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Association of TP53 Mutational Status and Gender with Survival After Adjuvant Treatment for Stage III Colon Cancer: Results of CALGB 89803

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Abstract

Purpose—The TP53 tumor suppressor is frequently mutated in colon cancer, but the influence of such mutations on survival remains controversial. We investigated whether mutations in the DNA binding domain of TP53 are associated with survival in stage III colon cancer.

Experimental Design—The impact of TP53 genotype was prospectively evaluated in CALGB 89803, a trial that randomized stage III colon cancer patients to receive adjuvant 5-fluorouracil/leucovorin (5FU/LV) or 5FU/LV with irinotecan.

Results—TP53 mutations were identified in 274 of 607 cases. The presence of any TP53 mutation did not predict disease free survival or overall survival with either adjuvant regimen when men and women were considered together or as separate groups. However, outcome differences among women became apparent when tumor TP53 genotype was stratified as wild-type vs. zinc binding or non-zinc binding mutations in the TP53 DNA binding domain. DFS at 5 years was 0.59, 0.52, and 0.78 for women with TP53 wild-type tumors, and tumors with zinc-binding, or non zinc-binding mutations, respectively. Survival at 5 years for these same women

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was 0.72, 0.59, and 0.90, respectively. No differences in survival by TP53 genotype were observed in men.

Conclusions—The presence of any TP53 mutation within the DNA binding domain did not predict survival in stage III colon cancer. However, TP53 genotype was predictive of survival in women following adjuvant therapy. Future colon cancer therapeutic trials, with inclusion of correlative molecular markers, should be designed to permit evaluation of survival and/or response to treatment in women separately from men.

Keywords

colon cancer; TP53; gender; adjuvant therapy; biomarkers

Introduction

The TP53 tumor suppressor protein is induced by exposure of cells to DNA damage, hypoxia, aberrant growth signals, and chemotherapy. Such stresses can diminish the fidelity of DNA replication and increase the number of mutations that develop in cells. Following stress, the activated p53 protein induces cell cycle arrest, DNA repair, cellular senescence, or apoptosis, thereby suppressing the growth of tumors.(1)

Mutation of TP53 is the most common genetic alteration in human cancers. An estimated 43% of colon cancers harbor TP53 mutations.(1) Sequence-specific DNA binding, resulting in transactivation of gene expression, is a key function of p53, and this activity is altered to a variable degree by different TP53 mutations. Most mutations of TP53 are missense mutations that produce proteins with single amino acid changes and diminished tumor suppressor activity.(2) In colon cancer, the influence of TP53 genotype on survival in patients receiving adjuvant chemotherapy remains unclear.(3–5) Most studies investigating the prognostic and predictive impact of the loss p53 activities in colon cancer have been retrospective and varied across disease stage or chemotherapeutic regimens. For example, Munro et al reviewed 168 reports involving over 18,000 patients and found that mutant TP53 was frequently prognostic for poor survival, particularly in patients with an otherwise better underlying prognosis; however, tumor TP53 status did not predict differential response to chemotherapy.(3) Furthermore, a large retrospective collaborative study found that patients with WT TP53 gained a survival benefit from 5FU--based chemotherapy after surgery compared to surgery alone.(4, 6) Patients with mutant TP53 did not appear to gain such benefit.

The TP53 gene and its protein product have been the subject of intensive structure-function studies. The active p53 protein exists in the cell as a tetramer of four identical subunits. Each monomer within the tetramer is comprised of several well-characterized domains, including two tandem N-terminal transactivation domains, a highly conserved DNA binding domain, and a C-terminal tetramerization domain. Approximately 95% of mutations within p53 are in the DNA binding domain (7, 8); consequently, most analyses of the significance of TP53 mutations, including the present study, have focused on this region of the gene (exons 5–8).

Within the p53 DNA binding domain, conserved regions III and IV reside in two loops, L2 (codons 163–195) and L3 (codons 236–251), stabilized by coordination with a zinc atom (Fig. 1A). Nearly half of tumor-associated TP53 mutations are within the L2 and L3 regions, (9) and another 25% occur in region V, which forms a loop-sheet-helix (LSH) (codons 273–286) motif that contacts DNA.(9) Although mutations occur throughout the DNA binding domain, high frequency mutations in conserved regions are considered most likely to impact upon TP53 functions.(10) The site and type of TP53 mutation varies and specific mutation types or locations may exhibit varying clinical consequences. For example, mutations in conserved zinc-binding regions have been associated with poor survival in colon cancer and in breast and head and neck cancer.(11–16) In the analysis conducted here, all mutations in the DNA binding domain of p53 were first evaluated as a group. As in other studies,(12, 14–16) we also classified mutations in the L2, L3 or LSH regions as zinc binding (ZB) and those involving other amino acids in the DNA binding domain as non-zinc binding (NZB).

Cancer and Leukemia Group B (CALGB) 89803 is a phase III study that randomized patients following potentially curative resection of stage III colon cancer to receive adjuvant chemotherapy with either bolus 5FU/LV or IFL. The trial found no difference in disease free or overall survival (DFS or OS) between the 5FU/LV and IFL treatment arms.(17) Prospective assessment of the impact of TP53 status on outcome was a secondary objective of CALGB 89803 and investigating the effect of gender on survival was included in the plan for biomarkers. Although mutation of TP53 is common in colon cancer, and TP53 mutation types exhibit differential effects upon tumor biology, ours is the first planned analysis of TP53 genotype in a large, uniformly treated colon cancer patient cohort. We investigated the relationships between TP53 mutations and both disease free and overall survival in all patients, and the impact of sex on these relationships.

