Activation of the p53 pathway by MDM2 inhibition using the small-molecule compound DS-3032b as therapeutic option for neuroblastoma

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Background

Neuroblastoma (NB) is the most common extracranial childhood tumor. Despite an aggressive treatment strategy, the overall survival of high-risk NB patients is still below 50% [1].

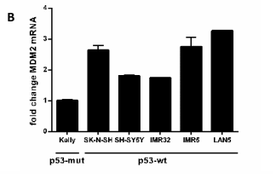
Deregulation of mouse double minute 2 homolog (*MDM2*) expression is one effective mechanism to impede activity of the crucial tumor suppressor p53. The E3 ubiquitin ligase MDM2 negatively regulates the transcriptional activity and stability of p53 via binding to its transcriptional domain and promoting its proteasomal degradation [2,3]. There is evidence that inhibition of wild-type p53 by aberrantly activated MDM2 is a possible mechanism of cell death escape in NB, as indicated by a frequent occurrence of *MDM2* amplification in NB [4-6].

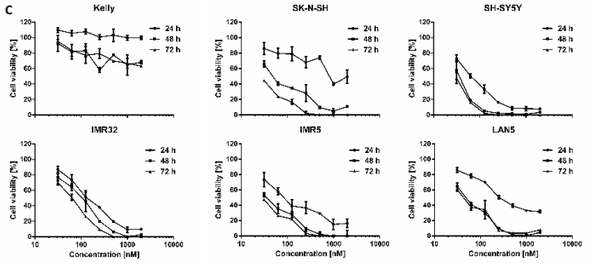
DS-3032b is a novel orally available small-molecule compound that impairs the binding of MDM2 to the transcriptional activation domain of p53 by binding to MDM2. Here, we report the first preclinical evaluation of the small-molecule MDM2 antagonist DS-3032b in NB.

Figure 1: DS-3032b selectively inhibits cell viability of NB cells with functional p53 independently of their MYCN copy number status.

A

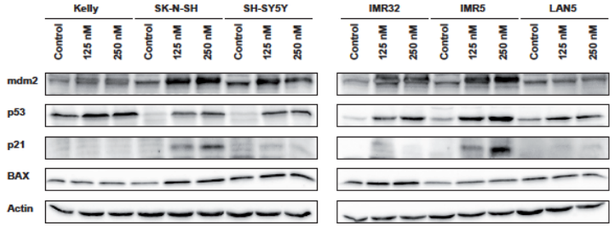
Cell line	*TP53*	*MYCN*	IC50 [nM]
Kelly	529C>T	amp	> 2000
SK-N-SH	wt	N	21.6
SH-SY5Y	wt	N	17.7
IMR32	wt	amp	52.6
IMR5	wt	amp	25.7
LAN5	wt	amp	44.1





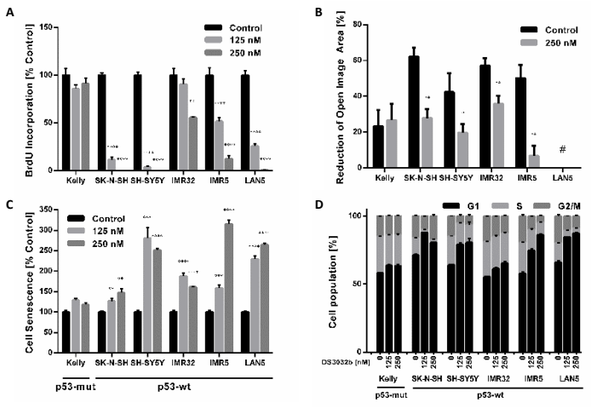
A. *TP53* mutational status, *MYCN* copy number status, and IC50 of DS-3032b after 72 h exposure in NB cell lines studied. B. Basal *MDM2* mRNA expression in NB cell lines. C. Dose-response curves for NB cell lines treated with DS-3032b for 24, 48, and 72 h. Data represent mean values and SD (n = 3 per group).

Figure 2: DS-3032b stabilizes p53 and selectively induces expression of p53 target genes in NB cells with wild-type *TP53*.



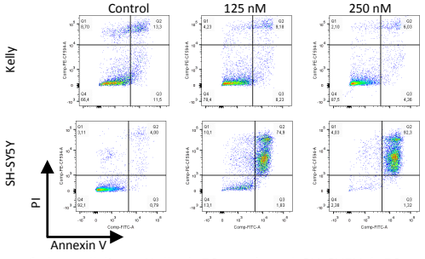
Activation of the p53 pathway shown by induction of mdm2, p21, and BAX protein in cell lines with wild-type *TP53*, in comparison to the p53 mutant cell line Kelly.

