The interplay between BcR signalling and the p53 pathway upon DNA damage in primary CLL cells

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Background

- Constitutively activated pro-proliferative B cell receptor (BcR) signalling and deregulated DNA damage response are major drivers in CLL.
- Inhibitors targeting the BcR signalling were considered to act independently of the p53 pathway that is part of DNA damage response in cells.
- Different frequencies of *TP53* gene defects in different BCR stereotyped subsets and subgroups based on IGVH usage (PMID: 24725250) suggested a potential interplay between p53 and BcR signalling.

Objective

- **To investigate whether a therapeutical inhibition of BcR signalling alters p53 level, phosphorylation and its transcriptional activity.**

Materials and methods

- ***In-silico* analysis** of publicly available data (PMID: 33833385) was used to track transcription programmes after BcR activation in primary CLL cells.
- **Patients cohort:** primary CLL cells with wild-type *TP53* gene,
  - pilot cohort: n=13 for combination with ibrutinib and n=12 for combination with idelalisib,
  - validation cohort: n=24.
- **Treatment** of primary CLL cells for 24 hours:
  - 1.5 μM doxorubicin or 15 μM fludarabine – DNA damaging agents stabilizing p53 in cells,
  - 10 μM ibrutinib or idelalisib – tyrosine kinase inhibitors targeting BcR signalling,
  - treatment combinations are indicated in the results section.
- Analysis of p53 level and phospho-patterns by SDS-PAGE and Zn(II)Phos-Tag method followed by western blots using anti-p53 antibody.
- qRT-PCR of p53 target genes *BAX*, *BBC3*, *CDKN1A*, *GADD45A*.

Results

1. Activated BcR signalling changes expression of p53 targets

- *In-silico* analysis of transcriptomic data from 3 proliferative primary CLL samples with unmutated IGHV and wt-*TP53* (PMID: 33833385).
- Focus on significant changes (FDR<0.05; logFC≥|2|) in the expression of typical p53 targets (list of targets – PMID: 28288132).
- **After 24 hours, activation of BcR signalling led to significant changes in the expression of 13 out of 116 typical p53 targets.**
- Genes listed in the table are mostly involved in regulating cell proliferation, cell death and stress response, including DNA-damage response.

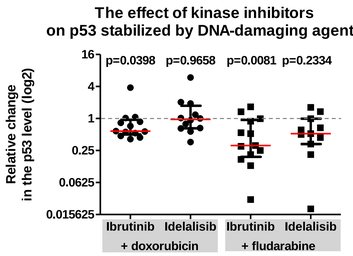
Differentially expressed p53 targets after BcR activation

p53 targets	logFC	FDR	p53 targets	logFC	FDR
FAS	4.475785	2.10E-18	***SESN2***	2.749924	5.16E-05
CSF1	5.483876	1.81E-14	***EPS8L2***	-3.52068	7.05E-05
ENC1	-7.80791	3.44E-12	***NINJ1***	3.598083	0.000152
TRIM22	-3.69048	4.10E-11	***SLC12A4***	2.415349	0.000236
ATF3	5.025042	8.54E-08	***PTP4A1***	-2.27854	0.000321
BTG2	-2.75325	1.27E-07	***PLK2***	-3.0477	0.001079
LAPTM5	-3.28526	9.97E-07			

2. Ibrutinib, but not idelalisib, decreases p53 level under DNA-damaging conditions

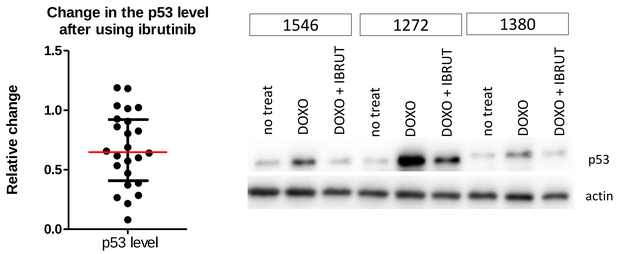
RIGHT PANEL

- Graph shows changes in the p53 level where combinatorial treatment with tyrosine kinase inhibitors and DNA damaging agents was compared to DNA damaging agents used alone.
- The grey dashed line represents no change in the p53 level, red lines represent the median, whiskers 1st and 3rd quartile. Indicated p-values were calculated by Wilcoxon Signed Rank Test.
- **Ibrutinib, but not idelalisib, treatment led to a significant decrease in the level of p53 protein with a more homogeneous effect in the doxorubicin group.**



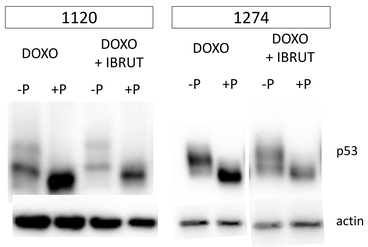
BOTTOM PANEL

- Validation of ibrutinib effect on p53 protein stabilized by doxorubicin.
- Representative western blots and a graph show a **34% median decrease in p53 level.**



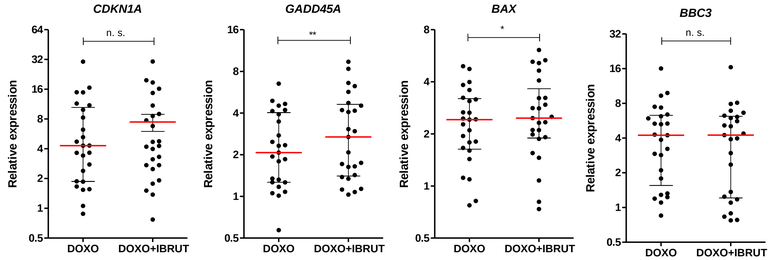
3. Ibrutinib decreases p53 level but doesn't change p53 phosphorylation pattern

- Stability and function of p53 are highly dependent on p53 phosphorylations that are tightly regulated.
- Zn(II)Phos-Tag method (representative western blots shown) revealed **that ibrutinib does not change the p53 phosphorylation pattern**, only the level of total p53 protein.



- +P/-P with/without phosphatase treatment.
- Each band in -P lines represents differentially phosphorylated p53 protein creating specific phospho-pattern.
- A band in +P samples represents the total p53 level.

4. Decrease in p53 level is not mirrored in the expression of its target genes



- The relative expression of four p53 downstream effectors did not decrease after adding ibrutinib to doxorubicine compared to doxorubicin alone.
- We hypothesize that p53 targets are either activated by **p53-independent mechanism** or potential effect of ibrutinib on p53 transcriptional **function is masked by persisting DNA damaging conditions**.
- p<0.05, **p<0.01, n.s. – non-significant, Wilcoxon test. Red lines represent median, whiskers 1st and 3rd quartile.

Conclusion

- **We documented the interplay between p53 and BcR signalling in primary CLL cells.**
- **Ibrutinib decreases the level of p53 in cells without a detectable effect on p53 transcriptional activity.**

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