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Combination Therapy with the Farnesyl Protein Transferase Inhibitor SCH66336 and SCH58500 (p53 Adenovirus) in Preclinical Cancer Models

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Abstract

SCH66336 is a p.o.-active, farnesyl protein transferase inhibitor. SCH66336 inhibits farnesylation of RAS and other proteins in tumor cells and suppresses tumor growth in human xenograft and transgenic mouse cancer models *in vivo*. SCH58500 is a replication-deficient, recombinant adenovirus, which expresses the human p53 tumor suppressor. In preclinical models, SCH58500 has therapeutic efficacy against a wide range of human tumor types containing nonfunctional p53 and enhanced activity in combination with many chemotherapeutic drugs. Here we report that combination therapy with SCH66336 and SCH58500 has synergistic or additive antiproliferative effects on a panel of tumor cells lines *in vitro*. The efficacy of the three-drug combination of SCH66336, SCH58500, and paclitaxel was also examined *in vitro*. Each two-drug interaction displayed such marked synergy, the addition of a third drug to the statistical model could only yield additivity. Greater combined efficacy for SCH66336 and SCH58500 was also observed *in vivo* in the DU-145 human prostate and *wap-ras/F* transgenic mouse cancer models.

Introduction

Oncogenic mutations in the *ras* gene are prevalent in human cancer, including up to 50% of colon cancers and >90% of pancreatic carcinomas (1). In normal cells, RAS switches between an inactive GDP-bound and an active GTP-bound state, which can initiate several intracellular signaling pathways (2). RAS signaling is terminated by the hydrolysis of GTP to GDP in a reaction that is stimulated by guanosine triphosphatase-activating proteins. As a consequence of specific mutational events in the *ras* gene, oncogenic RAS proteins have a greatly reduced capacity to hydrolyze GTP. This leads to constitutive activation of downstream signaling pathways and unregulated cellular proliferation (1, 3). Three *ras* genes encode four protein isoforms (*H-ras*, *N-ras*, *K-ras4A*, and *K-ras4B*), with *K-ras4A* and *K-ras4B* being splice variants of the same gene transcript (3). Although the functional differences of the four isoforms remain unknown, oncogenic mutations of different isoforms predominate in different tumors (4). *H-ras* mutations are generally found in carcinomas of the bladder, kidney, and thyroid. *N-ras* mutations are found in myeloid and lymphoid cancers, liver carcinoma, and melanoma. *K-ras* mutations predominate in colon, lung, and pancreatic carcinomas.

Many lines of evidence suggest that antitumor activity can be achieved by interfering with the function of oncogenic RAS proteins (5–8). Signal transduction by RAS is dependent on its plasma membrane localization. This localization is supported by a series of post-translational modifications, the first of which is farnesylation of a Cys residue near the COOH-terminus of RAS proteins. This reaction is catalyzed by FPT.[2] RAS prenylation is thought to be the critical modification for proper membrane localization and function (9–11). Therefore, FPT inhibition is a potential mechanism for interfering with RAS-driven tumor growth.

Prenylation of RAS proteins is complex. *In vitro*, both K- and N-RAS proteins can serve as substrates for a related protein prenyl transferase, geranylgeranyl protein transferase-1 (12, 13). Although this reaction occurs with a lower catalytic efficiency than the farnesylation of these proteins, geranylgeranylation of K- and N-RAS proteins occurs in cells treated with FTIs (14, 15). In contrast, the H-RAS protein is not a substrate for geranylgeranyl protein transferase-1 *in vitro* or in cells treated with FTIs. Despite this alternative prenylation of some RAS isoforms, inhibitors of FPT demonstrate *in vitro* and *in vivo* antitumor efficacy in a variety of preclinical cancer models (16–20). Therefore, the observed activity of FTIs may, in some cases, be due to the inhibition of farnesylation of proteins in addition to or other than RAS.

SCH66336 is a p.o.-active, potent, and selective inhibitor of the FPT enzyme (21, 22). This novel therapeutic agent has activity against a wide variety of human tumor xenografts and also causes regression of tumors in *wap-H-ras* transgenic mice. Enhanced antitumor activity has been reported in animal models when SCH66336 is combined with various cytotoxic chemotherapeutic agents, including cyclophosphamide, 5-fluorouracil, and vincristine (21). Presently, SCH66336 is undergoing phase I/II human clinical trials as an anticancer agent.

p53 is a DNA-binding protein, which acts as a transcription factor to control the expression of proteins involved in the cell cycle (23, 24). In response to DNA damage, the p53 protein accumulates in the cell nucleus causing cells to undergo cell cycle arrest and DNA repair or apoptosis, programmed cell death (25). Functional inactivation of p53 can occur by several mechanisms, including direct genetic mutation, binding to viral oncoproteins or cellular factors (*e.g.*, mdm-2), or alteration of the subcellular localization of the protein (23, 24). Although p53 is not essential for normal development, p53 "knockout" mice are susceptible to tumors early in life (26). Mutations in p53 have been reported in a majority of clinical cancers, and it has been estimated that p53 function is altered in at least half of all human malignancies (23, 24).

