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Failure of taxol-based combination chemotherapy for malignant glioma cannot be overcome by G_2/M checkpoint abrogators or altering the p53 status

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Abstract In an effort to develop more effective forms of adjuvant chemotherapy for malignant brain tumors, we sought to develop a taxol-based combination chemotherapy regimen for glioma cell lines in vitro. Here, we report that coexposure of LN-229 or T98G glioma cells to taxol and either doxorubicin, camptothecin, cytarabine, or VM26 resulted in antagonistic effects rather than additive or synergistic cytotoxicity. There were no interactions of taxol with the effects of carmustine (BCNU) or vincristine. Antagonism was more prominent in cytotoxic cell death assays than in clonogenic cell death assays and was not overcome by G_2/M checkpoint abrogators such as caffeine or pentoxyfilline. Ectopic expression of mutant and wild-type p53val^{135} attenuated taxol cytotoxicity in both T98G cells, which are mutant for p53, and LN-229 cells, which exhibit functional wild-type p53 activity. Interestingly, wild-type p53val^{135} abrogated the taxol-imposed G_2/M arrest in both cell lines. However, wild-type p53val^{135} did not promote glioma cell killing by combinations of taxol and any of the other drugs. Further, an analysis of a panel of 12 human glioma cell lines revealed no relationship between genetic or functional p53 status and taxol sensitivity. In summary, combination either with other chemotherapy drugs, with abrogators of the G_2/M checkpoint, with wild-type p53 gene transfer was not a promising approach for a taxol-based combination chemotherapy regimen in malignant glioma.

Key words Taxol · Glioma · Chemotherapy · Cell cycle · p53

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Introduction

Taxol is an antineoplastic agent that stabilizes microtubules and prevents their depolymerization. It reduces the formation time and the critical concentration of tubulin required for microtubule assembly. The intracellular target for taxol is β-tubulin, which contains two specific binding sites for taxol [27]. The interaction of taxol with the microtubule apparatus may result in a cell cycle arrest at the G_2/M checkpoint, the inhibition of cellular migration and locomotion [34], and the induction of apoptosis [1, 3, 21]. Induction of apoptosis by taxol is associated with the activation of different intracellular signaling pathways which include p34^{cdc2} kinase [11, 44] and the serine/threonine kinase c-Raf-1 [5]. Both inactivation and activation [11, 32] of p34^{cdc2} kinase have been associated with the induction of apoptosis by taxol. The activation of c-Raf-1, which depends on activated tyrosine kinases, seems to be necessary for the induction of p53 and p21 by taxol [4] and for the phosphorylation of Bcl-2 [5]. The strong association of taxol-induced apoptosis with phosphorylation of Bcl-2 led to the assumption that this phosphorylation is the cellular answer to the disruption of the microtubule apparatus which attributes to Bcl-2 the role of a guardian of microtubule integrity [22]. However, taxol-induced Bcl-2 phosphorylation has recently been linked to mitotic arrest rather than taxol-induced apoptosis [21].

The role of the taxol-induced G_2/M arrest in apoptosis is controversial. In a study of different murine tumors, cytotoxic effects of taxol were only correlated with baseline apoptosis within the tumors, but not with the degree of taxol-induced G_2/M arrest [23]. However, abrogation of wild-type p53 function by the HPV-16 E6 protein in human fibroblasts resulted in an increased G_2/M arrest induced by taxol which was correlated with increased apoptosis [39]. Modulation of the taxol-induced G_2/M arrest by activators or inhibitors of p34^{cdc2} kinase therefore seems to be an interesting experimental approach to improve the efficacy of taxol-based cancer

chemotherapy. The specific $p34^{cdc2}$ kinase inhibitor butyrolactone did not modulate taxol cytotoxicity in the lung cancer cell lines PC-9 and PC-14 cells, whereas MCF-7 cells were sensitized to taxol cytotoxicity by the $p34^{cdc2}$ kinase activator caffeine [31]. Moreover, increased apoptosis was associated with decreased G_2/M arrest, in apparent contrast to previous work [39].

