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Germline and somatic genetic variants in the p53 pathway interact to affect cancer risk, progression, and drug response

Running title: p53 pathway SNPs and mutations interact to affect cancer

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Declaration of Interests

The authors declare no competing interests.
Abstract

Insights into oncogenesis derived from cancer susceptibility loci (single nucleotide polymorphisms, SNP) hold the potential to facilitate better cancer management and treatment through precision oncology. However, therapeutic insights have thus far been limited by our current lack of understanding regarding both interactions of these loci with somatic cancer driver mutations and their influence on tumorigenesis. For example, while both germline and somatic genetic variation to the p53 tumor suppressor pathway are known to promote tumorigenesis, little is known about the extent to which such variants cooperate to alter pathway activity. Here we hypothesize that cancer risk-associated germline variants interact with somatic TP53 mutational status to modify cancer risk, progression, and response to therapy. Focusing on a cancer risk SNP (rs78378222) with a well-documented ability to directly influence p53 activity as well as integration of germline datasets relating to cancer susceptibility with tumor data capturing somatically-acquired genetic variation provided supportive evidence for this hypothesis. Integration of germline and somatic genetic data enabled identification of a novel entry point for therapeutic manipulation of p53 activities. A cluster of cancer risk SNPs resulted in increased expression of pro-survival p53 target gene KITLG and attenuation of p53-mediated responses to genotoxic therapies, which were reversed by pharmacological inhibition of the pro-survival c-KIT signal. Together, our results offer evidence of how cancer susceptibility SNPs can interact with cancer driver genes to affect cancer progression and identify novel combinatorial therapies.

Significance

These results offer evidence of how cancer susceptibility SNPs can interact with cancer driver genes to affect cancer progression and present novel therapeutic targets.
Introduction

Efforts to characterize the somatic alterations that drive oncogenesis have led to the
development of targeted therapies, facilitating precision approaches that condition treatment on
knowledge of the tumor genome, and improving outcomes for many cancer patients (1,2). However,
such targeted therapies are associated with variable responses, eventual high failure rates and the
development of drug resistance. Somatic genetic heterogeneity among tumors is a major factor
contributing to differences in disease progression and therapeutic response (1). Inter-individual
differences may arise not only from different somatic alterations, but also from differences in the
underlying genetic background. The maps of common germline genetic variants that associate with
disease susceptibility allow us to generate and test biological hypotheses, characterize regulatory
mechanisms by which variants contribute to disease, with the aim of integrating the results into the
clinic. However, there are challenges in harnessing susceptibility loci for target identification for
cancer, including limitations in (i) exposition of causative variants within susceptibility loci, (ii)
understanding of interactions of susceptibility variants with somatic driver mutations, and (iii)
mechanistic insights into their influence on cellular behaviors during and after the evolution of
somatic cancer genomes (3-5).

A key cancer signaling pathway known to harbor multiple germline and somatic variants
associated with cancer susceptibility is the p53 tumor suppressor pathway (6). It is a stress response
pathway that maintains genomic integrity and is among the most commonly perturbed pathways in
cancer, with somatic driver mutations found in the TP53 gene in more than 50% of cancer genomes
(7). Loss of the pathway and/or the gain of pro-cancer mutations can lead to cellular transformation
and tumorigenesis (8). Once cancer has developed, the p53 pathway is important in mediating cancer
progression and the response to therapy, as its anti-cancer activities can be activated by many
genotoxic anticancer drugs (9). These drugs are more effective in killing cancers with wild-type p53
relative to mutant p53 (10,11). While both germline and somatic alterations to the p53 pathway are
known to promote tumorigenesis, the extent to which such variants cooperate to alter pathway
activity and the effects on response to therapy remain poorly understood.

Most studies have separately examined the consequences of somatic and germline variation
affecting p53 activity to understand their roles in disease risk, progression or response to therapy.
Here we hypothesize that cancer-associated germline variants (single nucleotide polymorphisms,
SNPs) interact with TP53 somatic driver mutations to modify cancer risk, progression and potential
to respond to therapy. With a focus on a cancer-associated SNP that directly influences p53 activity,
we provide supportive evidence for this hypothesis, and go on to demonstrate how such germline-somatic interactions inform discovery of candidate drug targets.

**Materials and Methods**

**Assigning TP53 mutational status to breast, ovarian cancers and TCGA tumors**

We curated TP53 pathogenic missense mutations by integrating up-to-date functional evidence from both literature and databases as detailed in Supplementary Information. In total, we were able to find 218 out of 323 TP53 pathogenic mutations are oncogenic (Supplementary Table S1). All TP53 missense mutations in breast, ovarian cancers and TCGA primary tumors were extracted and matched with the curated lists of pathogenic and oncogenic TP53 missense mutations.

**Analysis for subtype heterogeneity SNPs with Breast and Ovarian cancer association studies**

Estimates of effect sizes [log(OR)s] for subtype-specific case-control studies and their corresponding standard errors were utilized for meta- and heterogeneity-analyses using METAL (2011-03-25 release) (12), under an inverse variance fixed-effect model. See Supplementary Information for details.

**Cancer GWAS SNPs**

We selected the GWAS significant lead SNPs (p-value <5e-08) in Europeans, and retrieved the associated proxy SNPs using the 1000 Genomes phase 3 data through the web server rAggr. See Supplementary Information for details.

**Enrichment analysis**

The hypergeometric distribution enrichment analysis was performed as described in (6). Significance was determined using PHYPER function as implemented in R and multiple hypotheses testing by Benjamini-Hochberg correction.

**Genotype imputation and population stratification**
Genotype data was obtained and filtered as described in (3). The genotype data of 7,021 TCGA patients were clustered tightly with Europeans. See Supplementary Information for details.

**TCGA survival analysis**

The omics datasets (gene mutation, copy number and mRNA expression) of the TCGA cohort were downloaded from the cBioPortal (https://www.cbioportal.org/). We considered those mutations with putative oncogenic properties (marked as ‘Oncogenic’, ‘Likely Oncogenic’ or ‘Predicted Oncogenic’ in OncoKB) as oncogenic mutations. TCGA clinical data was downloaded from the recently updated Pan-Cancer Clinical Data Resource (TCGA-CDR) (13). TCGA clinical radiation data was retrieved using R package TCGAbiolinks (V2.16.1). The patients with "Radiographic Progressive Disease" were defined as radiation non-responders. Patients with "Complete Response" or "Partial Response" were defined as responders. A Cox proportional hazards regression model was used to calculate the hazard ratio, the 95% confidence interval and p values for the two-group comparisons. The log-rank test was used to compare the differences of Kaplan-Meier survival curves. The clinical, gene expression and mutation data for the DFCI-SKCM cohort was downloaded from cBioPortal. The optimal cut-off of the gene expression for the survival analysis was determined using the survcutpoint function of the survminer R package, and used to stratify the patients into high- and low-risk groups.