SCH58500 is a replication-deficient, recombinant adenovirus, which expresses the human p53 tumor suppressor (27). In preclinical models, SCH58500 has therapeutic efficacy against a wide range of human tumor types containing nonfunctional p53, and it has enhanced activity in combination with many chemotherapeutic drugs (28–32). Adenovirus-mediated p53 gene therapy for cancer using SCH58500 is presently undergoing phase I/II clinical trials.

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[2] The abbreviations used are: FPT, farnesyl protein transferase; C.I.U., cellular infectious unit(s); FTI, FPT inhibitor; FBS, fetal bovine serum; PN, viral particle; MMTV, mouse mammary tumor virus.

Paclitaxel (Taxol™) inhibits cell replication by enhancing the polymerization of tubulin monomers into stabilized microtubule bundles that are unable to reorganize into the proper structures for mitosis (33, 34). This results in cell cycle blockage in mitosis and subsequent activation of an apoptotic pathway, which may be p53-independent (35, 36). Moasser *et al.* (37) reported synergy or additivity between paclitaxel and L744832, a peptidomimetic FTI with a molecular structure distinctly different from SCH66336. Nielsen *et al.* (32) reported synergy or additivity between SCH58500 and paclitaxel. Therefore, in the present work, we decided to examine the efficacy of combination therapy with SCH66336 and SCH58500, as well as the triple drug combination with paclitaxel.

Two-drug combination therapy using SCH66336 and SCH58500 had synergistic or additive antiproliferative efficacy in a panel of tumor cells lines *in vitro*. Greater combined efficacy was also observed *in vivo* in the DU145 human prostate and the *wap-ras/F* transgenic mouse cancer models. When the three-drug combination of SCH66336, SCH58500, and paclitaxel was tested, each two-drug interaction displayed such marked synergy that the addition of a third drug to the statistical model only produced additivity.

Materials and Methods

Cell Lines

All of the human tumor cell lines were purchased from ATCC (Rockville, MD). MDA-MB-231 human breast adenocarcinoma cells and PANC-1 human pancreatic epithelioid carcinoma cells were cultured in 90% DMEM (Life Technologies, Inc., Grand Island, NY) with 10% FBS (Life Technologies, Inc.). DU-145 human prostate carcinoma cells and PA-1 human ovarian teratocarcinoma cells were cultured in 90% Eagle's MEM plus 10% FBS. MIAPaCa2 human pancreatic carcinoma cells were cultured in DMEM with 10% fetal FBS and 2.5% horse serum (Life Technologies, Inc.). LNCap human prostate adenocarcinoma cells, DLD-1 human colorectal adenocarcinoma cells, and NCI-H460 human lung large cell carcinoma cells were cultured in RPMI 1640 (Life Technologies, Inc.) with 10% FBS. AsPC-1 human pancreatic adenocarcinoma cells were cultured in RPMI 1640 (Life Technologies, Inc.) with 20% FBS. MDA-MB-468 human breast adenocarcinoma cells were cultured in Leibovitz's L-15 medium plus 10% FBS. All of the cells were cultured at 37°C and 5% CO_2 except MDA-MB-468 cells, which were maintained in 37°C without CO_2.

Recombinant Adenoviruses

Construction and propagation of human wild-type p53 adenovirus (SCH58500; p53 Ad; rAd-p53; ACN53) and *E. coli* β-galactosidase adenovirus (β-gal Ad; rAd-β-gal) have been described previously (27). The Empty Ad construct consists of the Ad5 backbone and the promoter/enhancer regions from SCH58500 lacking only the p53 transgene. The concentration of total PNs was determined by measuring absorption at 260 nm (38). Infectious PNs were determined by measuring the concentration of viral hexon protein-positive 293 cells after a 48-h infection period. C.I.U. is defined as cellular infectious unit(s) (39). Adenoviruses were administered in a phosphate buffer [20 mM NaH_2PO_4 (pH 8.0), 130 mM NaCl, 2 mM $MgCl_2$, 2% sucrose].