Patients, Materials and Methods

Study population

In CALGB 89803, stage III colon cancer patients were randomized to receive 5FU/LV or IFL.(17) Patients on the 5FU/LV and the IFL arms received the same dose of 5FU and a description of the treatments and results have been published.(17) The primary endpoint was OS; DFS was a secondary survival endpoint. Secondary aims addressed the relationship between molecular features of tumors and host-associated risk factors, including gender, and outcome. The institutional review board of each center reviewed the protocol and patients gave written informed consent. The CALGB Statistical Center maintained the research database. See Figure S1 for the CALGB 89803 CONSORT diagram.

TP53 mutational analysis

Tumor DNA was extracted from macro-dissected H & E stained sections available from 616 patients. The UCSF Genomics Core Facility performed direct sequencing of TP53 exons 5–8 on 240 tumors. Sequencing by hybridization (18) was performed on 426 tumor samples (see Supplemental Methods). Tumors from fifty patients were genotyped for TP53 by both methods. There was 96% agreement between the sequencing methods. Sequencing was

incomplete for 7 tumors and consent was missing for 2 samples, which were omitted from the analysis.

Determination of microsatellite instability (MSI)

Immunohistochemistry (IHC) for MLH1 and MSH2 was performed as previously described. (19) Of the 607 patients whose tumors were genotyped for TP53, IHC data for MLH1 and MSH2 were available for 594. Similarly, as previously described, using a panel of ten microsatellite markers, extracted DNA was successfully amplified and the resultant data were available for 573 of the 607 tumors genotyped for TP53. Normal control tissue consisted of sections of non-neoplastic tissue from separate non-tumor tissue blocks. Cases with loss of either MLH1 or MSH2 expression were designated mismatch repair deficient (MMR-D). Tumors which demonstrated instability in at least 50% of the loci examined were designated MSI-high (MSI-H). Those with instability in at least one but fewer than 50% of the loci were designated MSI-intermediate (MSI-I), and those showing no instability in all loci were designated MSI-stable (MSI-S).(19)

Statistical analysis

In this biomarker correlative study, the primary endpoint was DFS measured from trial entry until documented disease progression or death from any cause. OS was measured from study entry until death from any cause. The presence or absence of a TP53 mutation was considered as a potential predictor of outcome in the subset of patients in which TP53 was sequenced. TP53 status was further defined as WT, or as containing either a ZB or a NZB mutation. Secondary goals included determining the impact of known prognostic factors (gender, age, number of lymph nodes sampled, number of positive lymph nodes, tumor stage, grade, location) on the relationship between TP53 status and outcome. Relationships between tumor marker status and clinicopathological factors were studied using the chi square and Satterthwaite t tests. The Kaplan-Meier method was used to estimate DFS and OS probability distributions. Point-wise confidence intervals for the 5 year Kaplan-Meier estimates obtained from the survival functions were based on Greenwood’s formula. The log rank test was used for survival comparisons among subsets defined by TP53 genotype, gender, and treatment arm. The proportional hazards model was used to test for interactions and to carry out survival comparisons controlling for treatment, and other clinicopathological factors. A separate multivariable analysis including sex, treatment, TP53, MMR, and associated interaction terms was conducted to determine the impact of MMR status on the relationship between TP53 and outcome. Data were analyzed with continued follow-up for survival and relapse until November 9, 2009. CALGB statisticians performed all statistical analyses.

Results

Characterization of TP53 mutations

Tumors from 607 of 1,264 patients were sequenced for TP53 DNA binding domain mutations (exons 5–8) and available for this analysis (Fig. 1A). We identified mutations in 45% (274/607) of the tumors; 69% (190/274) were missense (Fig. 1B). No tumor had more than one missense mutation. Eleven tumors had intronic and missense mutations; these were

categorized by missense mutation. There were 33 additional tumors with intronic mutations; 26 nonsense mutations; 24 silent mutations, and one deletion. Silent and intronic changes were classified as mutations because they may alter protein splicing or RNA stability.(10)

Association of TP53 status with survival

To investigate the relationship between TP53 status and survival, patients with any mutation in the TP53 DNA binding domain were first compared with the wild-type group. The 607 patients with TP53 mutational data were representative of the participants in CALGB 89803 (Table S1). 44.5% (562/1264) of CALGB 89803 participants were women and 44.5% (270/607) of patients with tumor TP53 sequence data were women. Mutational frequency was comparable between treatment arms (Table S1). Median follow-up among surviving patients with TP53 genotyping was 7.7 years (range 0, 9.8 years). The five-year estimates of DFS and OS by treatment arm for the 607 patients with TP53 data were similar to those of all participants in CALGB 89803 (Table 1, Rows 1 and 2). In these 607 patients, the presence of any TP53 mutation was not significantly related to DFS or OS (Fig. 2A and Table 1, Row 4). Similarly, DFS or OS in the presence or absence of any TP53 mutation showed no differences among women (Table 1, Row 5 and Fig. S2A) or men (Table 1, Row 6 and Fig. S2B).

Characterization of TP53 ZB and NZB mutations and their relationship to clinicopathologic variables

As some studies have found an inverse association between TP53 ZB mutations and survival, (12, 14–16) we next categorized the TP53 DNA binding domain mutations as ZB or NZB. ZB amino acids (in L2, L3 or the LSH loops of p53) comprise 35% of the DNA binding domain codons, but 69% (131/190) of the missense mutations (Fig. 1). Similar to reported frequencies (9), we found that 21% (39/190) of the missense mutations were in L2; 22% (41/190) were in L3; and 27% (51/190) were in the LSH motif. The most common missense mutations found here are known hotspots for colon cancer: R273H or R273C, R175H, R282W, R248Q, Y220C and G245S (Fig. 1B). All but one of the nonsense, silent and intronic mutations were in the NZB region.