**GDSC drug sensitivity analysis**

*TP53* mutation, copy number, RNAseq gene expression data, and drug IC50 values for the cancer cell lines were downloaded from Genomics of Drug Sensitivity in Cancer (GDSC; release-8.1). The classified cell lines based on *TP53* mutational status were further grouped based on the gene transcript levels: low (\(\leq 1\)st quartile), intermediate (> 1st quartile and < 3rd quartile), high (\(\geq 3\)rd quartile). The effects of the mutation status or transcript levels on drug sensitivity were then determined with a linear model approach. See details in Supplementary Information.

**Cell culture and their treatments**

Testicular cancer cell lines TERA1, TERA2, 2102EP, Susa-CR, GH, were cultured in RPMI medium containing 10% fetal bovine serum and 1% penicillin/streptomycin according to standard conditions. Susa cells were cultured in RPMI medium containing 20% fetal bovine and 1%
penicillin/streptomycin. GCT27 and GCT27-CR were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Hap1 cells were obtained from Horizon Discovery Ltd and cultured in IMDM (Sigma-Aldrich Co Ltd) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. FuGENE 6 Transfection Reagent (Promega) was used for DNA transfection. For transfection of siRNA, Lipofectamine RNAiMAX Transfection Reagent (ThermoFisher) was used. The cell lines were tested for Mycoplasma contamination every 3-4 weeks using MycoAlert™ mycoplasma detection kit (Lonza), and used for experiments at less than 20 passages. Cell line authentication was performed by STR (Short Tandem Repeat) analysis (Eurofins Genomics).

### CRISPR/Cas9-mediated genome editing

The Cas9 expression vector was obtained from Addgene (#62988). sgRNAs were designed and constructed as described previously (14). The oligo sequences for the sgRNA synthesis are listed in Supplementary Table S2. See Supplementary Information for details.

### RNA isolation, qRT-PCR and RNA-seq analysis

RNA isolation, qRT-PCR and RNA-seq analysis were performed as detailed in Supplementary Information.

### Drug screening

Cells were seeded in 384-well plates (flat bottom, black with clear bottom, Greiner) at density of about 2,000 cells per well in 81μl with cell dispenser (PerkinElmer) and liquid handling robotics (JANUS, PerkinElmer) and incubated overnight. Next, library compounds (Supplementary Table S3) were added to a final concentration of 10μM, 1μM, 100nM or 10nM. Dasatinib (1uM) was added as positive control and DMSO (Vehicle, 0.1%) was added as negative control. After 72 hours, cell were fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.5% Triton X-100 for 5 min, and then stained with 1:1000 dilution of 5mg/ml DAPI for 5 min. Next, the plates were imaged using a high-content analysis system (Operetta, PerkinElmer). The image data was analyzed by an image data storage and analysis system (Columbus, PerkinElmer). The cells with nuclear area>150 and nuclear intensity<700 were counted, and cell number was used as the viability readout. The screen was performed in duplicate. The Pearson Correlation Coefficient, a measurement for inter-
assay variability, averaged 0.98 and an average Z-factor, a measure employed in high throughput screens to measure effect size, of 0.69 for all plates was recorded, leading to high confidence in the primary screen positive hits (Supplementary Table S4).

SDS-PAGE and western blotting

SDS-PAGE and western blotting was performed as described in (15). The antibodies against p53 (sc-126), c-KIT (sc-17806), PARP1 (sc-7150), and β-Actin (sc-47778) were from Santa Cruz (Dallas, TX, USA). The antibodies against acetylated p53 (Lys382, #2525), cleaved Caspase 3 (Asp175, #9661) were from Cell Signaling. HRP-coupled secondary antibodies were from Dako.

IC50 and combination index CI analyses

To determine an IC50, 8 multiply diluted concentrations were used including a PBS control for 48 hour treatments and then cell viability was assessed by a MTT assay (see details in Supplementary Information). The IC50 was calculated using the Graphpad Prism software. A constant ratio matrix approach was used to determine the combination index CI values (16). Single drug data and combination data was entered into Compusyn software (http://www.combosyn.com) to compute CI50 and dose-reduction index (DRI). CI50 is (CX/IC50(X)) + (CY/IC50(Y)), where (CX/IC50(X)) is the ratio of the drug X’s concentration (CX) in a 50% effective drug mixture to its 50% inhibitory concentration (IC50(X)) when applied alone. The CI50 values quantitatively depict synergistic (CI<1), additive (CI=1), and antagonistic effects (CI>1).

In vivo study

All animal procedures were carried out under a UK Home Office project licence (PPL30/3395). Before submitting to the Home Office, the project licence was approved by the Oxford University Animal Welfare and Ethical Review Board (AWERB). Mice were housed at Oxford University Biomedical Services, UK. 6-8 week-old female BALB/c nude mice (Charles River, UK) were injected subcutaneously. See Supplementary Information for details.

Results

1. p53 regulatory cancer risk SNP rs78378222 associates with subtype heterogeneity
To represent germline effects, we focused on the cancer risk-associated SNP with the most
direct and most understood influence on p53 activity. This SNP, rs78378222, resides in the 3’-UTR
in the canonical TP53 polyadenylation signal (p53 poly(A) SNP). The minor C-allele is known to
associate with lower TP53 mRNA levels in different normal tissue types, such as in blood, skin,
adipose, esophagus-mucosa, and fibroblasts (17,18), and associate strongly with differential risk of
many cancer types (19-23).

We explored whether the p53 poly(A) SNP can differentially influence mutant and wild-type
TP53 (wtTP53) cancer risk by studying cancers with subtypes that differ substantially in TP53
mutation frequencies and for which susceptibility GWAS data are available. 18% of estrogen
receptor positive breast cancers (ER+BC) mutate TP53, in contrast to 76% of estrogen receptor
negative breast cancers (ER-BC) (24). Similarly, less than 10% of low-grade serous ovarian cancers
(LGSOC) mutate TP53, in contrast to 96% of high grade serous ovarian cancers (HGSOC) (25).
Over 85% of TP53 pathogenic missense mutations in breast and ovarian cancers are oncogenic
(either dominant negative or gain-of-function) (Fig. 1A) (see Methods). We analyzed data from
90,969 breast cancer patients of European ancestry (69,501 ER-pos BC, 21,468 ER-neg BC) (26)
and 105,974 controls, and 14,049 ovarian cancer patients of European ancestry (1,012 LGSOC,
13,037 HGSOC) and 40,941 controls (27).

