Missense mutations in p53 generate aberrant proteins with abrogated tumour suppressor functions that can also acquire oncogenic gain-of-function activities that promote malignant progression, invasion, metastasis and chemoresistance\(^1\)-\(^4\). Mutant p53 (mutp53) proteins undergo massive constitutive stabilization specifically in tumours, which is the key requisite for the acquisition of gain-of-functions activities\(^6\)-\(^8\). Although currently 11 million patients worldwide live with tumours expressing highly stabilized mutp53, it is unknown whether mutp53 is a therapeutic target in vivo. Here we use a novel mutp53 mouse model expressing an inactivatable R248Q hotspot mutation (floxQ) to show that tumours depend on sustained mutp53 expression. Upon tamoxifen-induced mutp53 ablation, allotransplanted and autochthonous tumours curb their growth, thus extending animal survival by 37%, and advanced tumours undergo apoptosis and tumour regression or stabilization. The HSP90/HDAC6 chaperone machinery, which is significantly upregulated in cancer compared with normal tissues, is a major determinant of mutp53 stabilization\(^9\)-\(^12\). We show that long-term HSP90 inhibition significantly extends the survival of mutp53 Q/− (R248Q allele\(^1\)) and H/H (R172H allele\(^3\)) mice by 59% and 48%, respectively, but not their corresponding p53\(^+\)/+ (also known as Trep53\(^−\)/−) littermates. This mutp53-dependent drug effect occurs in H/H mice treated with 17DMAG+SAHA and in H/H and Q/− mice treated with the potent Hsp90 inhibitor gane-tespib. Notably, drug activity correlates with induction of mutp53 degradation, tumour apoptosis and prevention of T-cell lympho- megasis. These proof-of-principle data identify mutp53 as an actionable cancer-specific drug target.

Tumour-promoting gain-of-function activities of missense mutp53 alleles are demonstrated by knock-in mouse models\(^1\)-\(^3\) and in patients with Li-Fraumeni syndrome carrying germline TP53 missense mutations. Li-Fraumeni syndrome patients with TP53 missense mutations have earlier cancer onset than patients with loss of p53 expression\(^3\)-\(^11\). Strikingly, p53R248Q\(^+\) Li-Fraumeni syndrome patients have higher tumour numbers and shorter tumour-free survival by 10.5 years than p53null/+ patients\(^6\). A key feature and prerequisite for gain-of-function is that mutp53 proteins undergo massive stabilization specifically in tumours\(^8\)-\(^12\).

RNA-interference-mediated depletion of mutp53 produces marked cytotoxic effects in human cancer cells in culture and xenograft models\(^13\). Notably, mutp53 knockdown in orthotopic allografts of Kras\(^{G12D}\) pancreatic murine cancer cells dramatically reduced their metastatic ability\(^14\). Together, this provides a strong rationale for testing mutp53 destabilizing cancer therapy in vivo. If confirmed, it might establish mutp53 as an important direct drug target in many cancer patients. To validate this rigorously, we generated conditional inactivatable p53 R248Q (floxQ) mice (Extended Data Fig. 1a–c) with a Rosa26CreERT2 (E2T) knock-in allele, which mirror the constitutive R248Q (Q) mice\(^2\) in tumour spectrum and survival (Extended Data Fig. 1d). Tamoxifen/4-hydroxytamoxifen (4OHT) treatment activates CreERT2, causing deletion of the floxQ allele (with up to 90% efficiency) and cell death in primary T-cell lymphoma cultures, but not in various controls (Fig. 1a and Extended Data Fig. 1e). Likewise, transplantation assays into immunocompromised hosts (subcutaneous and tail vein allografts, prophylactic and therapeutic treatments) showed that floxQ deletion markedly inhibited tumour growth in vivo (Fig. 1a–d and Extended Data Fig. 1f–h) and prolonged survival of recipients compared with controls (Fig. 1b and Extended Data Fig. 1f). These data strongly suggest tumour dependence on sustained high levels of mutp53.