No significant relationships were found between TP53 genotype (WT, ZB, NZB mutation) and age, the number of lymph nodes sampled, the number of positive lymph nodes, stage, or histologic differentiation (Table S1). Significant associations were identified between TP53 genotype and both tumor location and gender (Table S1, both $P < 0.001$). ZB mutations were more prevalent in tumors of the left colon: 28% versus 18% in tumors of the right colon (chi-square $P = 0.004$). TP53 mutations were more common in men (50%) than women (38%) (Chi-square $P = 0.003$), similar to a previous report.(15) Colon cancers in women less commonly harbored ZB mutations than colon cancers in men (16% versus 26%, respectively) (Table S1).

Survival differs by TP53 genotype among women but not men

Since gender can affect colon cancer patient outcomes, and different TP53 mutational frequencies are observed in men and women, we evaluated how gender and TP53 status may interrelate. A significant interaction between TP53 status and sex was observed for DFS

($P_{Interaction}$= 0.008) and OS ($P_{Interaction}$= 0.002). No significant difference in DFS or OS was observed among all genotyped patients by mutation type (Figure 2B and Table 1, Row 11) or in men (Figure 2D and Table 1, Row 13). However, in women TP53 genotype was a significant predictor of DFS (log rank P = 0.008) and OS (log rank P = 0.001) (Figure 2C and Table 1, Row 12). The estimated OS probabilities at 5 years for women with WT TP53, ZB and NZB tumors were 0.72, 0.59 and 0.90, respectively (Table 1, Row 12). Log rank tests for pairwise comparisons of DFS and OS among women were significant for WT TP53 versus NZB mutations (P = 0.006 and 0.003, respectively), and for ZB versus NZB mutations (P = 0.002 and 0.0002, respectively) (Table 2).

Survival differs according to TP53 genotype among women treated with 5FU/LV

We next explored the hypothesis that survival differences by TP53 genotype among women may be chemotherapy specific. Among women the P-values for tests of interaction between TP53 mutation status and treatment were $P_{Interaction}$= 0.06 and $P_{Interaction}$= 0.11 for DFS and OS respectively. Differences in DFS and OS based on TP53 genotype were prominent in women receiving 5FU/LV (log rank P = 0.0006 and 0.0001 for DFS and OS, respectively) (Fig. 3A and Table 1, Row 14) but not IFL (Fig. 3B and Table 1, Row 15). In a pairwise analysis, women whose tumors harbored NZB mutations experienced significantly better DFS and OS on the 5FU/LV arm compared to those with WT TP53 (P = 0.002 and 0.004, respectively) or ZB mutations (P = <0.001 and <0.001, respectively)(Table 2). No differences in survival according to TP53 genotype were observed among men treated with 5FU/LV or IFL (Table 1, Rows 16 and 17 and Fig. S3).

IFL may improve survival of women with TP53 ZB mutations

In a second exploratory analysis, we compared the 5FU/LV and IFL treatment arms among women stratified by TP53 genotype. Women with TP53 WT tumors experienced a statistically similar outcome on either treatment arm (Table S2; DFS, Fig. S4A). Women whose tumors harbored ZB mutations may have benefited marginally from IFL as compared to 5FU/LV: OS at 5 years was 0.49 for 5FU/LV versus 0.73 for IFL (log rank P = 0.07) (Table S2; DFS, Fig. S4B). Conversely, women with NZB mutations experienced a trend toward better survival with 5FU/LV compared to IFL: DFS at 5 year was 0.88 for 5FU/LV and 0.65 for IFL (log rank P = 0.08) (Table S2 and Fig. S4C).

Multivariable analyses

A multivariable analysis sought to determine whether relationships between TP53 genotype, sex, and outcomes could be explained by other clinical factors and general tumor characteristics (Table S3). For this analysis, in addition to sex, TP53 genotype, and interaction terms for sex by TP53 genotype, the variables considered were treatment arm, age, race, T stage, number of nodes sampled, number of nodes positive, differentiation, tumor location, extravascular invasion, peritumoral host lymphoid response, obstruction, extracellular mucin, performance status, and vessel invasion. The interaction of TP53 genotype and sex remained significant for DFS and OS in multivariable models containing prognostic factors significantly related to each outcome (DFS, $P_{interaction}$ =0.002 and OS $P_{interaction}$ =0.001) (Table S3). The simultaneous impact of potential prognostic markers on

the survival effect of TP53 genotype among women was also explored. Among women, NZB mutations remained significantly related to prolonged DFS and OS in the presence of the prognostic markers related to outcome.

A preliminary analysis of 594 patients genotyped for TP53 and assessed for MLH1 and MSH2 expression by immunohistochemistry showed that 16% of tumors containing wild-type TP53 were MMR deficient, consistent with previous reports (Table 3). Unexpectedly, nearly 13% (17/117) of tumors with NZB mutations were also MMR-D. A similar analysis was performed using a panel of microsatellite markers to determine microsatellite instability. Here, 21% of TP53 wild-type tumors were MSI-H, and nearly 16% of tumors harboring TP53 NZB mutations also had the MSI-H phenotype. In contrast, only 5% of the tumors with TP53 ZB mutations exhibited the MMR-D phenotype (Chi square P=0.0001). These observations led us to perform a second multivariable analysis. This multivariable analysis, which included only sex, treatment, TP53 genotype, MMR status, and associated interaction terms, determined whether MMR status impacts the significance of the TP53 genotype by sex interaction. The interaction of TP53 genotype and sex remained significant for DFS and OS (DFS, $P_{\text{interaction}}$ =0.005 and OS $P_{\text{interaction}}$ =0.001) in a final model including sex, TP53 genotype, treatment, MMR status, TP53 genotype by sex, and MMR by treatment. The MMR by treatment interaction was marginally significant at $P_{\text{interaction}}$ =0.04 for DFS but not significant for OS ($P_{\text{interaction}}$=0.29). No other interaction terms were found to be significant.