It is known that key regulatory pathway genes and stress signals, which can regulate wtTP53
levels and tumor suppressive activities, can also regulate mutant p53, including its oncogenic
activities (28,29). Thus, if the poly(A) SNP can influence both mutant and wtTP53 expression, the
minor C-allele (less TP53 expression) would be expected to have opposite associations with disease
subtype (Fig. 1B). That is, the minor C-allele would associate with increased cancer risk (OR>1) in
the subtypes with low TP53 mutation frequencies (ER+BC and LGSOC), and decreased cancer risk
(OR<1) in the subtypes with high TP53 mutation frequencies (ER-BC and HGSOC). Indeed, this is
the case, whereby we found an increase in the frequency of the minor C-allele in ER+BC and
LGSOC patients compared to healthy controls (OR=1.12, p=1.0e-03 and OR=1.59, p=0.016,
respectively) (Fig. 1C), but a decreased frequency in ER-BC and HGSOC patients compared to
controls (OR=0.80, p=2.3e-04 and OR=0.75, p=3.7e-04, respectively). Taken together, the
distribution of minor C-allele shows significant heterogeneity among the four cancer subtypes (p-
het=2.59e-09).

The above analysis supports a persistent effect for the p53 cancer risk SNP on tumors through a
possible influence on whether or not a tumor contains a somatically mutated TP53 locus. In order to
seek further and more direct support of this possibility, we performed similar analyses of the p53
poly(A) SNP in a cohort of 7,021 patients of European origin diagnosed with 31 different cancers and for whom the TP53 mutational status of their cancers could be determined (The Cancer Genome Atlas, TCGA). We partitioned the patients into two groups based on the presence or absence of the TP53 somatic alteration (mutation and CNV loss versus WT and no CNV loss; (Fig. 1D)). Interestingly, the p53 poly(A) SNP associated with allelic differences in minor allele frequencies between the groups of patients with either wtTP53 or mutant tumors (Fig. 1E). This is in line with the associations found with TP53 mutational status of breast and ovarian cancer subtypes, whereby the C-allele is more frequent in wtTP53 tumors.

2. A p53 regulatory cancer risk SNP can affect wild type and mutant TP53 in tumors, and associates with clinical outcomes.

As mentioned above, the minor C-allele of the p53 poly(A) SNP has been previously found to associate with lower p53 mRNA levels in many different normal tissues and cells (18). To investigate the activity of this SNP in tumors, we analyzed expression data from 3,248 tumors from the TCGA cohort, for which both germline and somatic genetic data are available and no somatic copy number variation of TP53 could be detected. Similar to results obtained in the normal tissues, we observed a significant association of the minor C-allele with lower TP53 expression levels in the tumors, estimated 1.5-fold per allele (p=1.7e-04, beta=-0.37; Fig. 2A). To test if the C-allele associates with lower levels of both wild type and mutant TP53, we divided the tumors into three groups based on their respective somatic TP53 mutational status (Supplementary Fig. 1A and Supplementary Table S5). We found 2,521 tumors with wtTP53, 448 with missense mutations, and, of those, 389 with oncogenic missense mutations. In all three groups, the C-allele significantly associates with lower TP53 expression levels (Supplementary Fig. 1B).

Next, we utilized Hap1 cells that contain a dominant-negative TP53 missense mutation (p.S215G), which results in a mutated DNA-binding domain (30). We generated clones with either the A-allele or the C-allele (Fig. 2B), and found significantly lower TP53 mRNA levels in cells with the C-allele relative to the A-allele (~2 fold, Fig. 2C). We also found the C-allele containing cells express less p53 protein (Supplementary Fig. 1C). The impairment of 3’-end processing and subsequent transcription termination by the minor allele of the p53 poly(A) SNP, have been proposed as a mechanism for the genotype-dependent regulatory effects on TP53 expression (17). Indeed, we observed significant enrichments of uncleaved TP53 mRNA in cells carrying the C-allele compared to the A-allele by qRT-PCR and 3’ RNA-sequencing (Supplementary Fig. 1D-E). Together, our data demonstrate that this cancer risk-associated SNP can influence the expression of both wild type and mutant TP53 in cancer cells and tumors.
To explore whether the p53 poly(A) SNP also associates with allelic differences in clinical outcomes, we stratified the TCGA cohort into two groups based on TP53 somatic alterations and the p53 poly(A) SNP genotypes. We found that in patients with wtTP53 tumors, those with the minor C-alleles have a significantly shorter PFI and worse OS compared to those without the minor alleles (Fig. 2D), but not in patients without stratification. An inverted, but not significant trend, among the patients with somatic TP53 mutations is noted. Similarly, significant TP53 mutational status-dependent, associations between the p53 poly(A) SNP and PFI can be found when we restrict our analyses to breast cancer patients only (Fig. 2E).

It is well documented that p53 somatic mutations antagonise cellular sensitivity to radiotherapy (31), an important component of current cancer treatments. Indeed, we see not only TP53 mutations, but also the p53 poly(A) SNP play roles in the radiation response phenotype in the TCGA cohort. Specifically, we focused on the 7021 patients for whom the SNP genotypes were available. Of these, 848 patients could be assigned with radiation response phenotypes (603 responders; 134 non-responders; see Methods). We determined that the radiation non-responders were significantly enriched in patients with TP53 somatic mutations (OR= 1.6, p = 0.021; Fig. 2F). The enrichment was further enhanced when we analysed those patients with both TP53 mutations and copy number loss (OR = 2.2, p = 0.0026). Importantly, we also found that in patients with wtTP53 tumors, but not with TP53 mutant tumors, radiation non-responders were greatly enriched in the C-allele of the p53 poly(A) SNP (less TP53 expression (OR = 5.6, p = 0.011 for risk allele; Fig. 2F).

3. Somatic copy number loss of TP53 can mimic effects of the p53 poly(A) SNP

Together, the results we have presented thus-far suggest that the relative 2-fold reduction of wtTP53 levels in tumors from patients with the minor allele of the p53 regulatory SNP can lead to worse clinical outcomes and treatment response. If true, we reasoned that we should be able to find similar associations in patients whose tumors lose a single copy of TP53. In the TGCA database, 1839 (26.6%) patients with wtTP53 tumors, and 2236 (59.3%) patients with mutant TP53 tumors show significant signs of loss at the TP53 locus (estimated one copy on average, GISTIC score -1). These tumors associate with 1.3-fold and 1.1-fold lower TP53 RNA expression respectively compared to the tumors without loss (Fig. 2G). In support of small reductions of TP53 expression affecting patient outcome, we found that wtTP53-loss associates with shorter PFI and worse OS compared to no wtTP53-losses (Fig. 2H), but are not found in patients with mutant TP53. These associations are independent of tumor type (adjusted p < 0.05; Fig. 2H). We also found in patients with wtTP53 tumors, that radiotherapy non-responders are significantly enriched in cancers with TP53 copy number loss (OR =1.6, p = 0.027; Fig. 2I).
We next sought to test whether the modest changes in TP53 expression (<2 fold) could predict chemosensitivities. We used the drug sensitivity dataset with both somatic genetic and gene expression data (GDSC; 304 drugs across 987 cell lines). Similar to what we observed in TCGA tumors, TP53 copy number loss in cancer cell lines associates with a modest reduction in TP53 expression (Fig. 3A). Strikingly, and as predicted, wtTP53 loss, but not mutant TP53-loss, significantly associates with reduced sensitivities to 31% of the drugs tested (Fig. 3B; Supplementary Table S6). Specifically, 93 out of the 304 drugs demonstrated reduced sensitivity in wtTP53 cell lines with TP53-loss compared to those without a loss (adjusted p < 0.05; Fig. 3B). These drugs included many known p53 activating agents including an MDM2 inhibitor (Nutlin3), as well as standard chemotherapeutics such as cisplatin, doxorubicin, and etoposide. Together, our observations clearly indicate that patients whose tumors have modest decreases in wtTP53 expression, mediated either through the regulatory SNP or somatic TP53 copy number loss, associate with poorer DNA-damage responses and clinical outcomes.