Importantly, clinically advanced autochthonous tumours in floxQ/− mice responded to mutp53 ablation with regression or stagnation (Fig. 2a–c and Extended Data Fig. 2a). Mechanistically, this was due to marked tumour apoptosis (Fig. 2d), but not cell cycle arrest (Extended Data Fig. 2b). Notably, mutp53 ablation was also associated with strong suppression of lung metastasis, contrasting with large metastatic nodules in controls (Fig. 2e). Moreover, mutp53 ablation in floxQ/− mice with early disease (10 weeks old) (Fig. 2f) extended median overall and T-lymphoma-specific survival by 37% from 128 to 175 days (Fig. 2g and Extended Data Fig. 2c). Notably, the improved overall survival of floxQ/− mice, which normally have a significantly shorter lifespan than p53-null littermates\(^2\) (Extended Data Fig. 1f), now resembled that of p53-null mice (Extended Data Fig. 2d), while their T-lymphoma-specific survival now extended beyond that of p53-null mice (Extended Data Fig. 2e). This further indicates that tumours driven by mutp53 depend on stabilized mutp53. In support, at endpoint (death), most tumours of all types (17/23, 74%) from floxQ/− mice that were tamoxifen-treated at 10 weeks were again composed of 100% mutp53-overexpressing cells (Fig. 2h and Extended Data Fig. 2f). This indicates strong selective pressure for the small minority of non-recombined mutp53-positive cells outcompeting the majority of recombined cells. It is tempting to speculate that complete allele removal would have further improved survival. Thus, these data establish for the first time that continued expression of stabilized mutp53 is essential for tumour maintenance in vivo.

The HSP90 chaperone machinery is highly activated in cancers compared with normal tissues and renders them resistant to proteotoxic stress by supporting proper folding of conformationally aberrant oncoproteins including mutp53 (refs 17, 18). Thus, cancer cells have a far smaller tolerance for HSP90 inhibition. We and others previously showed that HSP90 and its obligatory positive regulator, cytosolic HDAC6, are major determinants of mutp53 stabilization\(^9\)-\(^12\). Importantly, deletion of HSF1, the master transcriptional activator of the inducible heat-shock response including HSP90, dramatically suppresses oncogenicity in mutp53 H/− mice, but has no effect in p53-null mice\(^19\)-\(^20\). These data clearly indicate that tumorigenicity of the H allele—but not of p53-null—strongly depends on Hsf1-mediated chaperone support, mainly HSP90. 17AAG and its hydrophilic derivative 17DMAG are ansamycin-derived highly specific first-generation Hsp90 inhibitors (Hsp90)\(^22\). Likewise, histone...
deacetylase inhibitors, including FDA-approved SAHA, are promising anti-cancer drugs whose actions involve hyperacetylation of histone and select non-histone targets including HDAC6 substrate Hsp90, thus indirectly inhibiting Hsp90 (ref. 21).

The cytotoxicity of 17AAG/SAHA in mutp53 cancer cells, despite being pleiotropic drugs, is largely due to the destabilization of mutp53 protein via Hsp90/HDAC6 inhibition11,12. Moreover, owing to complementary drug targets, 17AAG/SAHA treatment caused synergistic cytotoxicity in human breast-cancer cells compared with monotherapy11. Likewise, 17AAG and SAHA synergized in T47D (p53L194F) cytotoxicity in human breast-cancer cells compared with monotherap
type. In addition, a high cytotoxicity of 17AAG/SAHA against parental MDA231 (p53R280K) cells, but lost their efficacy when excess ectopic mutp53 was present. Only the combination of both drugs overcame this block (Fig. 3a).

To translate the genetic proof-of-principle results from floxQ/– mice (Figs 1, 2) towards clinical application, we performed long-term treatments with 17DMAG+SAHA in mutp53R172H (H) mice3. Starting at 8 weeks when most H/H mice exhibited early intrathymic T-lymphoma (Fig. 3b), H/H and p53-null mice were treated lifelong with 17DMAG+SAHA versus vehicles. Strikingly, Hsp90 inhibition benefited only H/H but not p53-null mice, extending their overall survival from a median 140 to 182 days (P < 0.001, Fig. 3c). Moreover, drug treatment improved survival of H/H mice beyond that of p53-null mice (Fig. 3d). For T-lymphoma-specific survival, again only H/H but not p53-null controls benefited from 17DMAG+SAHA treatment (P < 0.001, Fig. 3e). These data strongly support the idea that tumours expressing mutp53 depend on its presence and fundamentally differ in their oncogenic wiring from p53-null tumours. Moreover, the mechanism of action of these pleiotropic HSP90 inhibitors is mutp53-dependent.

Analysis of vehicle- and drug-treated thymic tissues at death for evidence of drug activity showed HSPO9i activity (marked by transient induction of HSFI target Hsp70 (ref. 22)), but this did not significantly prevent tumours in p53-null mice (Fig. 3f). In contrast, in H/H mice, when the drug worked (Hsp70 induced, mutp53 undetectable) it apparently prevented T-lymphoma formation in every case since we found only normal thymic tissues (Fig. 3f, ‘p53H/H normal thymus’ mice 7–14, plus 12 mice analysed by autopsy). In contrast, in all six T-lymphomas that arose despite drug treatment, the drugs failed to inhibit Hsp90 (Hsp70 not induced) (Fig. 3f, 6/26 (23%) escapers). Mechanistically, NQO1 (NAD(P)H:quinone oxido-reductase) is required to activate 17DMAG by metabolizing its quinone moiety, and NQO1 downregulation is a predictive biomarker for resistance to this ansamycin class of Hsp90i23,24. Indeed, we observed reduced NQO1 expression in escaper versus responder T-lymphomas (Fig. 3g). In sum, Hsp90 inhibition prevented the development of T-lymphomas in mutp53 mice.