It has been suggested that there may be a TP53-body mass index (BMI) interaction that influences the prognostic effect of TP53 in colon cancer. (20) This observation prompted us to determine whether BMI might account for our observation of an interaction of TP53 status, gender and response to therapy in stage III colon cancer. Thus, baseline BMI was considered as a potential predictive factor, as a continuous variable and categorized at >= 30 or < 30 with TP53 mutational status and sex for both disease-free survival and cancer-specific mortality. Seven patients in our sample (n=607) were missing data on BMI at baseline. In a univariate analysis of relapse free survival (n=600; 218 RFS events), no significant interaction was found between BMI and TP53 genotype for BMI as a continuous measure or categorized at 30 (p_{int}=0.57 for both variables). Similarly, this interaction was not significant for disease free survival (DFS) (p_{int}=0.86 and 0.78, respectively for the continuous and categorized variables with n=600 and 255 DFS events), the primary endpoint in our manuscript. These two measures of BMI were also included individually in a model with BMI (continuous or categorical), sex, TP53 mutational status and the following interaction terms: BMI by sex; TP53 by sex; and BMI by TP53. Only TP53 by sex was significant. No significant impact due to BMI was otherwise observed.

The analyses described above show that neither MSI nor BMI accounts for the interaction of TP53 mutational status and gender with survival after adjuvant treatment for stage III colon cancer.

Discussion

Studies of the association of TP53 mutation to colon cancer prognosis have yielded inconsistent results. Among the reasons for this are that many studies have had insufficient statistical power to detect modest survival differences between patients with wild-type and TP53 mutant tumors, publication bias against negative results, the possibility that different types of TP53 mutations might lead to different outcomes, and the influence of adjuvant therapy. Some evidence suggests that patients with TP53 wild type, but not TP53 mutant, tumors gain a survival benefit from 5-FU based chemotherapy.(4)

The primary goal of CALGB 89803 was to determine if survival was improved in patients with stage III colon cancer who were treated with IFL vs. patients who received adjuvant 5FU/LV.(17) A secondary goal was to prospectively examine whether patients with colon cancers harboring mutations in the DNA binding domain of TP53 experienced differential survival compared to patients with TP53 WT cancers. An additional objective was to determine whether gender together with TP53 status affects outcome. Our analysis shows that when all genotyped patients were categorized as having any mutation or not, the presence of any DNA binding domain mutation in TP53 was not predictive of survival when compared to patients with TP53 WT tumors. Furthermore, this was true among women or men.

We extended our analyses to consider whether the type of TP53 DNA binding domain mutation affected outcome independently of adjuvant therapy treatment arm. We found an interaction between TP53 and sex reflecting differences in survival by type of mutation in women, but not men. In women, the presence of a NZB mutation was associated with prolonged survival compared to that of women whose tumors harbored ZB mutations when therapy consisted of 5FU/LV. In men, ZB and NZB mutations do not impact survival.

Several studies, together with data reported here, are consistent with congruent effects of TP53 status and gender. Women with germ-line TP53 mutations (Li-Fraumeni Syndrome) are more likely to develop cancer than men,(21) as are women with a germ-line variation in the p53 antagonist MDM2.(22) Women with colon cancer have a better prognosis than men (23–25), and exhibit differential survival after 5FU-based chemotherapy for stage III or advanced colon cancer.(26, 27) Also, ZB mutations in TP53 are less common in women than in men, and mutations in this domain may confer aggressive behavior in colon and other cancers. (11–16)

Clinically observed differences in the impact of various TP53 DNA binding domain mutations may be explained by divergent effects on gene transcription. Some mutants lose the capacity to activate genes essential to the tumor suppressor functions of WT p53. Other mutants bind the response elements of novel genes. Such “gain of function” mutants can acquire oncogenic activities by enhancing the expression of genes that are not typical TP53 targets.(28) A third group of mutants, including certain NZB mutants in the L1 loop, may bind DNA more avidly than wild type p53, transactivate genes utilized by WT p53 with increased efficacy, and possess enhanced tumor suppressor activities.(29) Additional complexity derives from observations that the activities of TP53 are subject to modification

by other transcription factors. Results that may be particularly relevant to our data show that transcriptional cooperation between the estrogen receptor and p53 enhanced sequence-dependent transcriptional potential in the FLT1 promoter motif.(30)

In addition to mutation of TP53, colon cancers accumulate other genetic changes as they develop from adenomas to invasive tumors. A subset of colon cancers develop somatic defects that lead to an inability to repair single-nucleotide DNA mismatches, a phenotype known as mismatch repair deficiency (MMR-D). Most reports from randomized clinical trials, including CALGB 89803, have associated MMR-D status with better prognosis after treatment of stage III colon cancer.(31,32) We previously reported that patients on the 5FU/LV arm of CALGB 89803 whose tumors were MMR-D did not do better than similarly treated patients with MMR-proficient (MMR-P) tumors.(19) Sinicrope and co-workers found that patients with somatic abnormalities leading to MMR-D do not benefit from 5FU/LV adjuvant chemotherapy.(33) In the present study, 16% of patients with tumors that were wild type for TP53 demonstrated MMR deficiency. Of the patients with NZB mutations in TP53, 13% were MMR deficient, while 5% of the tumors with ZB mutations showed the MMR-D phenotype. However, a multivariable analysis of sex, TP53 genotype, treatment, MMR status, and associated interactions showed consistent significance of the TP53 genotype by sex interaction for both DFS and OS. Thus, the relationship between TP53 genotype, sex and outcomes following 5FU-based chemotherapy that is reported here is not likely to be explained by MMR status.

The relationship between TP53 genotype and survival that we report here does not occur in a vacuum, but is likely to be context-dependent. In CALGB 89803, KRAS status has not been found to be prognostic for survival in either treatment arm or as a function of gender, (34) nor have we found that 18q LOH is prognostic or predictive in stage III MMR proficient colon cancer. (35) Going forward, we plan to test for interactions between additional activated oncogenes and other molecular markers of outcome seen in CALGB 89803 with p53 status, gender, and type of adjuvant therapy with the goal of classifying colorectal cancer into subsets with distinct clinical behaviors.