4. A drug-able p53 pathway gene with cancer risk SNPs associates with pathway inhibitory traits

Various therapeutic efforts have been designed around restoring wild-type p53 activity to improve p53-mediated cell killing (32). The identification of a p53 regulatory cancer risk SNP that affects, in tumors, TP53 expression levels, activity, TP53 mutational status, tumor progression, outcome and radiation responses (as demonstrated for the p53 poly(A) SNP) points to other potential entry points for therapeutically manipulating p53 activities guided by these commonly inherited cancer risk variants. We reasoned that p53 pathway genes with alleles which increase expression of genes that inhibit p53 cell-killing activities and increase cancer risk, would be potential drug targets to re-activate p53 through their inhibition.

In total, there are 1,133 GWAS implicated cancer-risk SNPs (lead SNPs and proxies) in 41 out of 410 annotated p53 pathway genes (KEGG, BioCarta and PANTHER and/or direct p53 target genes (33)) (Fig. 3C; Supplementary Table S7). To systematically identify those p53 pathway genes with cancer risk SNPs whose increased expression associates with inhibition of p53-mediated cancer cell killing, we looked to the above-described drug sensitivity dataset with both somatic genetic and gene expression data (34). In total, the transcript levels of 3 of the 41 p53 pathway genes that harbor cancer risk SNPs associate with Nutlin3 (the most significant compound associated with wtTP53 CNV status) sensitivities in cell lines with wtTP53 and no copy number loss compared to those with TP53 mutations (KITLG, CDKN2A and TEX9; adjusted p < 0.05; Fig. 3D). For all three of the significant associations, increased expression of these genes associates with increased
resistance to Nutlin3 treatment. In order to further validate these associations in terms of their dependency on p53 activation and not solely Nutlin3 treatment, we explored similar associations in the three noted DNA-damaging agents (Doxorubicin, Etoposide and Cisplatin) that demonstrated sensitivities to TP53 mutational status (Fig. 3B). Only for KITLG (Fig. 3E), did increased expression levels associate with increased resistance towards all four agents.

5. Increased expression of KITLG attenuates p53’s anti-cancer activities

There are multiple significant associations that are consistent with an inhibitory role of increased KITLG expression on p53’s anti-cancer activities in testicular germ cell tumors (TGCT), a cancer type that rarely mutates TP53. First, relative to other cancer types, KITLG copy gain (GISTIC score ≥1) is highly enriched in wtTP53 TGCT (3.7-fold, adjusted p = 2.9e-29; Fig. 4A). Second, the TGCT GWAS risk allele residing in KITLG is enriched in TGCT patients with wtTP53 tumors relative to the wtTP53 tumors of other cancer types (Fig. 4B). Third, patients with elevated expression of KITLG in wtTP53 TGCT progress faster (Fig. 4C). Fourth, the TGCT GWAS risk locus falls within an intron of KITLG occupied by p53 in many different cell types and under many different cellular stresses (Supplementary Fig. 2A). This region contains 6 common SNPs that are in high linkage disequilibrium (LD) in Europeans (r² >0.95) (red square, Fig. 4D) (35,36), including a reported polymorphic p53 response element (p53 RE SNP, rs4590952). The major alleles of this SNP associate with increased TGCT risk, increased p53 binding, transcriptional enhancer activity, and greater KITLG expression in heterozygous cancer cell lines wild type for TP53 (37). Third, higher grade, but not lower grade, wtTP53 TGCT patients carrying alleles associated with increased risk and KITLG expression also progress faster (Fig. 4E and Supplementary Fig. 2B-C; Supplementary Table S8).

In order to experimentally test the potential inhibitory role of increased KITLG expression on p53’s anti-cancer activities in TGCT, we deleted the risk locus in two TGCT-derived cell lines (TERA1 and TERA2) with wtTP53 and homozygous for the TGCT risk alleles (p53-REs+/+) (Fig. 4F and Supplementary Fig. S3A-C). As predicted from the above-described associations, we found significantly higher KITLG RNA levels in non-edited p53-REs+/+ clones, compared to either the heterozygous knock outs (KOs) p53-REs+/- clones or the homozygous KOs REs-/- clones (Fig. 4G). After Nutlin3 treatment, the p53-REs/- clones showed no measurable induction of KITLG relative to p53-RE+/+ cells (Fig. 4H, red bars versus grey bars). We found no significant differences between the p53-REs/- and p53-REs+/+ clones in other genes surrounding KITLG (±1Mbp; Supplementary Fig. S3D). Re-integration of the deleted regions into its original locus rescued basal expression, resulting in significantly higher KITLG RNA levels in the knock-in (KI) clones of both cell lines.
relative to the p53-REs/- (Fig. 4F and 4I; Supplementary Fig. S3E-G). The KI clones also rescued the p53-dependent induction of KITLG expression relative to the p53-REs/- (Fig. 4I).

KITLG is best known to act through the c-KIT receptor tyrosine kinase to promote cell survival in many cancer types (38). To determine if heightened KITLG/c-KIT signaling inhibits p53’s anti-cancer activities in TGCT, we explored its impact on cellular sensitivities to p53-activating agents. We found that deletion of the KITLG risk locus or c-KIT knock-down resulted in an increased sensitivity to Nutlin3, and increased levels of cleaved caspase3 and PARP1 (Fig. 5A-B; Supplementary Fig. S4A-B). We were able to rescue the increased Nutlin3 sensitivity and caspase3/PARP1 cleavage of p53RE-/ clones in KI cells (Fig. 5A and Supplementary Fig. S4C).

To further test the p53-dependence of these effects, we reduced TP53 expression levels and observed a reduced expression of cleaved caspase3 after Nutlin3 treatment (Supplementary Fig. S4D), and an overall insensitivity towards Nutlin3 in both p53-REs+/+ and p53-REs/- cells (Supplementary Fig. S4E).

