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CORRESPONDENCE

Loss of TRP53 reduces but does not overcome dependency of lymphoma cells on MCL-1

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TO THE EDITOR:

The gene encoding the anti-apoptotic BCL-2 family member MCL-1 is frequently amplified (~10%) in diverse human malignancies [1] and the finding that its inducible deletion impairs the growth of several types of tumours in vivo makes it an attractive target for anti-cancer therapy [2]. Lymphomas driven by c-MYC are particularly dependent on MCL-1, with loss of only one *Mcl-1* allele preventing expansion of such murine lymphomas in vivo [2]. Accordingly, MYC-driven lymphomas are highly sensitive to MCL-1 inhibitors [3] and deletion of one *Mcl-1* allele greatly delays c-MYC-driven lymphomagenesis in mice [4]. However, some c-MYC-driven lymphomas can tolerate the loss of one *Mcl-1* allele, which reduces MCL-1 protein levels by ~40% [5]. These lymphomas uniformly display mutations of the tumour suppressor *Trp53* [2]. Wild-type (wt) TRP53 function is required for optimal responses to BH3-mimetic drugs [6], and loss of *Trp53* overcomes the delayed lymphomagenesis seen in *Eμ-Myc;Mcl1*$^{+/-}$ mice [4]. These studies suggest that loss or mutation of *Trp53* diminishes the dependence of c-MYC-driven lymphomas on MCL-1. To explore this further, we conditionally deleted *Mcl-1* in TRP53 deficient *Eμ-Myc* mouse lymphomas in vivo, to determine whether the loss of TRP53 allows them to tolerate the complete absence of MCL-1. This is an important question given that several BH3-mimetic drugs targeting MCL-1 have entered clinical trials in haematological cancers, where defects in TRP53 function frequently underlie resistance to standard therapies.

We utilised *Eμ-Myc* transgenic mice carrying conditional *loxP* flanked (*floxed*) *Mcl-1* alleles [2] and a constitutively expressed but conditional tamoxifen-inducible Cre-recombinase (*Rosa-CreERT2*) (Supplementary Fig. S1). We engineered *Eμ-Myc* mice with germline loss of one *Trp53* allele and one or two *floxed Mcl-1* alleles plus the *CreERT2* transgene (Supplementary Fig. S1). *Eμ-Myc;Trp53*$^{+/-}$*;Mcl1*$^{fl/+}$*;RosaCreERT2* and *Eμ-Myc;Trp53*$^{+/-}$*;Mcl1*$^{fl/fl}$*;RosaCreERT2* mice rapidly developed pre-B/B lymphomas (median survival ~30 days), and all selected for loss of the wt *Trp53* allele, rendering them TRP53 deficient (Supplementary Fig. S2d). Ly5.2^{+} *Eμ-Myc;Trp53*$^{-/-}$*;Mcl1*$^{fl/+}$*;RosaCreERT2* ($N = 3$; $n = 29$), *Eμ-Myc;Trp53*$^{-/-}$*;Mcl1*$^{fl/fl}$*;RosaCreERT2* ($N = 6$; $n = 18$) and control *Eμ-Myc;Trp53*$^{-/-}$*;Mcl1*$^{+/+}$ *RosaCreERT2* ($N = 1$; $n = 3$) lymphomas were transplanted into immune-competent C57BL/6-Ly5.1 recipients that were treated with either vehicle or tamoxifen on days 5 and 6 post-transplantation to induce *Mcl-1*^{fl} deletion in the malignant cells in vivo (Supplementary Fig. S1b). Recipients bearing *Eμ-Myc;Trp53*$^{-/-}$*;Mcl1*$^{+/+}$*;CreERT2* control lymphomas exhibited a marginal survival advantage (3 days) following tamoxifen treatment (Supplementary Fig. S2e). In the absence of TRP53, removal of one *Mcl-1* allele in *Eμ-Myc;Trp53*$^{-/-}$*;Mcl1*$^{fl/+}$*;RosaCreERT2* lymphomas did not impact lymphoma growth or survival of recipient mice (Fig. 1a). Deletion of the floxed *Mcl-1* gene and encoded protein were confirmed in these lymphoma cells following treatment of mice with tamoxifen (Supplementary Fig. S2a-c). Remarkably, biallelic *Mcl-1* deletion in *Eμ-Myc;Trp53*$^{-/-}$*;Mcl1*$^{fl/fl}$*;RosaCreERT2* ($N = 6$; $n = 18$) lymphomas, resulting in complete removal of MCL-1, significantly prolonged tumour-free survival, with 14/18 (77.8%) of recipients cured (Fig. 1a). The four relapsed lymphomas may have selected against *Mcl-1*^{fl} recombination and hence loss of MCL-1 protein or acquired changes that compensated for MCL-1 loss.