In Vitro Drug Interaction Studies

SCH66336, (+) 4-{2-[4-(8-Chloro-3,10-dibromo-6,11-dihydro-5H-benzo cyclohepa {1,2-b} pyridin-11-yl)-1-piperidinyl]-2-oxoethyl}-1-piperidinecarboxamide, was synthesized by Schering-Plough, and its structure has been published (21). Paclitaxel (Taxol) was purchased from Calbiochem. Stock solutions of 100 mM SCH66336 in DMSO were diluted with culture media for *in vitro* studies. Tumor cells were seeded into culture wells of 96-well plates and allowed to attach for 3 h, then incubated with SCH66336 or vehicle for 7 days. SCH58500 was added to some cells for the last 3 days. For the three-drug combination study using SCH66336, SCH58500, and paclitaxel, DU-145 prostate tumor cells were aliquoted into culture wells and allowed to attach for 3 h. The cells were incubated with paclitaxel or vehicle for 4 h., then washed. SCH66336 or vehicle was added, and the incubation continued for 7 days. SCH58500, β-gal Ad, or Ad buffer was added after 4 days of the total incubation time (Note: β-gal Ad has no effect on cell proliferation in this model).

Cell proliferation was measured using the MTT assay (40). Briefly, 25 μl of 5 mg/ml MTT vital dye [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well and allowed to incubate for 3–4 h at 37°C and 5% CO_2. Then, 100 μl of 10% SDS detergent was added to each well, and the incubation was continued overnight. Fluorescence in each well was quantitated using a Molecular Devices microtiter plate reader. Cell proliferation data from drug interaction studies were analyzed using the Thin Plate Spline methodology of O'Connell and Wolfinger (41).

Tumor Therapy in Vivo

C.B.17/ICR-SCID mice were purchased from Taconic Farms (Germantown, NY). Nude mice were purchased from Charles River Laboratories (Wilmington, MA). Line 69–2F *wap-ras/F* transgenic mice [FVB/N-TgN(WapHRAS)69LlnYSJL; Ref. 42, 43] were from the Schering-Plough Research Institute breeding colony (also available from The Jackson Laboratory Induced Mutant Resource). All mice were maintained in a virus-antigen-free-barrier facility. Animal procedures were performed in accordance with the rules set forth in the NIH Guide for the Care and Use of Laboratory Animals and approved by the SPRI Animal Care and Use Committee.

i.p. DU-145 Model. Each male SCID mouse was given an i.p. injection of 2.5×10^6 DU-145 human prostate tumor cells on day 0. Mice were dosed i.p. with 1.5×10^{10} PN SCH58500 (4×10^8 C.I.U.) or 0.1 ml Ad buffer on days 7, 9, 11, 14, 16, and 18. Mice were also dosed p.o. with 40 mg/kg SCH66336 or 0.1 ml 20% hydroxyl-propyl-betacyclodexatrin twice daily on days 7–18. Tumors were harvested and weighed on day 29. There were 10 mice/group.

s.c. DU-145 Model. Male SCID mice received injections s.c. of 2.5×10^6 DU-145 human prostate tumor cells 23 days before the start of dosing on day 0. Mice were dosed intratumorally with 1×10^{10} PN recombinant Ad (3.5×10^8 C.I.U. SCH58500 or 1.7×10^8 C.I.U. β-gal Ad) or 0.1 ml Ad buffer on days 0, 1, 2, 5, 6, 7, 8, 9, 12, 13, 14, 15, and 16 (13 doses) once daily. Mice were also dosed p.o. with 40 mg/kg SCH66336 or 0.1 ml 20% hydroxyl-propyl-betacyclodexatrin twice daily on days 0–16. There were 10 mice/group. Tumor volumes for any two treatment groups on each day were compared using Student's *t* test (Statview software, SAS Institute, Cary, NC). Average percent inhibitions for groups were calculated using values significantly different from the vehicle control group at $P \leq 0.05$. The specific effects of p53 were distinguished from adenovirus vector effects by subtracting the average tumor growth in the β-gal Ad group from tumor growth in the SCH58500 group.

***Wap-ras/F* Transgenic Mouse Model.** Three studies were performed using *wap-ras/F* transgenic mice with mammary tumors. Due to the large heterogeneity in transgenic tumor volumes on day 0, tumor growth for each mouse was normalized to the starting volume for that particular tumor. Therefore, each mouse served as its own internal control, and the tumor growth curves show the rate of change in tumor volumes.

In the first study, mice with palpable tumors were randomized into three treatment groups. Group 1 received 0.1 ml Ad buffer/mouse/day, group 2 received 2.9×10^{10} PN β-gal Ad/mouse/day (4×10^8 C.I.U.), and group 3 received 2.9×10^{10} PN p53 Ad/mouse/day (8×10^8 C.I.U.). Mice were dosed on days 0–4, 7–11, and 14–18 (3 times per week for 3 weeks). All injections were intratumoral.