Thus-far, we have demonstrated that TGCT cells with increased expression of KITLG have increased pro-cancer survival traits previously attributed to KITLG/cKIT signaling in other cancer types. Moreover, these cells also have traits that suggest an inhibitory effect of KITLG on a p53-associated anti-cancer activity, namely the apoptotic response to p53 activation after MDM2 inhibition with Nutlin3 treatment. To further explore this, we screened 317 anti-cancer compounds to identify agents that, like Nutlin3, kill significantly more cells at lower concentrations in p53-RE-/- clones than in p53+/+ clones (Fig. 5C). We identified 198 compounds in the TERA1 screen and 112 compounds in the TERA2 screen that showed heightened sensitivity in p53-RE-/ cells in at least one of the 4 different concentrations tested (≥1.5 fold in both replicates; Supplementary Fig. S5A, blue dots). One hundred of these agents overlapped between TERA1 and TERA2 (1.7-fold, p = 1.1e-21; Supplementary Fig. S5A), suggesting a potential shared mechanism underling the differential sensitivities. For example, two MDM2 inhibitors in the panel of compounds, Nutlin3 and Cerdemetan, were among the 100 overlapping agents (Fig. 5D; Supplementary Table S3). We found a significant and consistent enrichment of topoisomerase inhibitors in both cell lines among 14 different compound classes (14 compounds in TERA1 [100%] and 10 compounds in TERA2 [71%] of 14 Topo inhibitors screened; Fig. 5D-E). To validate the genotype-specific effects of the topoisomerase inhibitors, we determined the IC50 values of three of them (Doxorubicin, Camptothecin. and Topotecan) using MTT measurements in multiple clones of TERA1 cells with differing genotypes. All three agents showed a significant reduction of IC50 values, increased sensitivities, in the p53-REs/- clones (lower KITLG) relative to the p53-REs+/+ clones (higher
KITLG) (Supplementary Fig. S5B). We were able to rescue this increased sensitivity to
topoisomerase inhibitors in the p53RE/- clones in KI cells (Supplementary Fig. S5B). Together,
these results demonstrate that TGCT cell lines with heightened KITLG expression mediated by the
risk locus, are less sensitive to 100 agents, most of which are known to activate p53-mediated cell
killing.

6. Inhibition of KITLG/c-KIT signaling and p53 activation interact to kill treatment resistant
cancer cells

There are many RTK inhibitors that are current therapeutic agents which inhibit c-KIT activity
(39). If p53-mediated KITLG-dependent pro-survival signaling can attenuate chemosensitivity to
p53-activating agents, RTK inhibitors should be able to interact synergistically with p53-activating
agents to kill TGCT cells. Indeed, co-modulation of these two pathways has shown promise in other
cancer types (40-42). We therefore tested which RTK inhibitor (known to inhibit c-KIT) kills TCGT
cells most efficiently. Of the five FDA-approved RTKs analyzed, Pazopanib, Imatinib, Nilotinib,
Suntinib and Dasatinib, the most potent was Dasatinib (Supplementary Fig. S5C). To determine
potential synergy of RTKs with Nutlin3 in TGCT, we treated cells with Dasatinib, and quantitated
potential drug-drug interactions by calculating Combination Indices (CI). We observed clear
synergistic interactions (CI <1) between Nutlin3 and Dasatinib in both TERA1 and TERA2 p53-
REs+/+ cells (Fig. 5F, grey bars), and enhanced levels of cleaved caspase3 and PARP1, relative to
single drug treatments without altering p53 stabilization (Supplementary Fig. S5D). Consistent with
the requirement of the p53-dependent activation of KITLG, no synergy between Dasatanib and
Nutlin3 was detected in p53-REs/- cells (CI>1; Fig. 5F, red bars).

We next explored the interaction between Dasatinib and multiple DNA-damaging
chemotherapeutics known to activate p53. We focused on the 3 topoisomerase inhibitors
(Doxorubicin, Camptothecin and Topotecan), as well as Cisplatin, a chemotherapeutic agent used to
treat TGCT, and which induces DNA damage and p53. Dasatinib demonstrated significant levels of
synergy with each of the DNA-damaging agents tested in p53-REs+/+ cells (Supplementary Fig.
S5E-F). Similar to Nutlin3, no synergy was detected in p53-REs/- cells of either cell lines for any
combination of agents (Supplementary Fig. S5E-F). Furthermore, the synergistic interaction
between Dasatinib and the p53-activating agents Nutlin3 and Doxorubin could be rescued by
knocking in the p53-bound germline TGCT-risk locus in KITLG (Fig. 5G, orange bars).

Thus, a more effective therapeutic strategy for TGCT patients could be to modulate both the cell
death and cell survival functions of p53, through co-inhibition of p53/KITLG-mediated pro-survival

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signaling together with the co-activation of p53-mediated anti-survival signaling. Such a therapeutic combination could provide an alternative for patients with treatment-resistant disease (43). To investigate this idea, we explored synergistic interactions between c-KIT inhibitor Dasatinib and p53 activators in cisplatin-resistant clones of GCT27 (GCT27-CR) and Susa (Susa-CR) (44), as well as in the intrinsically cisplatin-resistant TGCT cell line 2102EP (45) with wtTP53 and at least one copy of the haplotype containing the KITLG risk allele SNPs. Similar to the observations in the cisplatin-sensitive TGCT cell lines, Dasatinib and Doxorubicin interacted synergistically to kill all three cisplatin-resistant clones and cell lines (Fig. 5H). Moreover, co-treatment with Dasatinib and Doxorubicin of Susa-CR and 2102EP led to a significant reduction (~20-fold, on average) in the concentrations of Dasatinib and Doxorubicin used to achieve IC50 relative to when the drugs are used individually (Supplementary Fig. S5G). To determine if the combination treatment could show a greater efficacy in treating tumors, we generated a subcutaneous xenograft model using the 2102EP cell line, and treated the mice with two approved drugs Dasatinib and Doxorubicin either alone or in combination. Consistent with the observations made in cell culture, treatment of mice engrafted with 2102EP cells revealed stronger anti-tumoral effects with the Dasatinib/Doxorubicin pair relative to single drug treatments (Fig. 5I). This dosing regimen was well tolerated with no body weight loss in mice (Supplementary Fig. S5H).