We further assessed the impact of *Trp53* loss on MCL-1 dependency in *Eμ-Myc* lymphomas by using the MCL-1 inhibitor S63845. *Eμ-Myc* lymphoma-derived cell lines with spontaneous *Trp53* loss ($N = 5$) or mutation ($N = 4$) were compared with control *Eμ-Myc* lymphoma lines with intact TRP53 function ($N = 9$). Loss of TRP53 function conferred only minor resistance to MCL-1 inhibition (increase in IC_{50} value), with higher doses of MCL-1 inhibitor effectively killing *Trp53*-deficient lymphoma cells (Fig. 1b). In the human diffuse large B cell lymphoma (DLBCL) line DoHH2, CRISPR-mediated loss of TP53, validated by resistance to the MDM2 inhibitor Nutlin-3a (Supplementary Fig. S3a), did not impact the response to S63845 in short-term killing assays (Supplementary Fig. S3b). However, the *TP53* deficient DoHH2 cells had a competitive advantage over their wt *TP53* counterparts when cultured together in sub-optimal (~IC_{25}) doses of S63845 for 14 days (Supplementary Fig. S3c), consistent with previous observations [6].

Considering the potential underlying mechanism, there is a direct relationship between TRP53 and MCL-1 in their opposing functions in regulating apoptosis (Fig. 1c). TRP53 can induce apoptosis by increasing the expression of the pro-apoptotic BH3-only proteins, PUMA/*Bbc3*, NOXA/*Pmaip1* and BIM/*Bcl2l11* [7].

In conclusion, we demonstrate that loss/mutation of TRP53/TP53 renders mouse c-MYC-driven lymphomas and a human DLBCL cell line less dependent on MCL-1 for sustained survival and expansion. Importantly though, lymphomas with loss of TRP53/TP53 can still be effectively killed using either higher concentrations of the MCL-1 inhibitor or by homozygous deletion of *Mcl-1*. This is important for the clinical translation of MCL-1 inhibitors because it suggests that patients bearing cancers with loss/mutation of *TP53* may require higher doses of MCL-1 inhibitors or combination therapies to augment the levels of pro-apoptotic BH3-only proteins [6].

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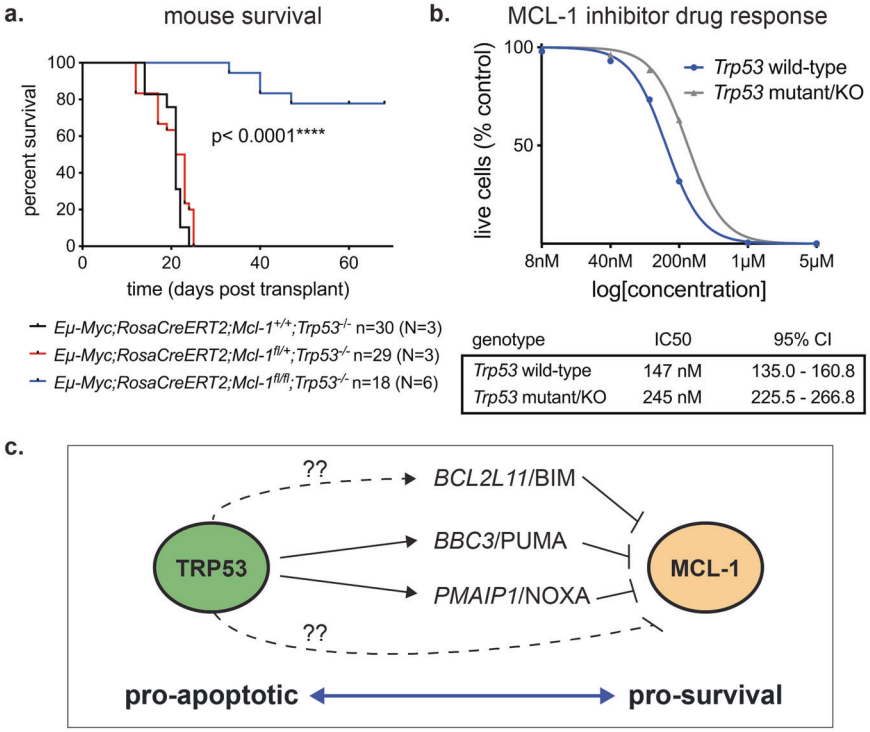