To reduce complexity and dosing of the combinatorial anti-HSP90 treatment, we eliminated HDAC inhibition and replaced 17DMAG with the highly potent synthetic Hsp90i ganetespib. Ganetespib has demonstrated a favourable safety profile in more than 1,200 cancer patients treated so far, and is currently being evaluated in 11 clinical trials including a phase 3 lung cancer trial (www.syntapharma.

LETTER

Figure 1 | Genetic ablation of mutp53 curbs tumour growth in allografts. a–d, Various prophylactic (a, b) and therapeutic (c, d) protocols of primary floxQ/– versus Q/– and p53-null T-lymphomas allotransplanted (black arrows on time axes) via subcutaneous (a, c, d) or tail vein (b) injections into nude mice, treated with daily intraperitoneal injections of tamoxifen (tam.) or corn oil (stars on time axes). a, Experimental diagram, allograft mass, representative tissue immunostaining and immunoblot at endpoint. Unpaired two-tailed Student’s t-test; mean ± s.e.m.; n, number of allografts. b, Deletion of mutp53 improves survival of host mice. Kaplan–Meier analysis, log rank test; n, number of mice. c, Two different therapeutic protocols (100 mg per kg tamoxifen for 5 days versus 150 mg per kg for 7 days) show the dose-dependence of allograft growth on mutp53 depletion. Unpaired two-tailed Student’s t-test; mean ± s.e.m.; n, number of allografts. d, Allograft growth using therapeutic protocol (Fig. 1c, endpoint 2) and mean fold changes at endpoint; n, number of allografts.

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stagnation (Fig. 4i). H/H mice, whose lifespan is normally identical to p53-null mice, responded to ganetespib with improved survival beyond stagnation (Fig. 4i). In two of two Q/–p53-null mice7, responded to ganetespib with improved survival beyond stagnation (Fig. 4i). H/H mice, whose lifespan is normally identical to p53-null mice, showed significantly shorter than p53-nulls2 (Extended Data Fig. 1d), showed improved survival now resembling p53-nulls (Extended Data Fig. 4l). Animals were treated once (arrow) at 10 weeks with tamoxifen or oil for 5 consecutive days. h, p53 immunostaining at endpoint (death) of representative T-lymphomas (see also Extended Data Fig. 2f).

Figure 2 | Mutp53 ablation in floxQ/– mice causes autochthonous tumour regression or stagnation and extends survival. a, Fold growth over time until endpoint of clinically advanced tumours in floxQ/–;ERT2/+ mice imaged by ultrasound and treated with tamoxifen or oil (beginning at day 0, arrowhead), normalized to initial tumour size. b, Daily growth rates of individual tumours during the first 5–12 days of tamoxifen/oil treatment (left), and mean ± s.e.m. of all tumours (right). Unpaired two-tailed Student’s t-test, #, sarcomas; all others are T-lymphomas. c, Representative examples of sagittal ultrasound images of T-lymphomas in tamoxifen-treated floxQ/–;ERT2/+ and control mice. d, Genetic ablation of mutp53 in autochthonous tumours induces apoptosis. Immunostaining for mutp53 and cleaved caspase 3 in representative control and mutp53-ablated T-lymphomas. e, Lung metastasis in these mice by haematoxylin and eosin (H&E) and p53 immunostaining. f, Organ-confined disease in young floxQ/– mice indicated by p53 immunostaining of malignant thymic cell clones. WT, wild type. g, Kaplan–Meier analysis and log rank statistics comparing cancer-related overall survival of floxQ/–;ERT2/+ versus Q/–;ERT2/+ and p53–/–;ERT2/+ mice. Animals were treated once (arrow) at 10 weeks with tamoxifen or oil for 5 consecutive days. h, p53 immunostaining at endpoint (death) of representative T-lymphomas (see also Extended Data Fig. 2f).
Figure 3 | Pharmacological inhibition of the mutp53 stabilizing HSP90/HDAC6 axis with 17DMAG+SAHA prolongs survival of H/H mice in a mutp53-dependent manner. (a), 17AAG and SAHA synergize in degrading mutp53 and suppressing growth of subcutaneous xenografts of MDA231 cells expressing excess ectopic p53R280K. Mean ± s.e.m.; n, number of xenografts, unpaired one-tailed Student’s t-test. (b), Organ-confined disease in young H/H mice, indicated by p53 immunostaining of malignant thymic cell clones. (c–e), cancer-related overall survival (c, d) and T-lymphoma-specific survival (e) of 17DMAG+SAHA-treated mutp53 H/H and p53-null mice, Kaplan–Meier analyses, log rank statistics. (f), Immunoblot of p53 and Hsp70 in thymic tissues at endpoint from mice shown in e; each lane represents a different vehicle- or drug-treated mouse. (g), Real-time PCR analysis of NQO1 in representative responder and escaper H/H T-lymphomas from f.