7. KITLG/c-KIT signaling interacts with p53 to affect cancer progression and drug response in melanoma

Our results clearly support a model, whereby increased expression of KITLG mediated by the region with the TGCT cancer risk SNP(s) heightens KITLG/c-KIT signaling and attenuates p53 activity, thereby allowing for the retention and re-activation of wtTP53 in testicular cancer cells. The KITLG testicular cancer risk SNP(s) have yet to be found to associate with other cancer types (46), suggesting a tissue-specificity of this locus with transcriptional enhancer activity. However, other genetic variants that elevate KITLG/c-KIT signaling could also attenuate p53 activity, and thus allow for the retention and ultimate re-activation of wtTP53 in cancer cells. To test this, we focused on known somatic driver mutations of c-KIT in the TCGA cohort. If our model is correct, we would expect the majority of tumors with activating c-KIT mutations to retain a wtTP53 locus. Indeed, 43 out of 6,997 (0.61%) patients with wtTP53 tumors also have oncogenic c-KIT mutations relative to just 10 out of 3,735 (0.27%) of TP53 mutant tumors (Fig. 6A; OR = 2.3, p = 0.014).

As expected, the tumor types enriched in c-KIT oncogenic mutations in the TCGA cohort are cancers known to be driven by KIT signaling (38). Testicular cancers (TGCT; 13.6%; 20 out of 147), skin cutaneous melanoma (SKCM; 3.9%; 14 out of 356) and acute myeloid leukemias (AML;
2.8%; 5 out of 181) have proportionally more c-KIT mutations than all wtTP53 tumors (0.61%) (adjusted p < 0.05; Fig. 6B left panel). It is important to note that these enrichments are only significant when wtTP53 without TP53-loss, but not TP53 loss or mutant tumors are considered (Fig. 6B). If our model is correct and inhibition of c-KIT signaling will re-activate p53’s ability to kill the wtTP53 cancers, we would expect, like in TGCT, that elevated KITLG levels will associate with faster progression and/or poorer survival of the cancers with both wild-type TP53 and c-KIT. Indeed, in both melanoma and AML, we observed the association between heightened KITLG expression and poorer clinical outcomes (Fig. 6C, the TCGA-SKCM cohort; Fig. 6D the TCGA-AML cohort). Consistent associations were observed in an independent cohort (DFCI-SKCM) of 35 wtTP53 melanoma patients (Fig. 6E), for which both the somatic genetic and expression data are available (47). Importantly, we found that in melanoma and AML patients with wtTP53 and no copy number loss tumors, those with heightened KITLG expression have significantly poorer outcomes, but not in patients with TP53 mutant or copy number loss (Fig. 6F-G). Together these observations, suggest that heightened KITLG/cKIT signaling in AML and melanoma could attenuate p53 activity allowing for wt TP53 retention and re-activation using cKIT inhibitors. In further support of this, in AML, it has been shown that the c-Kit inhibitor Dasatinib does enhance p53-mediated cell killing (40). Similarly, when we treated melanoma cells (SKMEL5 with wild type TP53 and c-KIT) with Dasatinib and the p53 activating agents Nutlin3 or Doxorubicin, we observed clear synergistic interactions (Fig. 6H, CI <1; p = 0.0013 between Nutlin3 and Dasatinib and p= 0.00066 between Doxorubicin and Dasatinib).

Discussion

In this study, we demonstrate that germline cancer-risk SNPs could influence cancer progression and potentially provide information guiding precision medicine therapy decisions. Our work highlights that even small relative reductions in wtTP53 expression, mediated either by the minor allele of the p53 poly(A) SNP or through loss of at least one copy of TP53, can reduce relative p53 cellular activity in cancer cells and overall survival of patients. Patients with either of these genetic variations represent a large proportion of cancer patients. Patients with the minor allele of the SNP and wtTP53 in their cancers are found in 2.6% of the total TCGA cohort, with up to 5.9% in certain cancer types. Overall, in the TCGA, 26.6% of patients have cancers wherein at least one copy of wtTP53 is losted with up to 73.1% in certain cancer types. In terms of including TP53 status in prognosis for patients, TP53 mutation is often what is looked at most. Our work suggests that wtTP53 copy number loss could also add additional information to those patients that retain wtTP53.
Indeed, patients with tumors that express lower wtTP53 levels will be interesting to study more in depth to understand how to increase wtTP53 levels to improve treatments, such as increasing transcription of wtTP53, inhibiting miRNAs or blocking alternative polyadenylation.

The p53 stress response pathway inhibits cell survival, mediating both tumor suppression and cellular responses to many cancer therapeutics (48). p53 also targets pro-survival genes. Activation of these genes in tumors retaining wtTP53 provide a survival advantage (49). We provide human genetic evidence that also supports a tumor-promoting role of p53 pro-survival activities and, in the case of the TGCT risk locus, points to the development of more effective therapy combinations through the inhibition of these pro-survival activities in tumors that retain p53 activity. Although TGCTs are one of the most curable solid tumors, men diagnosed with metastatic TGCT develop platinum resistant disease and die at an average age of 32 years (43). There have been few new treatments developed in the last two decades, and current therapeutic approaches can, importantly in context of a cancer of young men, result in significant survivorship issues, including sustained morbidities and delayed major sequelae (43). Our observations suggest the TGCT KITLG risk allele in the polymorphic p53 enhancer leads to increased p53-dependent activation of the pro-survival target gene, KITLG, which increases TGCT survival rather than senescence/apoptosis in the presence of active p53. We demonstrate that co-inhibition of c-KIT and p53 activation interact synergistically to kill platinum-resistant TGCTs with a drug combination (Dasatinib and Doxorubicin) that had limited toxicity in a Phase II clinical trial (50), suggesting that an effective therapeutic strategy for treatment-resistant TGCTs could be to modulate both the cell-death and cell-survival functions of wtTP53 cancers.

Using the most well-studied somatic mutation known to enhance KITLG/c-KIT signalling (c-KIT mutations), we were able to identify SKCM as another potential repurposing opportunity for combination therapies which inhibit KITLG/c-KIT signalling and activate p53. The role of c-KIT signalling in the skin is well established with the pathway of crucial importance for the development of melanocytes (51). In line with previous work, we found wtTP53 SKCM to be enriched for c-KIT mutations (52,53). Furthermore, we found high KITLG expression to associate independently with poorer overall survival in wtTP53 SKCM patients. Our data provides molecular support for targeting of KITLG/c-KIT in melanoma. Melanoma rarely mutates TP53 and expresses high levels of p53 protein, in line with the fact that SKCM is enriched for wtTP53 and no TP53 copy number loss (54). Melanomas are hardwired to be resistant to p53 dependent apoptosis, perhaps because melanocytes are programmed to survive UV light (55). Several mechanisms have been proposed for this inhibition of p53 triggered apoptosis, including the action of iASPP, deletion of the CDKN2A locus,
aberrant phosphorylation of p53 and activation of MDM2 by downstream c-KIT signalling (55,56). More recently, it has been shown that WNT5a signalling and wild-type p53 might co-operate in melanoma to drive cells into a slow cycling state which is therapy resistant (57). It is possible that KITLG/c-KIT-mediated inhibition of the p53-apoptotic response adds a further mechanism through which wtTP53 can be inhibited in melanoma without mutation, and opens up the possibility of harnessing the pro-apoptotic function of p53 by inhibiting the KITLG/c-KIT pathway. Indeed, we showed that the combination of Dasatinib and Nutlin-3a and Dasatinib and Doxorubicin are synergistic in a wild-type TP53 and c-KIT SKCM cell-line.