Fig. 1 *Eμ-Myc* lymphoma cells with loss of TRP53 remain dependent on anti-apoptotic MCL-1. a Kaplan–Meier survival curve for mice transplanted with *Eμ-Myc*$^{T/+}$;*CreERT2*$^{KI/+}$;*Trp53*$^{-/-}$ lymphomas bearing either wt *Mcl-1*, a single floxed *Mcl-1* allele or two floxed *Mcl-1* alleles and treated with tamoxifen on days 5 and 6 to delete the floxed *Mcl-1* alelle(s) in the lymphomas. *p* value determined by Log-rank (Mantel–Cox) test. N indicates number of independent lymphomas of a given genotype tested; n indicates the number of recipient mice transplanted with the indicated lymphomas examined. **b** Response of *Eμ-Myc* lymphoma-derived cell lines to the MCL-1 inhibitor, S63845, comparing a panel of cell lines with deficient vs wild-type TRP53 function; $N = 9$ of each. Data represent non-linear regression of the means with IC_{50} and 95% confidence interval (CI) indicated. **c** Schematic depicting the intersecting and opposing roles of TRP53 and MCL-1 in the regulation of apoptosis.

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DATA AVAILABILITY

Data sharing is not applicable to this paper as no datasets were generated or analysed during the current study. Reagents are available on request.

REFERENCES

1. Beroukhim R, Mermel C, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010;463:899–905.
2. Kelly GL, Grabow S, Glaser SP, Fitzsimmons L, Aubrey BJ, Okamoto T, et al. Targeting of MCL-1 kills MYC-driven mouse and human lymphomas even when they bear mutations in p53. Genes Dev. 2014;28:58–70.
3. Kotschy A, Szlavik Z, Murray J, Davidson J, Maragno AL, Le Toumelin-Braizat G, et al. The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. Nature. 2016;538:477–82.
4. Grabow S, Delbridge AR, Aubrey BJ, Vandenberg CJ, Strasser A. Loss of a Single Mcl-1 Allele Inhibits MYC-Driven Lymphomagenesis by Sensitizing Pro-B Cells to Apoptosis. Cell Rep. 2016;14:2337–47.
5. Brinkmann K, Grabow S, Hyland CD, Teh CE, Alexander WS, Herold MJ, et al. The combination of reduced MCL-1 and standard chemotherapeutics is tolerable in mice. Cell Death Differ. 2017;24:2032–43.
6. Thijssen R, Diepstraten ST, Moujalled D, Chew E, Flensburg C, Shi MX, et al. Intact TP-53 function is essential for sustaining durable responses to BH3-mimetic drugs in leukemias. Blood. 2021;137:2721–35.
7. Villunger A, Michalak EM, Coultas L, Mullauer F, Bock G, Ausserlechner MJ, et al. p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. Science. 2003;302:1036–8.

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AUTHOR CONTRIBUTIONS

BJA, MSB, STD, ZW, CC, MJH, GLK and AS conceptualised and planned the study, performed experiments, analysed data and wrote the paper.

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COMPETING INTERESTS

AS, GLK, STD, ZW, CC and MJH are currently, and BJA and MSB have previously been, employees of the Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, which receives Royalties and Milestone payments related to the BCL-2 inhibitor, Venetoclax/ABT-199. AS, GLK and MH have received research funding from Servier.

ETHICS APPROVAL

All experiments with mice followed the guidelines of the Melbourne Directorate Animal Ethics Committee, according to The Walter and Eliza Hall Institute of Medical Research Ethics Committee.

ADDITIONAL INFORMATION

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