In the second transgenic study, mice with palpable tumors were randomized into six treatment groups. Group 1 received oral 20% HPβCD and intratumoral Ad buffer, group 2 received oral 20% HPβCD and intratumoral Empty Ad vector, group 3 received oral 20% HPβCD and intratumoral SCH58500, group 4 received oral SCH66336 and intratumoral Ad buffer, group 5 received oral SCH66336 and intratumoral Empty Ad vector, and group 6 received oral SCH66336 and intratumoral SCH58500. The recombinant Ad dose was 1×10^{10} PN (3.5×10^8 C.I.U. SCH58500 or 1.6×10^8 C.I.U. Empty Ad) once daily on days 1–4 and 7–10. SCH66336 was dosed at 40 mg/kg twice daily on days 2–10.

In the third transgenic study, mice with palpable tumors were randomized into six treatment groups. Group 1 received intratumoral Ad buffer and i.p. paclitaxel vehicle, group 2 received intratumoral Empty Ad vector and i.p.

Table 1 *Analysis of in vitro drug interactions between SCH66336 (farnesyl protein transferase inhibitor) and SCH58500 (p53 adenovirus)*

Cell line	Tumor type	p53 protein	Ras mutation	Isobole analysis ($P \leq 0.05$)
MDA-MB-231	Human breast	Mutant	Mutant	Mixed synergy and additivity
DLD-1	Human colorectal	Mutant	K-ras	Synergy
SK-OV-3	Human ovarian	Null	Wild-type	Additive
BxPC-3	Human pancreatic	Mutant	Wild-type	Synergy
MIAPaCa2	Human pancreatic	Mutant	K-ras	Synergy
PANC-1	Human pancreatic	Mutant	K-ras	Additive
DU-145	Human prostate	Mutant	Wild-type	Synergy
LNCap	Human prostate	Wild-type	Wild-type	Additive

paclitaxel vehicle, group 3 received intratumoral SCH58500 and i.p. paclitaxel vehicle, group 4 received intratumoral Ad buffer and i.p. paclitaxel, group 5 received intratumoral Empty Ad vector and i.p. paclitaxel, and group 6 received intratumoral SCH58500 and i.p. paclitaxel. The recombinant Ad dose was 1×10^{10} PN (3.5×10^{8} C.I.U. SCH58500 or 1.6×10^{8} C.I.U. Empty Ad) once daily on days 0–4, 7–11, and 14–18. The paclitaxel dose was 5 mg/kg once daily on days 0–4, 7–11, and 14–18.

Results

***In Vitro* Drug Interaction Studies.** The results of the *in vitro* drug interaction assays are summarized in Table 1. SCH66336 and SCH58500 had synergistic or additive antiproliferative efficacy against a panel of tumor cell lines independent of p53 mutational status, ras mutational status, or tissue of origin. Fig. 1 shows representative models for the DU-145 cell line ($p53^{mut}$, ras^{wt}) generated using Thin Plate Splines (41).

The three-drug combination of SCH66336, SCH58500, and paclitaxel was tested in DU-145 prostate tumor cells. Each two-drug interaction was so markedly synergistic that the addition of a third drug to the statistical model could only produce additivity ($P = 0.5725$ for synergism). SCH66336 and SCH58500 had synergistic efficacy ($P = 0.0128$; Fig. 2*A*), SCH58500 and paclitaxel had synergistic efficacy ($P = 0.0015$; Fig. 2*B*), and SCH66336 and paclitaxel had synergistic efficacy ($P = 0.0193$; Fig. 2*C*). Importantly, no antagonism was observed.

Tumor Therapy *in Vivo*. In the i.p. DU-145 prostate model, the two-drug combination of SCH58500 and SCH66336 had greater efficacy than either drug alone ($P \leq 0.0002$; Fig. 3*A*). Treatment with SCH66336 alone or SCH58500 alone resulted in 35% and 45% reductions in total tumor burden, respectively. Combination treatment resulted in a 69% decrease in total tumor burden at the end of the study.

Fig. 3*B* shows results from the s.c. DU-145 model. Tumors in this model were large at the initiation of dosing, with a mean value of 764 mm^3. Treatment with SCH66336 alone or SCH58500 alone resulted in tumor growth inhibition. However, the combination of SCH66336 and SCH58500 had the greatest efficacy of the treatments tested, and the effect of this combination was significantly different from either single agent ($P \leq 0.05$; days 16–19). Unfortunately, the study had to be stopped early because of the very high tumor burden in the control groups.