Unlike other tumor suppressors, complete loss of p53 activity is not a requirement for cancer initiation. Reduction of p53 activity below a critical threshold through mutations is apparently necessary and sufficient for cancer development (58). These mutations are primarily missense mutations that affect p53’s ability to bind to DNA in a sequence-specific manner and regulate transcription of its target genes. These same mutations when found constitutionally result in Li-Fraumeni Syndrome: a syndrome comprising dramatic increase in cancer risk in many tissues types. These missense mutations may benefit cancers not simply through loss of p53 function, but also through dominant-negative and gain-of-function activities (59). In mice, knock-in TP53 gain-of-function mutants displayed a more diverse set of, and more highly metastatic tumors than TP53 knock-out mutants (60,61). Many of the factors that regulate wild-type p53 tumor suppression can also regulate mutant p53, including its pro-cancer activities. For example, wild-type p53 mice that express lower levels of MDM2 show increased p53 levels, a better p53 stress response, and greater tumor suppression, resulting in later and reduced tumor onset in many tissue types. Mutant p53 levels are also increased in these murine models, but cancers are found to arise earlier and harbor gain-of-function metastatic phenotypes (62).

We go on to discuss that our SNP association with inverted cancer risk and somatic TP53 mutational status in humans reveal a similar scenario. Specifically, we demonstrated that the C-allele of the p53 poly(A) SNP which can lead to decreased wild type and mutant p53 levels in tumors, associates with an increased risk of wtTP53 cancers, but decreased risk of sub-types with primarily mutant TP53. For example, women with the minor allele associated with an increased risk for the more TP53 wild-type breast and ovarian subtypes and a decreased risk for the more mutant subtypes. We also demonstrated that the TCGA pan-cancer or breast patients with wtTP53 tumors and carrying the C allele have shorter PFI compared to patients with wtTP53 tumors without the C allele. Of note, an inverted trend was found for mutant TP53 tumors. Together, these observations support a role for germline p53 pathway SNPs not only in modulating risk of disease and tumor biology in wtTP53
cancers but also in TP53 mutant cancers, wherein alleles that increase mutant p53 levels would also increase its pro-cancer activities.

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References


51. Wehrle-Haller B. The role of Kit-ligand in melanocyte development and epidermal homeostasis. Pigment cell research / sponsored by the European Society for Pigment Cell Research and the International Pigment Cell Society 2003;16(3):287-96

55. Box NF, Vukmer TO, Terzian T. Targeting p53 in melanoma. Pigment cell & melanoma research 2014;27(1):8-10
Figure Legends

**Figure 1. A p53 regulatory cancer risk SNP associates with subtype heterogeneity risk.** (A) Pie charts of the percentages of oncogenic and loss-of-function p53 mutations found amongst all known pathogenic p53 missense mutations in breast and ovarian cancers. (B) A proposed model of how the p53 poly(A) SNP could modify the ability of mutant p53 to drive cancer and of wild type p53 to suppress it. (C) Forest plots illustrating the associations of the p53 poly(A) SNP with breast cancer and ovarian cancer subtypes. The odd ratios (OR) are plotted for the SNP and subtype, and the error bars represent the associated 95% confidence intervals (CI). (D) A schematic overview of the association testing between the SNP and p53 mutational status in TCGA tumors. (E) A bar plot of the minor allele frequencies (MAFs) of the p53 poly(A) SNP in patients with either wtTP53 tumors or mutant TP53 tumors.

**Figure 2. A p53 regulatory cancer risk SNP and somatic copy number loss of p53 associates with clinical outcomes.** (A) A box plot of TP53 mRNA expression levels in 3,248 tumors from individuals with differing genotypes of the p53 poly(A) SNP. The fold change of median TP53 expression between genotypes, the p-value (linear regression) and beta coefficients of the association of the genotype with mRNA levels are depicted. (B) A schematic diagram of the TP53 mutational status and CRISPR-editing strategy in Hap1 cells. (C) A bar plot of TP53 mRNA levels for each genotype in Hap1 cells, measured using qRT-PCR normalized to GAPDH. Error bars represent SEM of 3 independent experiments. p-values were calculated using a two-tailed t-test. (D) A forest plot of the PFI and OS of cancer patients (pan-cancer TCGA cohort) stratified by the somatic TP53 mutational status. Hazard ratios (HR) and p values were calculated using Cox proportional hazards model. (E) Kaplan-Meier survival curves for PFI in a total of 381 breast cancer patients carrying either the major or the minor allele of the p53 poly(A) SNP and/or somatic TP53 mutations. Curves were truncated at 10 years, but the statistical analyses were performed using all of the data (logrank test). (F) A bar plot showing the percentage of non-responders in each group stratified by the somatic or germline TP53 alterations as indicated on the x axis. Numbers of patients (number of non-responders / total number of patients) in each group are indicated within the bars. p values were calculated by two-tailed Fisher’s exact test (*p<0.05, **p<0.005). (G) Box plots of TP53 mRNA expression levels in wtTP53 tumors (left panel) and mutant TP53 tumors (right panel) from individuals with differing TP53 copy number status. (H) A forest plot of PFI and OS of TCGA cancer patients stratified by the somatic TP53 mutational status. HR comparing PFI and OS in patients with or without TP53 copy number loss are indicated on the right. (I) A bar plot showing the
percentage of non-responders in each group stratified by the TP53 mutations and copy number loss as indicated on the x axis.

**Figure 3.** Copy number loss of TP53 and increased expression of a druggable pathway gene with cancer risk SNPs dampens p53’s anti-cancer activities. (A) Box plots of p53 mRNA expression levels in wtTP53 cells (left panel) and mutant TP53 cells (right panel) with differing TP53 copy number statuses. (B) Volcano plots of 304 drugs and their association with differential sensitivities in cancer cell lines with TP53 copy number loss relative to cell lines without TP53 copy number loss (left: wtTP53 cells; right: mutant TP53 cells). -Log\(_{10}\) adjusted p-values (linear regression and FDR-adjusted) are plotted against the beta coefficient. The horizontal dashed lines represent the FDR-adjusted p value of 0.05. (C) A Chord Diagram of 102 cancer GWAS lead SNPs in 41 p53 pathway genes that associate with differential risk to a total of 19 different cancer types. The width of the connecting bands indicate the number of lead SNPs for each association. A dot plot of the odds ratios for each association is presented in the inner circle and with red dots. The median odd ratio for each association is presented in parentheses next to the gene name. (D) Volcano plots of the associations between the transcript levels of the 41 p53 pathway cancer GWAS genes and Nutlin3 sensitivities in cancer cell lines with either wtTP53-no.loss (upper panel) or TP53 mutant-loss (lower panel). (E) Box plots of the Log\(_2\) IC50 values of p53 activating agents in cells either with low, intermediate or high KITLG mRNA levels and wtTP53-no.loss.