Hypothesis generating analyses tested for interactions of TP53 status, gender, and adjuvant therapy treatment arm (Figure 3). A marginally significant three-way interaction of TP53 genotype by sex by treatment was found for DFS ($P_{\text{interaction}}$ =0.05) though this result is based on a small number of events and requires validation in additional studies More favorable survival of women with NZB mutant tumors compared to those with WT TP53, or ZB mutant tumors, was observed on the 5FU/LV, but not the IFL adjuvant therapy arm. Furthermore, the overall survival of women with ZB mutations was reduced in comparison to women with WT TP53 on the 5FU/LV arm; in contrast, there was a trend toward benefit from IFL in women with those aggressively behaving tumors harboring ZB mutations. The limited patient numbers in these subset analyses prevent us from drawing firm conclusions regarding the relationship between TP53 mutation type, gender, and response to a particular adjuvant regimen, but our observations warrant further investigation.

An important follow-up to the work presented here will be to assess survival stratified by TP53 genotype and gender in a similar group of patients who participated in the PETACC-3

study,(36) in which stage III patients were randomized to receive infusional 5FU alone or in combination with irinotecan as adjuvant therapy following colon cancer resection. Subsequent to completion of CALGB 89803, the MOSAIC and NSABP C-07 trials indicated that the combination of oxaliplatin with 5FU/LV (FOLFOX) is superior to 5FU/LV alone as an adjuvant treatment of stage III colon cancer.(37,38) It may then be revealing to study the effect of TP53 genotype, sex and specific treatment in these clinical trials.

The National Institutes of Health (NIH) Revitalization Act mandated that women and minorities should be included in clinical research, because treatments may have different effects in different populations. In 2010, the Institute of Medicine of the National Academies of Sciences found that even when women are included in clinical trials the results are often not analyzed separately by sex, and this has slowed progress in healthcare. (39) The present report is consistent with the conclusions of the NIH and the Institute of Medicine by showing that the interaction of TP53 genotype and gender is a determinant of colon cancer survival. Our work reinforces the view that future studies of molecular markers of outcome in the treatment of colon cancer should be designed to permit evaluation of men and women as separate groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance

The association of TP53 genotype with survival from colon cancer is unclear. We prospectively evaluated the association of mutations in TP53 and survival of 607 patients who participated in CALGB 89803, an Intergroup trial of adjuvant chemotherapy for stage III colon cancer. TP53 genotype was predictive of survival of women, but not men, following adjuvant therapy when patients were stratified based on their tumor TP53 genotype (wild-type or mutant in either the zinc binding or non-zinc-binding regions of the DNA-binding domain). The Institute of Medicine of the National Academies of Sciences reports that even when women are included in clinical trials, the results are often not analyzed separately by sex, and concluded that this has slowed progress in healthcare. This study shows that future studies of molecular markers of outcome in the treatment of colon cancer should be designed to permit outcome evaluation of men and women as separate groups.