Figure 4 | Treatment of H/H and Q−/− mice with ganetespib suppresses tumour growth and extends survival in a mutp53-dependent manner. (a, b), Prophylactic (a) and therapeutic (b) treatment of subcutaneous Q−/− allografts with ganetespib (ganet.) or vehicle (DMSO). (a), Arrowhead, tumour cell injection. (b). Initial allograft volume and mass at endpoint, mean ± s.e.m., unpaired two-tailed Student’s t-test; n, number of allografts. (c, d), Time course of mutp53 levels and apoptosis (cleaved caspase 3) in ganetespib-treated (arrow) Q−/− subcutaneous T-lymphoma allografts, analysed by immunoblot (c) and immunostaining (d). Asterisks mark non-specific bands (c). Enlarged panels after the first and second doses (d, far right) immunostained for p53 (arrows), dead cells stain blue. (e), Growth of subcutaneous Q−/− T-lymphoma allografts treated once (arrow) with ganetespib or cyclophosphamide alone or in combination. Mean ± s.e.m., unpaired two-tailed Student’s t-test; n, number of allografts. **P < 0.05, ***P < 0.01 (single drugs versus combination). (f–h), Kaplan–Meier analysis and log rank statistics of cancer-related overall survival (f, g) and T-lymphoma-specific survival (h) of mutp53 H/H, Q−/− and their respective p53−/− controls. (i), Fold growth over time of clinically advanced Q−/− and p53−/− T-lymphomas imaged by ultrasound and treated with ganetespib. Mean ± s.e.m., unpaired two-tailed Student’s t-test; n, number of tumours, *P < 0.05. (j), Comparison of overall survival of ganetespib-treated mutp53 H/H and their respective p53−/− controls, shown in f. (k, l), Immunoblot analysis of thymic tissues from same-litter siblings (k) and from Q−/− mice from h at endpoint. Each lane represents individual DMSO- or ganetespib-treated mice.
Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to U.M.M. (Ute.Moll@stonybrookmedicine.edu).

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METHODS

Generation of floxQERT2 mice. The floxed HUPKI (humanized p53 knock-in) p53R248Q allele (referred to as floxQ) was generated by introducing loxP sites flanking exons 2–10 of the p53 locus where mouse exons 4–9 replaced with human exons 4–9 containing a p53 R248Q mutation in exon 7, as described in Extended Data Fig. 1a–c. The p53floxQERT2 mice were crossed with p53–/– mice on a 129SvImJ/C57Bl6 background mixed as previously described to generate p53floxQ–/– animals. The p53floxQ–/– animals were then intercrossed with p53–/+ animals (previously described in ref. 2) to generate p53floxQ–/– mice on a 129SvImJ/C57Bl6 background. Rosa26ERT2/+ERT2 mice, referred to as ERT2 on a pure C57Bl6 background were also previously described2,28.

Response and our approved protocol, the maximum tumour burden per mouse was 4 cm³, time of euthanasia was considered for 5 consecutive days (Fig. 2f–h and Extended Data Fig. 2c–f). As a pre-set criterion, animals that died from non-cancer-related causes (the minority) were excluded from subsequent analyses. To treat clinically advanced tumours for 5 days a week until endpoint (death). As a pre-set criteria, animals that died from non-cancer-related causes (the minority) were excluded from subsequent analyses.

For ganetespib treatments of H/H and Q/– mice and their corresponding p53–/– control, ganetespib (aka STA-9090, Synta Pharmaceuticals) was dissolved in 10% DMSO/18% cremophor/3.6% dextrose (‘10/18 DRD’) as previously described36. For references, rodents safely tolerate doses of ganetespib of up to 150 mg per kg once weekly or 75 mg per kg twice weekly36. Randomly assigned animals received freshly prepared ganetespib or the corresponding volume of 10/18 DRD vehicle by tail vein injection as follows: 50 mg per kg ganetespib once a week starting at 8 weeks of age until death for the treatment of autochthonous tumours (including the sibling analysis in Fig. 4); 75 mg per kg ganetespib twice a week for the treatment of T- and B-lymphoma allotypes (beginning 1 day before lymphoma transplantation in the prophylactic protocol, or beginning when at least two out of four palpable tumours per mouse had appeared in the therapeutic protocol); 150 mg per kg of ganetespib once or twice for acute high-dose treatment (Fig. 4c, d). As a pre-set criterion, animals that died from non-cancer-related causes (the minority) were excluded from subsequent analyses. For the sibling analysis (Fig. 4), the sisters were killed at 90 days and the brothers at 148 days, each 24 h after their last treatment, and their thymic tissues were subjected to immunoblot analysis. Cyclophosphamide was dissolved in PBS and injected intraperitoneal at indicated doses.