In line 69–2F transgenic mice [FVB/N-TgN(WapHRAS)69LlnYSJL], an activated human *H-ras* oncogene is carried on the Y chromosome in the FVB mouse strain background (42, 43). Male mice spontaneously develop mammary tumors at 6–9 weeks of age, and the host immune system is intact in this model. We previously demonstrated the sensitivity of this transgenic strain to the farnesyl protein transferase inhibitor, SCH66336 (21). In the first *wap-ras/F* study reported here, intratumoral SCH58500 caused tumor regression in all treated mice, whereas tumors in mice treated with β-gal Ad or Ad buffer continued to grow ($P \leq 0.006$; Fig. 4*A*). This study demonstrates the sensitivity of this transgenic strain to p53 gene therapy. In our second *wap-ras/F* study, oral SCH66336 and intratumoral SCH58500 had enhanced antitumor efficacy when combined ($P \leq 0.001$; Fig. 4*B*). Control vector (Empty Ad) had no effect on tumor growth rates when administered by itself or with SCH66336 ($P > 0.05$). In contrast, combination therapy with SCH66336 and SCH58500 induced rapid tumor regression. After 4 days of treatment, tumors were 49% smaller than on day 0, and after 10 days of therapy, they were 87% smaller than on day 0. Tumors treated with SCH66336 alone or SCH58500 alone had grown by 19% and 43%, respectively after the first 4 days of treatment and did not start to regress until after 8 days of therapy. Once treatment was terminated on day 10, all tumors resumed growing, except for the tumors treated with both SCH66336 and SCH58500. These tumors remained essentially dormant for an additional 2 weeks before resuming growth.

Transgenic *wap-ras/F* tumors were previously shown to be resistant to paclitaxel therapy (44), and this finding was confirmed in the present study (Fig. 4*C*). SCH58500 inhibited tumor growth ($P \leq 0.05$) but was not able to sensitize the tumors to paclitaxel chemotherapy ($P > 0.05$).

Discussion

The FTI SCH66336 has activity against a wide variety of human tumor xenografts and also causes regression of tumors in *H-ras*^{mut}

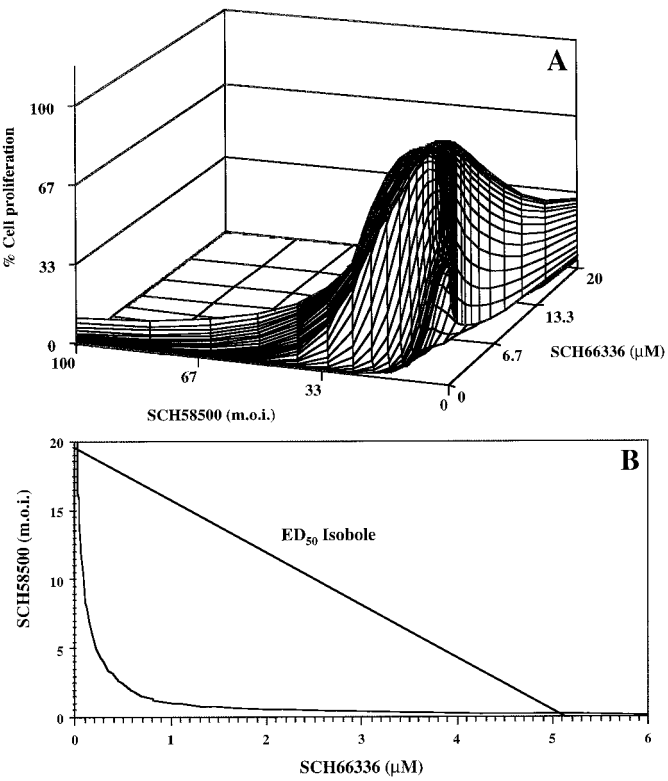

Fig. 1. Graphical representations of the statistical analysis performed in DU-145 human prostate tumor cells *in vitro*. *A*, three-dimensional surface response model of SCH58500 and SCH66336 interactions. *B*, isobole generated from the three-dimensional model shown in *A*.

transgenic mice (21). Regression of the transgenic tumors is attributable to increased apoptosis and a decreased mitotic index. Gene therapy with the p53 tumor suppressor also induces apoptosis in a wide variety of tumor models (30). Combination therapy using SCH66336 and SCH58500, a recombinant adenovirus expressing p53, had synergistic or additive efficacy against tumor cell proliferation. This result was independent of p53 status, ras status, or tissue of origin for the tumor cells. Combination therapy with SCH66336, SCH58500, and paclitaxel had overall additive efficacy in DU-145 prostate tumor cells due to the pronounced synergy observed for each two-drug combination. Combination therapy with oral SCH66336 and intratumoral SCH58500 had enhanced efficacy compared to either drug alone in both i.p. and s.c. DU-145 human prostate tumor xenograft models. Mammary tumors in *wap-ras/F* transgenic mice were sensitive to treatment with SCH66336 or SCH58500 but not to paclitaxel therapy. Combination therapy with oral SCH66336 and intratumoral SCH58500 was highly effective. However, p53 gene therapy was not able to sensitize these tumors to paclitaxel therapy.