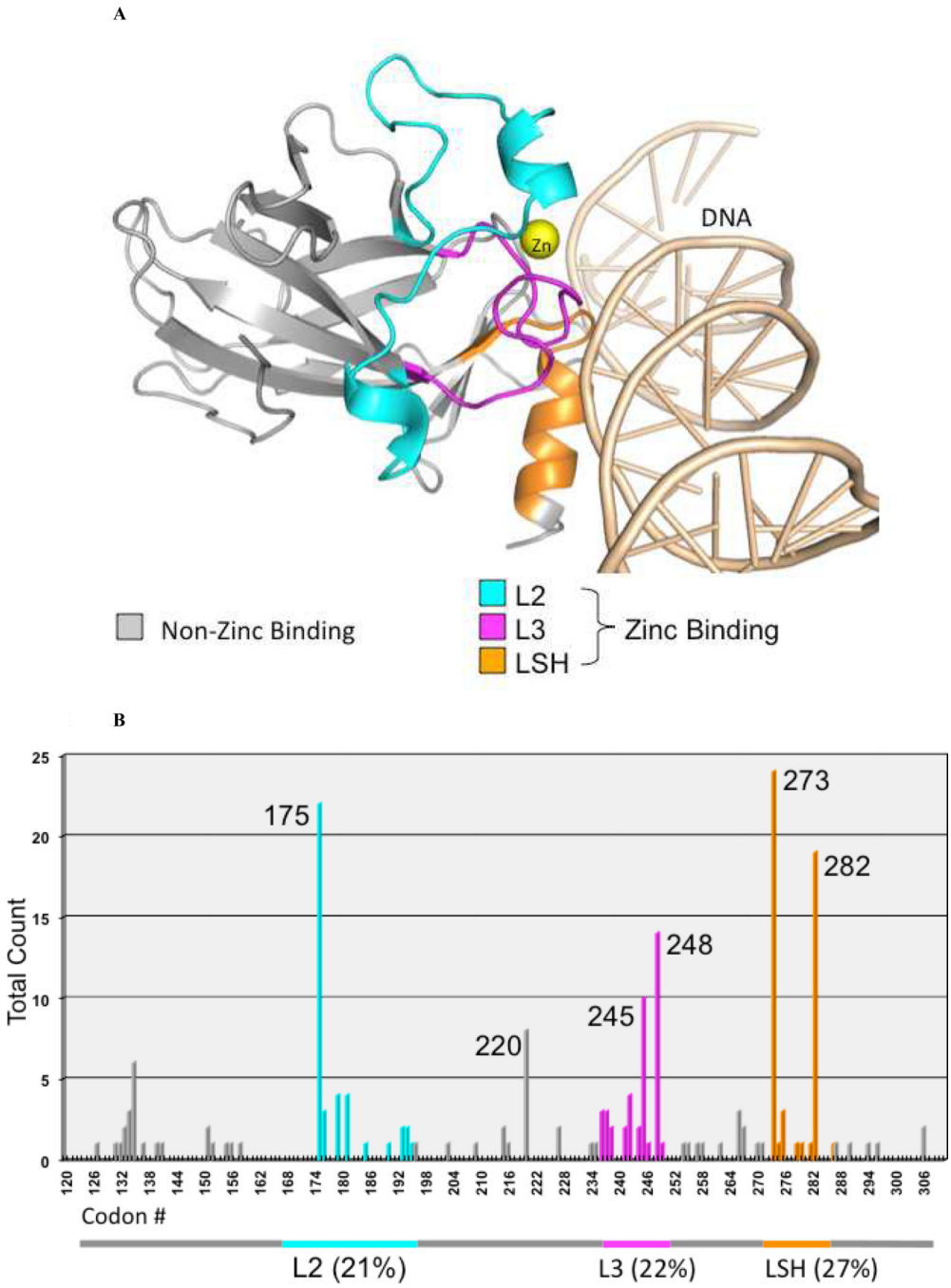

Figure 1.

(A) Structure of the p53 DNA binding domain. The zinc binding regions are cyan for L2, magenta for L3, and orange for LSH; non-zinc binding regions are gray; the zinc atom (Zn) is yellow. Image was rendered using PyMOL 1.4.1 software and p53 crystal structure PDB ID: 1TSR. (B) Missense mutations in the DNA binding domain. Codons encompassing zinc binding (ZB) mutations are denoted by colored horizontal bars spanning L2 (codons 163–195), L3 (codons 236–251) and LSH (codons 273–286); non-zinc binding (NZB) mutations are denoted by gray bars. Codon numbers are provided for hotspots.

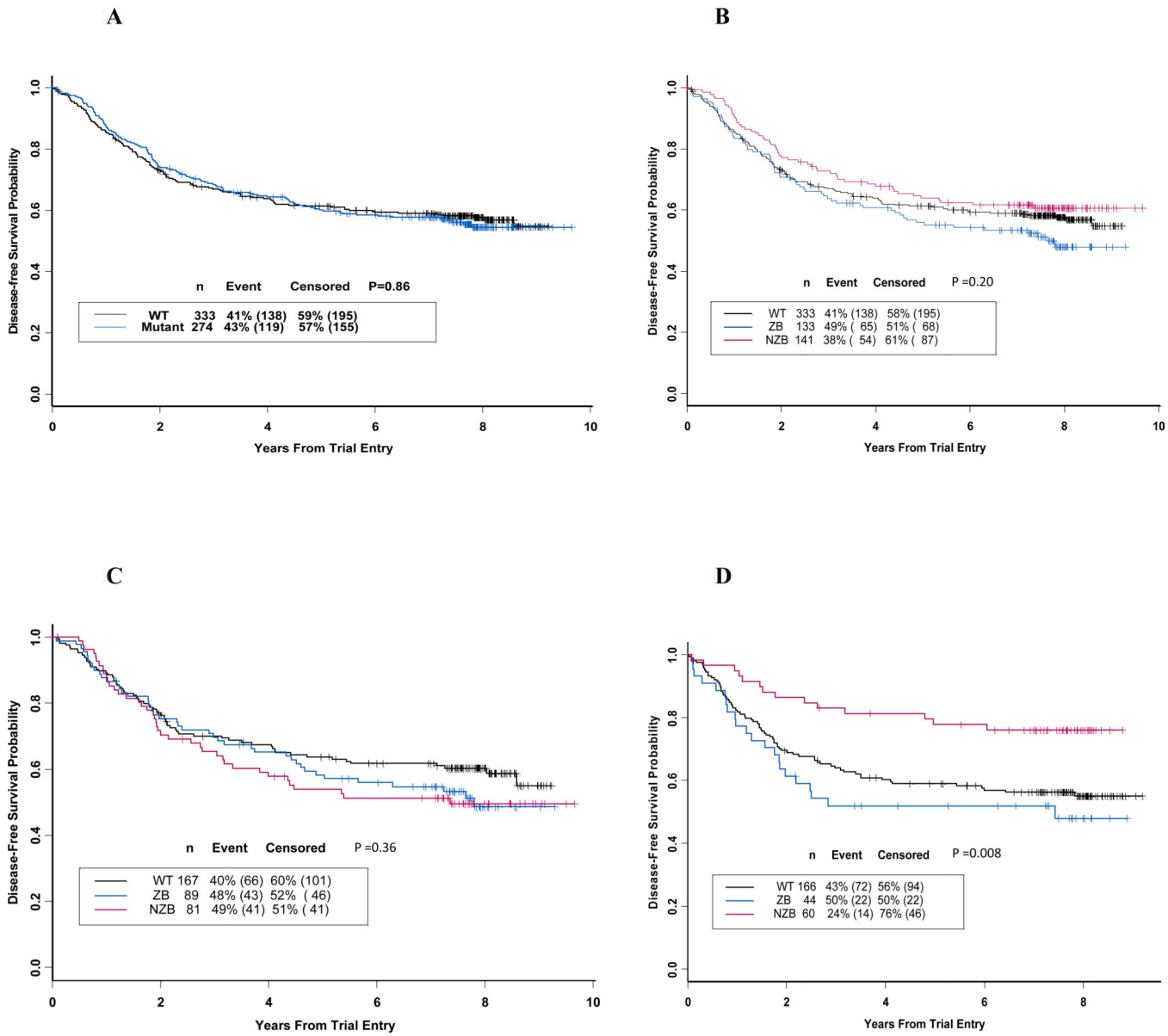

Figure 2.