Figure 3: DS-3032b selectively leads to a decrease of proliferation and migration NB cells with functional p53 by inducing senescence and cell cycle arrest.



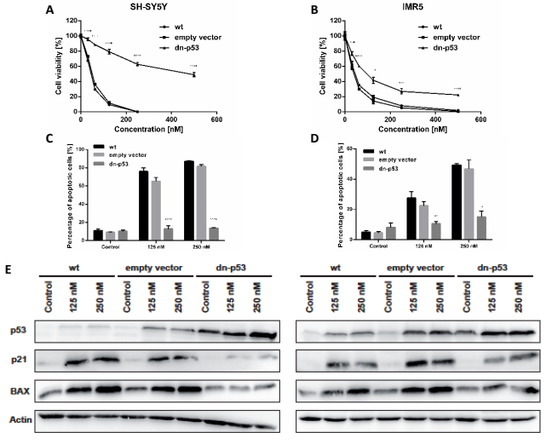
A-C. Proliferation (BrdU ELISA), migration (scratch assay), and senescence (SA-β-gal activity assay) for cell lines treated with DS-3032b for 48 h. D. Cell cycle distribution (FACS after PI staining) for NB cell lines treated with DS-3032b for 48 h. Data represent mean values and SD (n = 3 per group). Hash indicates that the scratch assay was technically unfeasible for LAN5 due to a low cell adherence to the plate.

Figure 4: DS3032b selectively induces apoptosis in neuroblastoma cells with wild-type *TP53*.



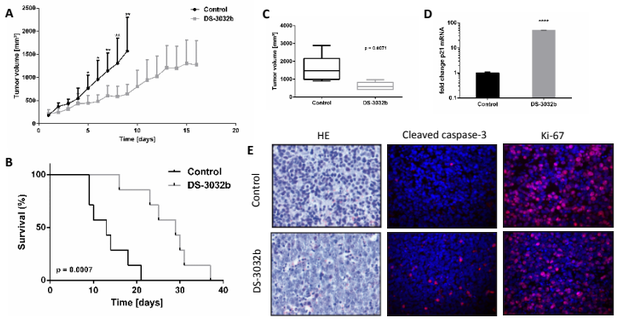
Dot-plots of the cell lines Kelly (p53-mut) and SH-SY5Y (p53-wt) after treatment with DS-3032b for 48 h.

Figure 5: Stable, ectopic expression of dominant negative p53 (dn-p53) abrogates antiproliferative activity of DS-3032b in SH-SY5Y and IMR5 cells.



SH-SY5Y and IMR5 were transfected with a plasmid encoding dn-p53 or an empty vector. A - D. Viability (XTT assay) and apoptosis (FACS after annexin V-FITC/PI staining) for cell lines SH-SY5Y and IMR5 treated with DS-3032b for 48 h. E and F. Protein expression in SH-SY5Y and IMR5 after treatment with DS-3032b for 48 h.

Figure 7: DS-3032b causes tumor growth delay in wild-type p53 NB xenograft mice and improves overall survival.



A and B. Tumor growth and overall survival in mice treated with DS-3032b (n = 7), compared to controls (n = 7). C. Tumor volume on day 9 of treatment in mice treated with DS-3032b, compared to controls. D and E. Expression analysis of p21 mRNA and immunohistochemical analysis of apoptosis (cleaved Caspase-3) and proliferation (Ki-67) in a representative tumor from mice treated with 4 doses of DS-3032b over a course of 36 h, compared to controls.

Summary

1. Targeted inhibition of MDM2 using DS-3032b leads to a selective activation of the p53 pathway in NB cells with wild-type *TP53*.
2. DS-3032b showed antiproliferative and cytotoxic activity by inducing G1 cell cycle arrest und apoptosis independent of *MYCN* amplification status.
3. In contrast, no effect of DS-3032b was detected in NB cells harboring *TP53* mutations or expressing dominant negative p53.
4. Oral treatment with DS-3032b resulted in tumor growth inhibition and prolonged survival in a murine subcutaneous NB xenograft model.

Conclusion: DS-3032b is a promising therapeutic option for NB patients independent of *MYCN* amplification status

References

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