In the *wap-ras/F* transgenic model, an activated *H-ras* oncogene is expressed. Tumor models expressing mutated *H-ras* are generally among the most sensitive to FTI treatment. This is explained, at least in part, by the observation that cells treated with FTIs exhibit an accumulation of H-RAS as an unprenylated protein (15). This is in contrast to the other RAS isoforms, which are subject to alternative prenylation by geranylgeranyl protein transferase-1 in FTI-treated cells (14, 15). FTI-induced tumor regressions have been observed in *H-ras* transgenic models and in the EJ bladder carcinoma xenograft model, which also expresses an activated *H-ras* (21). Furthermore, *H-ras*-transformed cells are driven into apoptotic death *in vitro* when treated with an FTI in combination with either forced suspension growth (45) or a MEK inhibitor.[3] Treatment of *H-ras*-transformed cells *in vitro* with FTI alone results in accumulation of cells in the G_0/G_1 phase of the cell cycle[4]. Similarly, expression of wild-type p53 in a variety of transformed cells can induce either a G_0/G_1 cell cycle arrest or an apoptotic response (32). Therefore, the enhanced efficacy of SCH66336 and SCH58500 in *H-ras*^{mut} transgenic tumors is likely

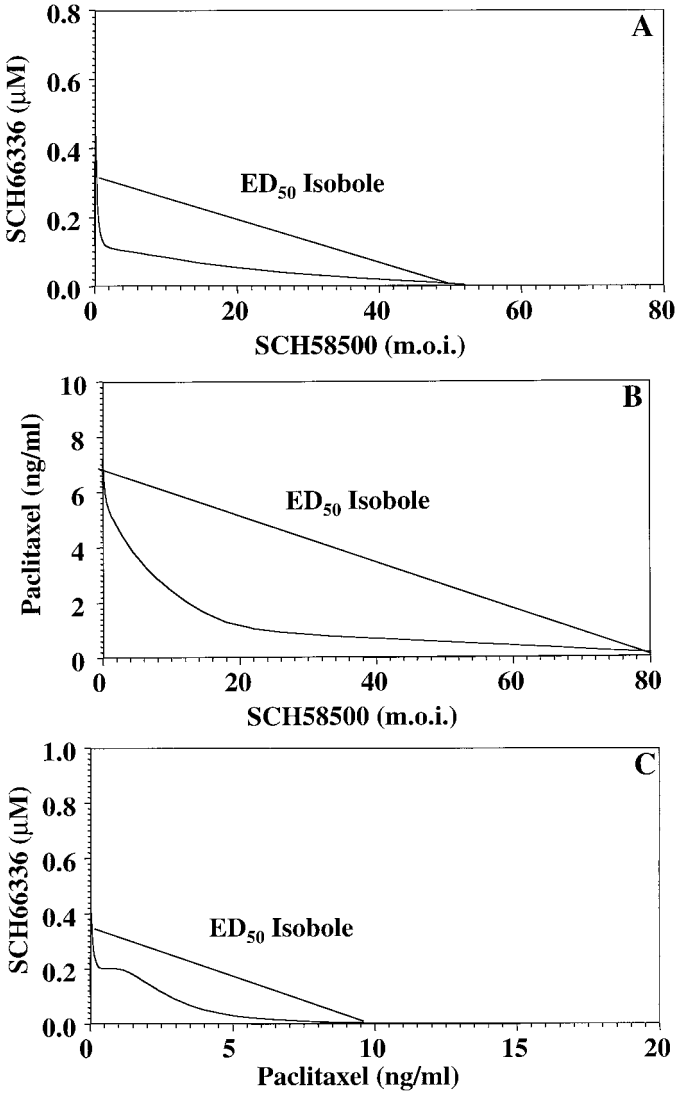

Fig. 2. Antiproliferative effects of the three-drug combination of SCH58500, SCH66336, and paclitaxel in DU-145 prostate tumor cells. *A*, isobole for SCH58500 and SCH66336. *B*, isobole for SCH58500 and paclitaxel. *C*, isobole for SCH66336 and paclitaxel.