Kaplan-Meier estimate of disease-free survival related to (A) TP53 genotype (WT = wild type; Mutant = any TP53 mutation in exons 5–8) among all patients evaluated, *P* = 0.86. (B-D), Kaplan-Meier estimates of disease-free survival related to TP53 status (WT = wild type; ZB = zinc binding mutation; NZB = non-zinc binding mutation) among: (B) all patients evaluated, *P* = 0.20; (C) women, *P* = 0.008; and (D) men, *P* = 0.36. Corresponding 5 year survival estimates are in Table 1, Rows 11–13.

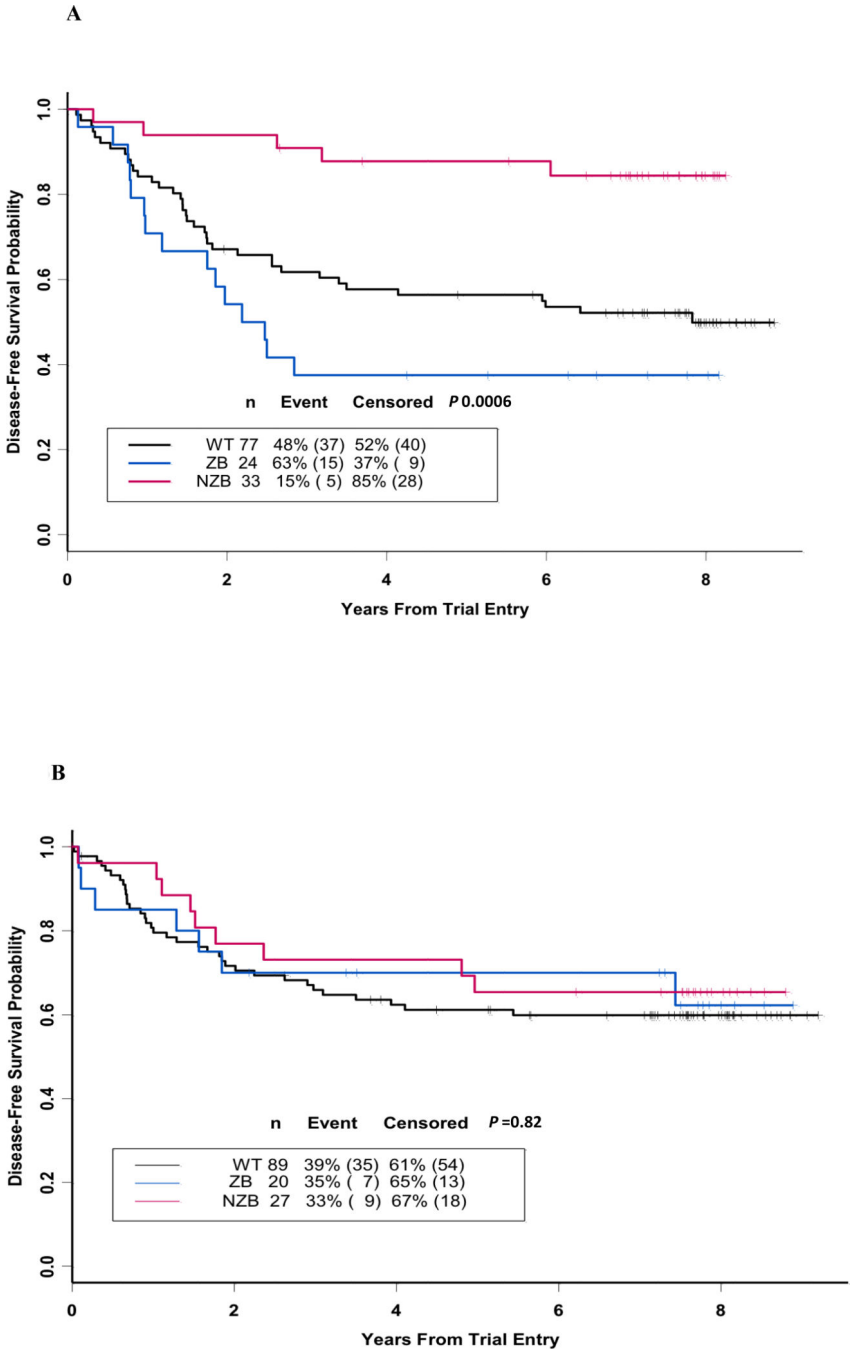

Figure 3.

Kaplan-Meier estimate of disease-free survival related to TP53 genotype (WT = wild type; ZB = zinc binding mutation; NZB = non-zinc binding mutation) among (A) women treated with 5FU/LV, $P = 0.0006$; and (B) women treated with IFL, $P = 0.82$. Corresponding 5 year survival estimates are in Table 2, Rows 14–15.

Table 1

Kaplan-Meier estimates of 5 year DFS and OS with corresponding 95% confidence intervals and *P*-values as a function of p53 genotype, gender and treatment.