Hsp90 inhibitor studies. HSP90 is crucial for cancer cell survival18. Cultured cells were treated with 2.5 μM or 5 μM 17-allylamino-17-demethoxygeldanamycin (17AAG, LC Laboratories, A–6880) or 5–500 nM ganetespib in DMSO for 24 h or 48 h. For xenografts, human cancer cells were subcutaneously injected into nude mice (0.5 mice per site in 100 μl Matrigel, four sites per mouse). Tumours became visible around day 10. For Fig. 3a and Extended Data Fig. 3, treatment of 10 mg per kg 17AAG and/or 25 mg per kg SAHA (vorinostat/suberoylanilide hydroxamic acid, LC Laboratories, V-8477) in DMSO or vehicle control was switched at day 24 and every other day with endpoint at 44 or earlier in the few cases where tumours became ulcerated.

For 17DMAG+SAHA treatments of H/H and corresponding p53–/– mice, 17DMAG (17-dimethylaminoethyleno-17-demethoxygeldanamycin, LC Laboratories D-3440), a hydrophilic derivative of 17AAG19, was dissolved in 5% (w/v) hydroxypropyl-β-cyclodextrin (HOP-β-CD) (Sigma 332607), and SAHA (LC Laboratories V-8477) was dissolved in a 5 M equivalent of HOP-β-CD as previously described20. Equal volumes of 17DMAG and SAHA (or both vehicle solutions) were combined and frozen in aliquots until use. Starting at 8 weeks of age, randomly assigned animals received 100 μl intraperitoneal injections of 10 mg per kg 17DMAG plus 25 mg per kg SAHA, or 100 μl HOP-β-CD vehicle, for 5 days a week until endpoint (death). As a pre-set criteria, animals that died from non-cancer-related causes (the minority) were excluded from subsequent analyses.

For genetespib treatments of H/H and Q/– mice and their corresponding p53–/– control, ganetespib (aka STA-9090, Synta Pharmaceuticals) was dissolved in 10% DMSO/18% cremophor/3.6% dextrose (‘10/18 DRD’) as previously described36. For references, rodents safely tolerate doses of ganetespib of up to 150 mg per kg once weekly or 75 mg per kg twice weekly36. Randomly assigned animals received freshly prepared ganetespib or the corresponding volume of 10/18 DRD vehicle by tail vein injection as follows: 50 mg per kg ganetespib once a week starting at 8 weeks of age until death for the treatment of autochthonous tumours (including the sibling analysis in Fig. 4); 75 mg per kg ganetespib twice a week for the treatment of T- and B-lymphoma allotypes (beginning 1 day before lymphoma transplantation in the prophylactic protocol, or beginning when at least two out of four palpable tumours per mouse had appeared in the therapeutic protocol); 150 mg per kg of ganetespib once or twice for acute high-dose treatment (Fig. 4c, d). As a pre-set criterion, animals that died from non-cancer-related causes (the minority) were excluded from subsequent analyses. For the sibling analysis (Fig. 4), the sisters were killed at 90 days and the brothers at 148 days, each 24 h after their last treatment, and their thymic tissues were subjected to immunoblot analysis. Cyclophosphamide was dissolved in PBS and injected intraperitoneal at indicated doses.

Human cancer cell lines. Breast cancer MDA231 (p53R280K, HTB-26), MDA646 (p53R273K, HTB-132), T47D (p53L194F, HTB-133), SKBR3 (p53R273H, HTB-30), prostate cancer DU145 (p53P223L and p53V274F, HTB-81), non-small-cell lung cancer H1975 (p53R273H, CRL-5908) and ovarian cancer cell line ES2 (p53S241F, CRL-1978) were purchased from the American Type Culture Collection. The ovarian cancer lines EFO21 (p53C124R, ACC-235) and COLO704 (p53wt, ACC-198) were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). The ovarian cancer cell line COV344 (p53wt) was purchased from Sigma-Aldrich (07071909). The ovarian cancer cell line HOCH7 (p53C275F) was a gift from N. Concini. Parental parental MDA231 and MDA231+R280K cells stably overexpressing pcDNA3 vector or pcDNA3-p53R280K plasmids, respectively, were generated by transfection and selection. All cells were cultured in DMEM/10% FCS and tested negative for mycoplasma. No further authentication of cell lines was performed.

