FRAME* VARIANTS IN *TP53* GENE:
13 LABORATORIES OF ERIC (European Research Initiative on CLL)




- The sample set: 54 unique variants, 5 variants detected 2x and 1 variant 3x.
- Variant location: DNA-binding (DBD; N=51) or tetramerization domain (N=3).
- For the interpretation of many variants, no data were available → Figure 1.
- In-frame variants were frequently accompanied with 2nd allele inactivation (not shown) and are selected during therapy → Figure 2.

**Figure 1. Lack of evidence for interpretation of analyzed variants**

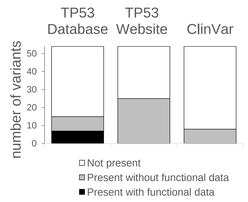
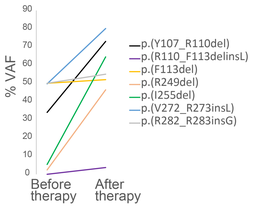


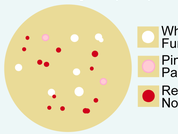

**Figure 2. In-frame *TP53* variants were clonally selected in relapse**



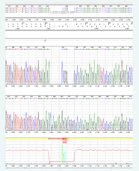

### Functional analysis in yeast (54 variants)

*TP53* gene amplification: exons 4–10 / codons 42–374

Cloning of PCR product into a modified *Saccharomyces cerevisiae* strain with a reporter gene *ADE2* under control of promoter with p53 binding site (RGC): FASAY method [3]



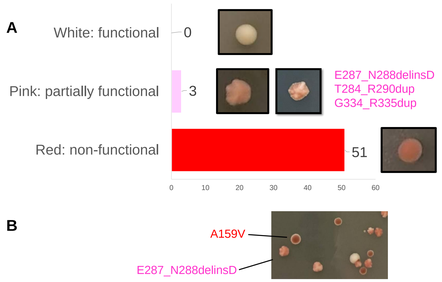

Phenotype of the colony: ability of the clonned variant to activate the transcription of a reporter gene



Confirmation of the variant phenotype: Sanger sequencing of *TP53* in individual colonies

- Yeast assay: 51 variants - complete loss of function (red colonies), 3 variants - partially retained function (pink colonies) → Figure 3A.
- Among variants with partially retained function, p.A287_N288delinsD co-occurred with another missense variant in a compound heterozygous state → Figure 3B.

**Figure 3: Yeast assay: complete or partial loss of function**




### Functional analysis in human cells (7 variants)

Variants partially functional (N=3),and non-functional in yeast (N=4)
negative control (wt p53), positive control (Arg248Gln)

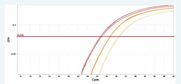
RPE1 TP53-knockout human cell lines expressing doxycyclin-inducible p53 variants

Doxycycline: expression induction / Nutlin-3a: p53 protein stabilization

Quantification of p53 targets

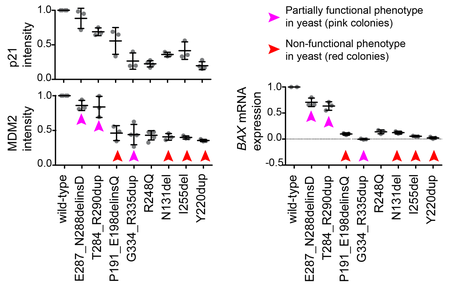
ScanR microscopy
p21 and MDM2 protein expression

qRT-PCR
BAX mRNA expression

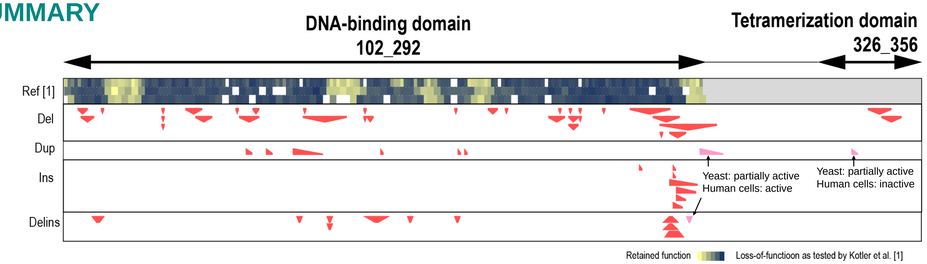


- Human cell assay: two variants with a partially functional phenotype in yeast located at the DBD boundary retained >70% of wild-type p53 activity; the tetramerization domain variant showed complete LOF → Figure 4.

**Figure 4: Human cell assay**




## SUMMARY




**Summary of in-frame variants reported and tested in the study and their positions in p53 protein**

The tested variants (lower part) compared to the heatmap showing the effect of 1AA (1st line) and 2 AA (2nd and 3rd line) deletions on growth-suppression in in-vitro experimental system published by Kotler et al. [1].
The color of the tested variants shows the impact on p53 transactivation capacity in yeast test: red color - complete loss of function; pink – partial loss of function.
For variants with partial activity in yeast (pink), the activity in human cell line assay is described in detail.


## REFERENCES

1. Kotler et al. 2018, PMID: 29979965; 2. Funk et al., PMID: 39774325; 3. Flaman et al. 1995, PMID: 7732013.