Row	Factor	N	5 yr. DFS (95% CI)	P*	5 yr. OS (95% CI)	P*
1	Treatment (89803)					
	5FU/LV	629	0.62 (0.58, 0.65)	0.36	0.73 (0.69, 0.76)	0.48
	IFL	635	0.58 (0.54, 0.62)		0.69 (0.66, 0.73)	
2	Treatment (study)					
	5FU/LV	303	0.60 (0.54, 0.66)	0.55	0.73 (0.68, 0.78)	0.51
	IFL	304	0.61 (0.55, 0.66)		0.71 (0.66, 0.76)	
3	Sex					
	F	270	0.62 (0.56, 0.67)	0.61	0.74 (0.68, 0.79)	0.28
	M	337	0.60 (0.54, 0.65)		0.70 (0.65, 0.75)	
4	TP53 Genotype					
	WT	333	0.61 (0.56, 0.66)	0.86	0.73 (0.68, 0.78)	0.91
	Mutant (ZB+NZB)	274	0.60 (0.54, 0.66)		0.71 (0.65, 0.76)	
5	TP53 Genotype (F)					
	WT	166	0.59 (0.51, 0.66)	0.16	0.72 (0.65, 0.78)	0.22
	Mutant (ZB+NZB)	104	0.67 (0.57, 0.75)		0.77 (0.67, 0.84)	
6	TP53 Genotype (M)					
	WT	167	0.64 (0.56, 0.71)	0.16	0.74 (0.66, 0.80)	0.30
	Mutant (ZB+NZB)	170	0.56 (0.48, 0.63)		0.68 (0.60, 0.74)	
7	TP53 Genotype (F, 5FU/LV)					
	WT	77	0.56 (0.44, 0.67)	0.15	0.72 (0.60, 0.81)	0.47
	Mutant (ZB+NZB)	57	0.67 (0.53, 0.77)		0.77 (0.63, 0.86)	
8	TP53 Genotype (F, IFL)					
	WT	89	0.61 (0.50, 0.70)	0.55	0.72 (0.62, 0.81)	0.24
	Mutant (ZB+NZB)	47	0.67 (0.51, 0.79)		0.77 (0.62, 0.87)	

Row	Factor	N	5 yr. DFS (95% CI)	P*	5 yr. OS (95% CI)	P*
9	TP53 Genotype (M, 5FU/LV)					
	WT	87	0.58 (0.47, 0.68)	0.94	0.70 (0.59, 0.79)	0.72
	Mutant (ZB+NZB)	82	0.62 (0.51, 0.72)		0.75 (0.64, 0.83)	
10	TP53 Genotype (M, IFL)					
	WT	80	0.69 (0.58, 0.78)	0.06	0.78 (0.67, 0.86)	0.07
	Mutant (ZB+NZB)	88	0.51 (0.40, 0.61)		0.61 (0.50, 0.70)	
11	TP53 Genotype					
	WT	333	0.61 (0.56, 0.66)	0.20	0.73 (0.68, 0.78)	0.18
	ZB	133	0.56 (0.47, 0.64)		0.66 (0.57, 0.73)	
	NZB	141	0.64 (0.55, 0.71)		0.76 (0.68, 0.82)	
12	TP53 Genotype (F)					
	WT	166	0.59 (0.51, 0.66)	0.008	0.72 (0.65, 0.78)	0.001
	ZB	44	0.52 (0.36, 0.65)		0.59 (0.42, 0.72)	
	NZB	60	0.78 (0.65, 0·86)		0.90 (0.78, 0.95)	
13	TP53 Genotype (M)					
	WT	167	0.64 (0.56, 0.71)	0.36	0.74 (0.66, 0.80)	0.48
	ZB	89	0.58 (0.47, 0.68)		0.69 (0.58, 0.78)	
	NZB	81	0.54 (0.42, 0.64)		0.66 (0.54, 0.75)	
14	TP53 Genotype (F, 5FU/LV)					
	WT	77	0.56 (0.44, 0.67)	0.0006	0.72 (0.60, 0.81)	0.0001
	ZB	24	0.37 (0.19, 0.56)		0.49 (0.27, 0.67)	
	NZB	33	0.88 (0.71, 0.95)		0.97 (0.80, 0.99)	
15	TP53 Genotype (F, IFL)					
	WT	89	0.61 (0.50, 0.70)	0.82	0.72 (0.62, 0.81)	0.42
	ZB	20	0.70 (0.45, 0.85)		0.73 (0.46, 0.88)	
	NZB	27	0.65 (0.44, 0.80)		0.81 (0.60, 0.92)	
16	TP53 Genotype (M, 5FU/LV)					

Row	Factor	N	5 yr. DFS (95% CI)	P*	5 yr. OS (95% CI)	P*
	WT	87	0.58 (0.47, 0.68)	0.65	0.70 (0.59, 0.79)	0.72
	ZB	43	0.67 (0.51, 0.79)		0.79 (0.63, 0.88)	
	NZB	39	0.56 (0.39, 0.70)		0.71 (0.54, 0.83)	
17	TP53 Genotype (M, IFL)					
	WT	80	0.69 (0.58, 0.78)	0.14	0.78 (0.67, 0.86)	0.18
	ZB	46	0.50 (0.35, 0.63)		0.61 (0.45, 0.73)	
	NZB	42	0.52 (0.36, 0.66)		0.61 (0.44, 0.74)	

Table 2

Log rank*P*-values for pairwise comparisons of overall survival by TP53 genotype and treatment

	***P*-value WT vs. ZB**		***P*-value WT vs. NZB**		***P*-value ZB vs. NZB**	
Patient Subset	**(DFS)**	**(OS)**	**(DFS)**	**(OS)**	**(DFS)**	**(OS)**
Women	0.36	0.13	0.006	0.003	0.002	0.0002
Men	0.29	0.58	0.21	0.23	0.75	0.53
Women, 5FU/LV	0.16	0.02	0.002	0.004	<0.0001	<0.0001
Women, IFL	0.80	0.72	0.53	0.18	0.84	0.50
Men, 5FU/LV	0.66	0.48	0.55	0.89	0.34	0.42
Men, IFL	0.05	0.13	0.24	0.11	0.64	0.86

WT, wild-type; ZB, zinc-binding; NZB, non-zinc binding

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Table 3

Association of TP53 genotype with microsatellite instability measured by immunohistochemistry

Marker	TP53 genotype			
Frequency **Row Percent** **Column Percent**	**WT**	**Zinc binding**	**Non-zinc binding**	**Total**
MLH1 (+) and MLH2 (+)	276	125	117	518
	53.28	24.13	22.59	87.21
	84.15	94.7	87.31	
MLH1 (−) or MLH2 (−)	52	7	17	76
	68.42	9.21	22.37	12.79
	15.85	5.30	12.69	
Total	328	132	134	594
	55.22	22.22	22.56	100.00

Chisquare *P*=0.009