Cell death. Cell death was determined by CellTiter-Blue Cell Viability Assay (Promega G8080A) according to the manufacturer’s instructions, in six-well plate format with five Micromaxa cells per well or 150,000 human cancer cells per well. Cells were treated as indicated and fluorescence was detected by FILTERmax 3 Mio lymphoma cells per well or 150,000 human cancer cells per well. Cells were treated as indicated and fluorescence was detected by FILTERmax 3 Mio lymphoma cells per well or 150,000 human cancer cells per well. Cells were treated as indicated and fluorescence was detected by FILTERmax 3 Mio lymphoma cells per well or 150,000 human cancer cells per well. Cells were treated as indicated and fluorescence was detected by FILTERmax 3 Mio lymphoma cells per well or 150,000 human cancer cells per well.
antibodies: mutp53 (FL-393, Santa Cruz, sc-6243; DO1, Santa Cruz sc-126), Hsp70 (Enzo C92F3A-5), PARP (Cell Signaling 9542), cleaved PARP (Cell Signaling 9541), cleaved caspase 3 (Cell Signaling 9661), Chk1 (Cell Signaling 2360), CDK1 (Millipore MAB8878), MAPK (Millipore 05-157), actin (Thermo Scientific MS-1295-P0), Hsc70 (Santa Cruz, sc-7298), GAPDH (Santa Cruz, sc-25778).

Quantitative PCR analysis. Total RNA was isolated using TRizol reagent (Invitrogen) and 2 μg was reverse-transcribed with random primers and SuperScript II Reverse Transcriptase (Invitrogen 18064-014). Real-time PCR was performed in duplicates with QuantiTect SYBR Green Mix (Qiagen 204143) using the MJ Research DNA Engine Opticon 2 machine. Primers for mouse NQO1 were: 5’ TGGCCGAACACAAGAAGCTG 3’ (forward), 5’ GCTACGAGCACTCTCTCAAACC 3’ (reverse). NQO1 expression was normalized to the housekeeping gene HPRT.

Statistics. Statistical tests with appropriate underlying assumptions on data distribution and variance characteristics were used. Kaplan–Meier analyses with log rank statistics for animal survival curves were generated using SigmaPlot-Systat software version 11. Note: in Figs 3e and 4h, dots represent censored animals, namely mice dead owing to cancer types other than T-lymphomas. Each line ends when the last animal dies (if caused by T-lymphoma, the line goes down to zero; if caused by another cancer type, the animal is censored and the line stops with a dot).

An unpaired two-tailed Student’s t-test was used to analyse tumour measurements. No statistical method was used to predetermine sample size. No blinding was used. All n numbers indicate biological replicates, unless indicated otherwise.