**Figure 4.** The p53-bound cancer risk locus in KITLG associates with patient outcome and attenuates p53’s anti-cancer activities. (A-B) Dot plots showing the enrichment of KITLG copy number gains (A) and risk allele frequencies (B) across TCGA cancer types. -Log\(_{10}\) adjusted p-values are plotted against the Log\(_2\) fold change of the percentage of tumors with KITLG gains/risk alleles in a given cancer type vs. the other cancers combined. (C) A Kaplan-Meier survival curve for PFI in p53wt testicular cancer patients with high or low KITLG mRNA expression. p value was calculated using log-rank test. (D) Genetic fine mapping identified 6 SNPs with the strongest TGCT GWAS signal and which are in high linkage disequilibrium (r\(^2\)) in Europeans (red square). (E) A Kaplan-Meier survival curve for PFI in high-stage p53wt testicular cancer patients carrying either the risk (orange) or the non-risk allele (grey) of the KITLG risk SNP. (F) A diagram of the CRISPR-editing utilized. (G) KITLG gene expression in CRISPR-edited clones using qRT-PCR normalized to GAPDH. In total, 2 to 3 clones of each genotype were analyzed in 3 independent biological replicates. p-values were calculated using a one-way ANOVA, followed by Tukey’s multiple
comparison test. (H) A bar graph of the fold change in KITLG expression after Nutlin3 treatment, Error bars represent SEM of 2 clones for each genotype and in 2 independent experiments. p-values were calculated using a two-tailed t-test. (I) Dot plots of KITLG expression in CRISPR-edited clones.

Figure 5. p53/KITLG pro-survival signaling can attenuate responses to p53-activating agents. (A) Bar plots of the IC50 values for Nutlin3. p-values were calculated using a two-tailed t-test and error bars represent SEM in at least 3 independent biological replicates. (B) Western blot analysis of cells that were treated with or without Nutlin3 for 6 hours, lysed and analyzed for p53, acetylated p53, Parp1 and cleaved-caspase3 protein expression. (C) Schematic overview for the microscopy-based high-content drug screening. (D) Bar plots depicting the number of hits and “non-hits” for each of the 14 drug classes examined. (E) Scatter plots of the fold enrichment of hits amongst each drug class relative to the total compounds in the 14 drug classes. The horizontal dashed lines represent the FDR-adjusted p value of 0.05. (F-G) Bar plots of combination indexes of Dasatinib with Nutllin3 (F) or Doxorubincin (G) in p53-RES+/+ (grey bars, two clones), p53-RES/- (red bars, two clones) and knock-in clones (orange bars, one clone) of TERA1 and TERA2 cells. (H) Bar plots of combination indexes of Dasatinib with Nutllin3 or Doxorubincin in panel of TGCT cell lines. (I) Growth curves of 2102EP xenograft tumors treated with vehicle, Doxorubicin, Dasatinib or the combination of Doxorubicin and Dasatinib. Error bars represent means ± SEM (n=6).

Figure 6. KITLG/c-KIT signaling interacts with p53 to affect cancer progression and drug response in melanoma. (A) A bar graph of the percentage of oncogenic c-KIT mutations in wtTP53 tumors relative to TP53 mutant tumors. (B) Scatter plots of the fold enrichment of oncogenic c-KIT mutations in a given cancer type relative to all cKIT mutation in pan-cancer. The horizontal dashed lines represent the FDR-adjusted p value of 0.05. (C-E) Kaplan-Meier survival curves for OS (C, left panel) and PFI (C, right panel) in TCGA-SKCM patients, for OS (D) in TCGA-AML patients, and for OS (E, left panel) and DFS (E, right panel) in DFCI-SKCM patients stratified based on KITLG mRNA levels. (F-G) Two forest plots of PFI and OS of TCGA cancer patients (F: SKCM; G: AML) stratified by the somatic TP53 mutational status. HR and p values were calculated using Cox proportional hazards model. (H) A bar plot of combination indexes of Dasatinib with Nutllin3 or Doxorubincin in melanoma cells. p values were calculated by one-sample t-test. Error bars represent means ± SEM (n=3).
**Figure 1**

**A**

TP53 pathogenic missense mutation

- Oncogenic: 90.9%
- Loss-of-function: 8.3%
- Unknown: 0.8%

Breast cancer

Ovarian cancer

- Oncogenic: 87.7%
- Loss-of-function: 11.9%
- Unknown: 0.4%

**B**

p53 poly(A) SNP

\[ \text{mut-p53} \rightarrow \downarrow \rightarrow \text{Decrease cancer risk} \]

\[ \text{WT-p53} \rightarrow \downarrow \rightarrow \text{Increase cancer risk} \]

**C**

Breast cancer

- ER+ (○)
- ER- (●)

rs78378222 (TP53)

- p = 2.3e-4
- p = 1.0e-3

Ovarian cancer

- LGSOC (○)
- HGSOC (●)

rs78378222 (TP53)

- p = 3.7e-4
- p = 0.016

**D**

TCGA Case-only association testing

TP53 mut & CNV loss

n = 3,168

TP53 WT & no CNV loss

n = 1,457

**E**

- MAF

- MAF [TP53 mut & CNV loss]
- MAF [TP53 WT & no CNV loss]

- p = 0.012
Figure 5

A. TERA1 vs TERA2

B. Western blot analysis of p53, β-Actin, Acetyl-p53, Parp1, Cleaved Parp1, Cleaved Caspase3 in TERA1 and TERA2.

C. I. Cell Seeding
TERA (p53-RES+/+) vs (p53-RES−/−)
TERA2 (p53-RES+/+) vs (p53-RES−/−)

II. 317 compounds
TDI COMPLETE Oncology Drug Set:

III. Quantification of cell viability
DAPI staining / Imaging

D. Heatmap showing hits and non-hits for TERA1 and TERA2.

E. volcano plot showing log adjusted p-value against log fold change for TERA1 and TERA2.

F. CI values for TERA1 and TERA2 for Dasatinib + Nutlin3.

G. Comparison of CI values for TERA1 and TERA2 for Dasatinib + Doxorubicin.

H. Tumour volume (mm³) over time for GCT27, GH, Susa, Susa-CR, 2102EP, and 2102EP-CR for vehicle, Dasatinib, Doxorubicin, and Dasatinib + Doxorubicin.

I. Comparison of tumour volume (mm³) over time for GCT27, GH, Susa, Susa-CR, 2102EP, and 2102EP-CR for vehicle, Dasatinib, Doxorubicin, and Dasatinib + Doxorubicin.