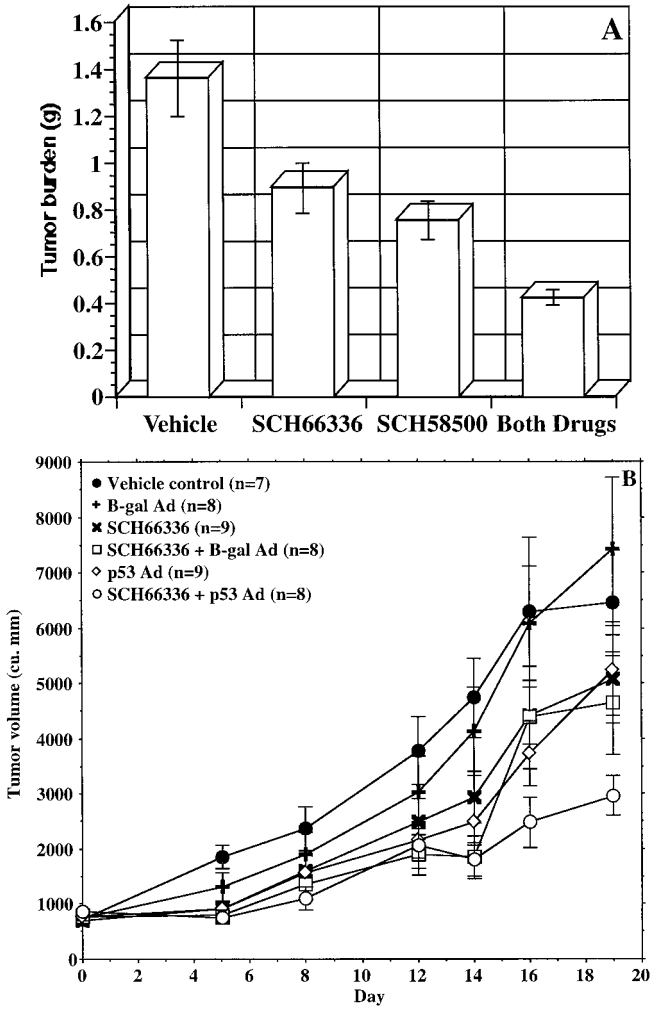

Fig. 3. SCH58500 and SCH66336 in the DU-145 human tumor xenograft model. *A*, i.p. tumors. Mean tumor weights ± SEM are shown. *B*, s.c. tumors. Mean tumor volumes ± SE are shown.

[3] D. L. Brassard, J. M. English, M. Malkowski, P. Kirschmeier, T. L. Nagabushan, and W. R. Bishop. Combination of a farnesyl protein transferase inhibitor (SCH 66336) and a MEK inhibitor demonstrate differential effects in H-Ras, H-Ras (CVLL), and K-Ras transformed cells, submitted for publication.

[4] H. R. Ashar, L. James, K. Gray, D. Carr, M. McGuirk, E. Maxwell, L. Armstrong, R. J. Doll, A. G. Taveras, W. R. Bishop, and P. Kirschmeier. The farnesyl transferase inhibitor SCH66336 induces p53 and alters the cell cycle distribution of human tumor cell lines, submitted for publication.