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Extended Data Figure 1 | Generation and characterization of the conditionally inactivatable p53 flox R248Q allele. a, Mouse exons 4–9 were replaced with human exons 4–9 (called HUPKI) containing a p53 R248Q mutation in exon 7 (marked by a star). Exons 2–10 were flanked with loxP sites in introns 1 and 10 (red arrows) to allow for Cre-targeted removal of the mutp53 allele upon addition of tamoxifen/4OHT. A deletable Neo selection box was flanked by FRT sites (green). Knock-in mice were mated with FLP mice to delete the Neo cassette in vivo, leaving behind the distal loxP site. The 'floxQ' allele thus has two loxP sites for subsequent Cre deletion. b, For genotyping, the Neo-deleted floxQ knock-in allele produces a 657-base-pair amplicon, in contrast to the 490-base-pair amplicon derived from the wtp53 allele. c, Normal mouse embryo fibroblasts (MEFs) from floxQ/+ embryos, which as non-malignant cells express non-stabilized mutp53, were adenovirally infected with empty vector (−Cre) or Cre-expressing vector (+Cre). Cre-mediated deletion of the mutp53 allele was confirmed by immunoblot analysis. d, The floxQ and constitutive Q ('Q') alleles behave identically in all aspects of gain-of-function including overall survival and tumour spectrum (not shown). Both floxQ and Q mice predominantly develop aggressive T-lymphomas, with some additional B-lymphomas and sarcomas. Also, the RosaCreERT2 allele has no discernable impact (data not shown). Kaplan–Meier analysis comparing overall survival of floxQ/− (red), Q/− (blue) and p53−/− (black) mice. Significance was assessed by log rank and Wilcoxon tests. e, Deletion of the mutp53 allele induces cell death in vitro. Viability of primary T-lymphoma cells freshly harvested from floxQ/−;ERT2/+ mice (n = 4) and Q/−;ERT2/+ control mice (n = 3), untreated or treated once with 4OHT or vehicle (EtOH) in short-term culture for 3–6 days. CellTiter-Blue (CTB) assay, unpaired two-tailed Student’s t-test; mean ± s.e.m.; n, number of independent T-lymphomas. Bottom, corresponding immunoblots of representative T-lymphomas at day 6. f, Deletion of mutp53 improves survival of host mice. Therapeutic protocol with primary floxQ/− T-lymphomas allotransplanted (black arrow on time axis) via subcutaneous injections into SCID mice. After visible tumours appeared, SCID mice were treated with daily intraperitoneal injections of oil or tamoxifen (star on time axis). Mice were killed when allowable endpoint size (1.5 cm³) was reached. Kaplan–Meier analysis, log rank test. Tamoxifen-induced allele deletion was strong but incomplete, shown by representative p53 immunofluorescence staining of tumours at endpoint (4′,6-diamidino-2-phenylindole (DAPI) counterstain). g, Initial tumour volumes measured before treatment was started in the therapeutic protocol of the various groups shown in Fig. 1c. Unpaired two-tailed Student’s t-test; mean ± s.e.m.; n, number of allografts. h, Control for Fig. 1d. Therapeutic treatment of nude mice allografted with p53−/−;ERT2/+ T-lymphoma cells and treated with tamoxifen (150 mg per kg for 7 days) as indicated in the scheme in Fig. 1c (endpoint 2). No response to tamoxifen. Time course, initial allograft volume and tumour mass at endpoint. Unpaired two-tailed Student’s t-test; mean ± s.e.m.; n, number of allografts; NS, not significant.
Extended Data Figure 2 | Mutp53 ablation in floxQ/− mice causes autochthonous tumour regression or stagnation and extends T-lymphoma-specific survival. a, Time course of imaged tumours, normalized to their initial tumour size (same as Fig. 2a but zoomed into the first 12 days of treatment). Stagnation or regression of floxQ/−;ERT2/+ tumours treated with tamoxifen, in contrast to treated control tumours (floxQ/−;ERT2/+ on oil and Q/−;ERT2/+ on tamoxifen) which grow robustly. Symbol #, sarcomas; all others are T-lymphomas. b, Similar mitotic index supports the idea that cell cycle arrest and senescence are not significantly affected upon genetic mutp53 ablation in autochthonous T-lymphomas (from Fig. 2a–c) and subcutaneous allografts (from Fig. 1c, d). Instead, apoptosis is the main mechanism of tumour regression/stagnation. Quantification of phospho-S28 histone H3 (pH3)-positive cells in individual autochthonous tumours or allografts. Five (left) or three (right) random 340 high-power fields (with no or only minimal apoptosis) were counted for each tumour. Mean ± s.d. are plotted.

Representative pH3 immunostainings are shown. c–e, Kaplan–Meier analyses and log rank statistics comparing tamoxifen-treated T-lymphoma-specific survival (c, e) and overall (d) survival of floxQ/−;ERT2/+ mice versus constitutive Q/−;ERT2/+ and p53−/−;ERT2/+ control mice from Fig. 2g. Animals were treated once (arrow) at 10 weeks with oil or tamoxifen by intraperitoneal injections for 5 consecutive days. f, At endpoint (death), like T-lymphomas (Fig. 2h), most sarcomas in tamoxifen-treated floxQ/−;ERT2/+ mice are also entirely composed of p53-positive cells. This indicates strong selective pressure for mutp53-positive tumour cells in that the small minority of non-recombined malignant cells outcompeted the vast majority of recombined mutp53-deleted cells and with time took over the tumour mass, supporting tumour addiction to high levels of mutp53. Out of ten sarcomas, nine (90%) stained positive for p53 and only one (10%) was negative for p53. Immunostainings for p53 of representative fibro- and angiosarcomas are shown. Note, the blue cells in oil-treated osteosarcoma are normal stromal cells.
Extended Data Figure 3  | Synergistic action of 17AAG + SAHA in subcutaneous xenografts of mutp53-harbouring T47D (p53 L194F) human breast cancer cells. Representative images of nude mice and their dissected tumours with one Mio cell injected per site.
Extended Data Figure 4 | Ganetespib kills mutp53 human and mouse cells in a mutp53-dependent manner.  a–c, On a molar basis, ganetespib is >50-fold more potent than 17AAG in degrading mutp53 and killing human mutp53 cancer cells. MDA468 (p53R280K) (a) and T47D (p53L194F) (c) breast cancer cells, as well as ES2 (p53S241F) ovarian cancer cells (b), were seeded into six-well plates and treated for 24–48 h. After incubation, dead cells were washed off and total protein lysates from only live cells were immunoblotted as indicated. CTB assays on parallel cultures for cell viability were performed at the same time point. Mean ± s.e.m. of four (b) or three (c) technical replicas, unpaired two-tailed Student’s t-test. p-Akt and p-Erk are also Hsp90 clients; cleaved PARP indicates activated apoptosis.

**Extended Data Figure 4**

b, Ganetespib destabilizes mutp53 but not wtp53 in cultured human ovarian carcinoma cells EFO21 (p53C124R) and HOC7 (p53C275F) (d), wtp53 COV434 and COLO704 (e) and in human non-small-cell lung cancer xenografts H1975 (p53R273H) (f). Nude mice bearing tumour xenografts (each lane is an independent tumour) were treated with a single bolus of DMSO or ganetespib (50 mg per kg intravenously), tumours were harvested at baseline (30 min), 48 h and 72 h and cells were lysed and immunoblotted as indicated (f). Chk1 and CDK1 are other Hsp90 clients; cleaved PARP indicates activated apoptosis. g, h, Ganetespib decreases stabilized mutp53 levels in live Q/H T-lymphoma cells within 24 h, associated with induction of apoptosis. Freshly isolated live Q/H T-lymphoma cells were treated with DMSO or 50 nM ganetespib for 24 h, followed by immunoblots as indicated. Hsp70 indicates drug activity, Hsc70 is the loading control (g). Death curves of freshly isolated Q/H or p53−/− T-lymphoma cells treated with DMSO or 50 nM ganetespib for the indicated times, CTB and trypan blue exclusion assays (h). All values are relative to DMSO treatment at the same time point. Mean ± s.e.m., unpaired two-tailed Student’s t-test, n = 4 independent isolates per genotype for every time point. *P < 0.05, **P < 0.01. i, j, Ganetespib suppresses growth of subcutaneous allografts of Q/H– B-lymphoma. Prophylactic protocol; treatment days are indicated in red, tumour cell injection is marked by arrow, time course of allograft growth; n, number of allografts (l). Therapeutic protocol (same as in Fig. 4b) and tumour mass at endpoint (j). Mean ± s.e.m.; unpaired two-tailed Student’s t-test; n, number of allografts. k, Ganetespib yields synergistic anti-tumour effects in combination with cyclophosphamide. Subcutaneous allografts of Q/H and H/H T-lymphoma cells were treated once (arrow) with the indicated doses of ganetespib or cyclophosphamide alone or in combination. The mean ± s.e.m. allograft size for Q/H and H/H at the start of treatment was 274 ± 36 mm3 and 323 ± 44 mm3, respectively. Unpaired two-tailed Student’s t-test; n, number of allografts. Each single drug is compared with the combination. *P < 0.05, **P < 0.01. l, Comparison of ganetespib treatment of floxQ/H− versus corresponding p53−/− control mice from Fig. 4g. The floxQ/H− mice, which normally have a significantly shorter lifespan than p53−/− littermates (median 139 days versus 195 days, respectively, see also Extended Data Fig. 1d) respond to ganetespib with significantly longer survival (right shift) and now resemble that of p53−/− mice. Kaplan–Meier analysis, log rank statistics. m, n, Ganetespib monotherapy once a week improves overall survival more efficiently than either genetic mutp53 ablation or 17DMAG + SAHA given five times a week. Comparison of Kaplan–Meier survival curves (log rank statistics) of tamoxifen-treated floxQ/H− versus animals from Fig. 2g and ganetespib-treated floxQ/H− animals from Fig. 4g (m). Note, on the basis of their phenotypic identity (see Extended Data Fig. 1d), floxQ/H− mice were used in Fig. 4g in lieu of Q/H− to ensure direct comparability with tamoxifen treatment. Comparison of Kaplan–Meier survival curves (log rank statistics) of 17DMAG + SAHA-treated H/H animals from Fig. 3c and ganetespib-treated H/H animals from Fig. 4f (n).
Corrigendum: Improving survival by exploiting tumour dependence on stabilized mutant p53 for treatment

E. M. Alexandrova, A. R. Yallowitz, D. Li, S. Xu, R. Schulz, D. A. Proia, G. Lozano, M. Dobbelstein & U. M. Moll

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In this Letter on page 353, the words 'substrate Hsp90' should have been included in this sentence as follows: "Likewise, histone deacetylase inhibitors, including FDA-approved SAHA, are promising anticancer drugs whose actions involve hyperacetylation of histone and select non-histone targets including HDAC6 substrate Hsp90, thus indirectly inhibiting Hsp90 (ref. 21)". This has been corrected in the online versions.