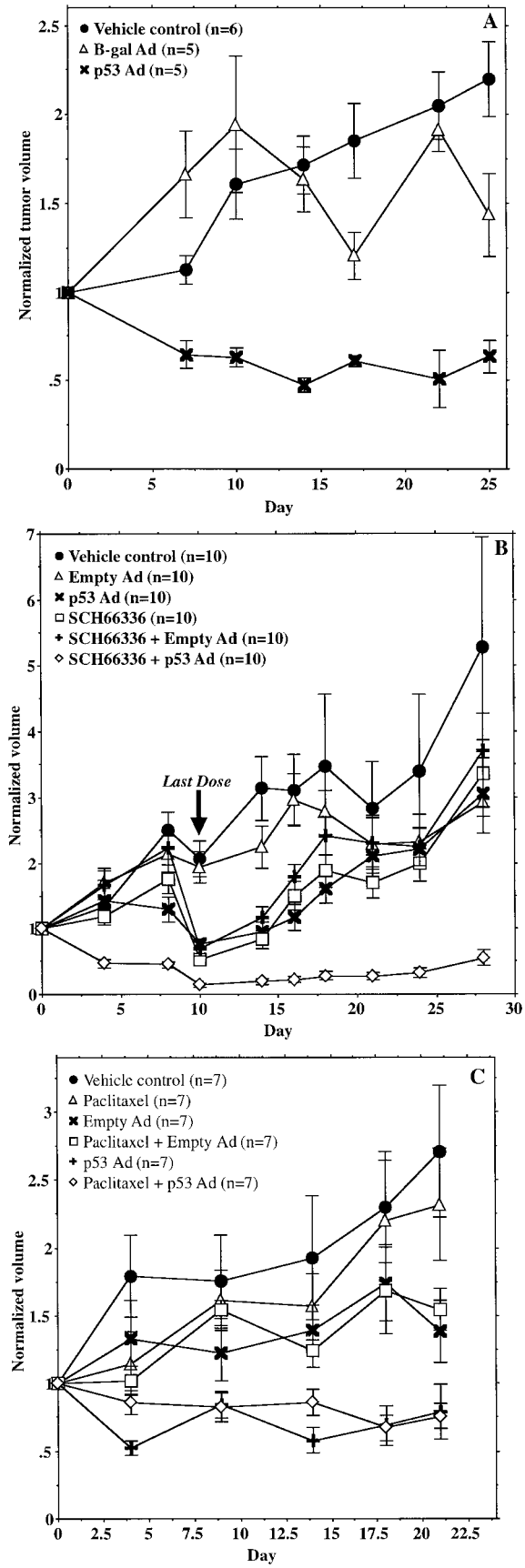

Fig. 4. SCH58500 and SCH66336 in *wap-ras/F* transgenic mice. *A*, SCH58500 alone. Tumor volumes on day 0 ranged from 236–1494 mm^3. *B*, SCH58500 and SCH66336 combination therapy. Tumor volumes on day 0 ranged from 216–926 mm^3. *C*, SCH58500 and paclitaxel combination therapy. Tumor volumes on day 0 ranged from 392–1481 mm^3. Means of normalized tumor volumes ± SE are shown.

explained by a combination of cell cycle effects and an increased propensity to undergo apoptosis.

In the *MMTV-v-ras* transgenic mouse model, it was reported that p53 status had no effect on FTI response (46). MMTV-*v-ras* × p53 −/− transgenic mouse tumors regressed after FTI therapy at a rate equivalent to parental MMTV-*v-ras* mice. However, the induction of apoptosis by FTI was significantly lower in $p53^{null}$ tumors (8-fold *versus* 16-fold), and there was a shift in this *H-ras*^{mut} tumor cell population toward G_0/G_1 arrest after FPT inhibition.

Other models used here both *in vitro* and *in vivo* are tumor cells expressing either wild-type RAS or mutant K-RAS. As indicated above, alternative prenylation of K-RAS allows this protein to membrane-associate even upon FTI treatment (15). Alternatively, prenylated RAS proteins appear to be competent both in signal transduction and cellular transformation. This has led to the suggestion that in cells that are mutant for *K-Ras* or cells that express wild-type RAS, other farnesylated proteins can mediate the biological response to FTI treatment (47).

Recently, it was reported that FTI treatment induces p21 in human tumor cell lines in a p53-dependent manner (48). We have also observed that SCH66336 treatment of $p53^{WT}$ tumor cell lines (*e.g.*, HCT116; NCI-H460; MCF7) induces p53 expression and subsequently p21 expression.[4] This suggests a role that p53 may have in the cellular response to FTI therapy. It has been further suggested that farnesylated proteins may be involved in regulating the cellular activity of p53 (48). A role for p53 in FTI response is consistent with the observations reported here that reintroduction of wild-type p53 into tumor cells using adenoviral delivery enhances the antiproliferative and antitumor activity of SCH66336.

Although the work of Sepp-Lorenzino and Rosen (48) suggests a role for p53 in cellular responses to FTI treatment, it also reports that the inhibition of tumor cell proliferation did not require wild-type p53. Along these lines, in a number of tumor cell lines expressing wild-type RAS or activated K-RAS, SCH66336 treatment led to accumulation in the G_2-M phase of the cell cycle, independent of p53 status.[4] The molecular mechanism of this cell cycle effect remains to be elucidated. However, these data suggest that FTIs likely exert both p53-dependent and p53-independent effects on tumor cell proliferation.

Moasser *et al.* (37) reported that a peptidomimetic FTI could synergize with paclitaxel in blocking tumor cell proliferation. We have found that SCH66336, a tricyclic compound, also has a synergistic interaction with paclitaxel. This synergy might be partially explained by the G_2-M accumulation observed following FTI treatment.[4] Paclitaxel is most effective during the G_2-M phase of the cell cycle (35), and the combination of these two agents is likely to result in an enhanced mitotic block.

Farnesyl transferase inhibitors and p53 tumor suppressor gene therapy represent two novel, experimental approaches for the clinical treatment of cancer. The precise biological mechanisms by which these two therapies induce their antitumor effects are not fully elucidated. However, the work presented here suggests that these two therapeutic approaches have synergistic antitumor activity when used in combination. Furthermore, combining either of these approaches with the microtubule stabilizing agent paclitaxel also results in synergistic effects on tumor cells. Because use of combination treatment has become a mainstay in cancer treatment, the observations reported here suggest new combinations that should be explored in clinical cancer care.

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