Activity of taxol against malignant glioma cell lines in vitro has been shown in several studies [6, 15, 29]. However, despite the remarkable in vitro potency of taxol, phase II clinical studies of taxol for primary or recurrent malignant gliomas showed no relevant activity [8, 9, 26]. These results were in part attributed to the poor ability of taxol to cross the blood–brain barrier [14], even though taxol concentrations in the tumor could reach concentrations in the therapeutic range, provided there was no protective blood–tumor barrier [18]. Incorporation of taxol in biodegradable polymer implants for surgery [40] or in lipid-coated microbubbles for i.v. injection [19] are promising attempts to solve this problem.

In the present study, we sought to identify suitable drug partners for taxol-based combination chemotherapy of malignant glioma and asked whether taxol cytotoxicity of malignant glioma cells could be enhanced by G_2/M checkpoint abrogators or p53 gene transfer strategies. Further, the influence of the endogenous p53 status on glioma cell susceptibility to taxol-induced cell death was investigated.

Materials and methods

Materials and cell lines

Vincristine, cytarabine, camptothecin, doxorubicin, caffeine, and pentoxyfilline (PTX) were purchased from Sigma (St. Louis, MO). Taxol, BCNU, VM26, and cisplatin were obtained from Bristol (Munich, Germany). T98G human malignant glioma cells were obtained from the American Type Culture Collection (Rockville, MD). A172, LN-229, LN-18, LN-308, LN-319, LN-428, U87MG, U138MG, U251MG, U373MG, and D247MG human malignant glioma cells were kindly provided by Dr. N. de Tribolet (Lausanne, Switzerland). LN-229 cells expressing murine temperature-sensitive $p53val^{135}$ have been described [35]. T98G cells expressing $p53val^{135}$ were generated for the present study by electroporation and limiting dilution cloning as outlined before. Transgene expression was assessed by immunoblot analysis as described [35].

Cell cycle analysis

The cells were treated as indicated. Detached cells were harvested from the supernatant by centrifugation and added to the nondetached cells, which were harvested by trypsinization. The cells were washed once with phosphate-buffered solution (PBS) and fixed in 80% ice-cold ethanol over night. The cells were then centrifuged and washed with PBS, and 10^6 cells per condition were stained with propidium iodide (50 μg/ml in PBS, containing 100 μg/ml RNase A). The cells were subjected to flow cytometric analysis of DNA content using a Becton Dickinson FACScalibur cytometer. Percentages of cells in the different cell cycle phases were calculated by CellQuest software (Becton Dickinson, Heidelberg, Germany).

Fig. 1A–D Taxol-based combination chemotherapy of human malignant glioma cells in vitro. **A, C** LN-229 or **B, D** T98G cells were exposed to increasing concentrations of taxol (*filled circles*, no taxol; *open circles*, 2 n*M*; filled squares, 20 n*M*; open squares, 200 n*M*) and either carmustine (*BCNU*), vincristine, doxorubicin, camptothecin, cytarabine, or VM26 in **A, B** cytotoxic cell death assays (72-h continuous exposure) or **C, D** clonogenic cell death assays (24-h pulse, plus further culturing for 5 generation times in drug-free medium). Survival or growth was assessed by crystal violet assay. Data are mean percentages of survival ($n = 3$, SEM $< 10\%$)

Viability assays

For acute cytotoxicity assays, the cells were seeded at 1×10^4 cells per well in 96-well plates, adhered for 24 h, and exposed to the drugs for 72 h. Survival was assessed by crystal violet staining. For clonogenic survival assays, the cells were seeded at 1×10^3 cells per well, adhered for 24 h, pulse-treated for 24 h with the drugs, and maintained drug-free for 5–10 generation times in complete medium. Proliferation was assessed by crystal violet staining. Briefly, the cell culture medium was removed and surviving cells stained with 0.5% crystal violet in 20% methanol for 20 min at room temperature. The plates were washed extensively under running tap water and air-dried, and optical density values were read in an enzyme linked immunosorbent assay (ELISA) reader at 550-nm wave length [42]. For the determination of EC_{50} values, the drug effects were monitored in serial dilutions over a broad range of concentrations.

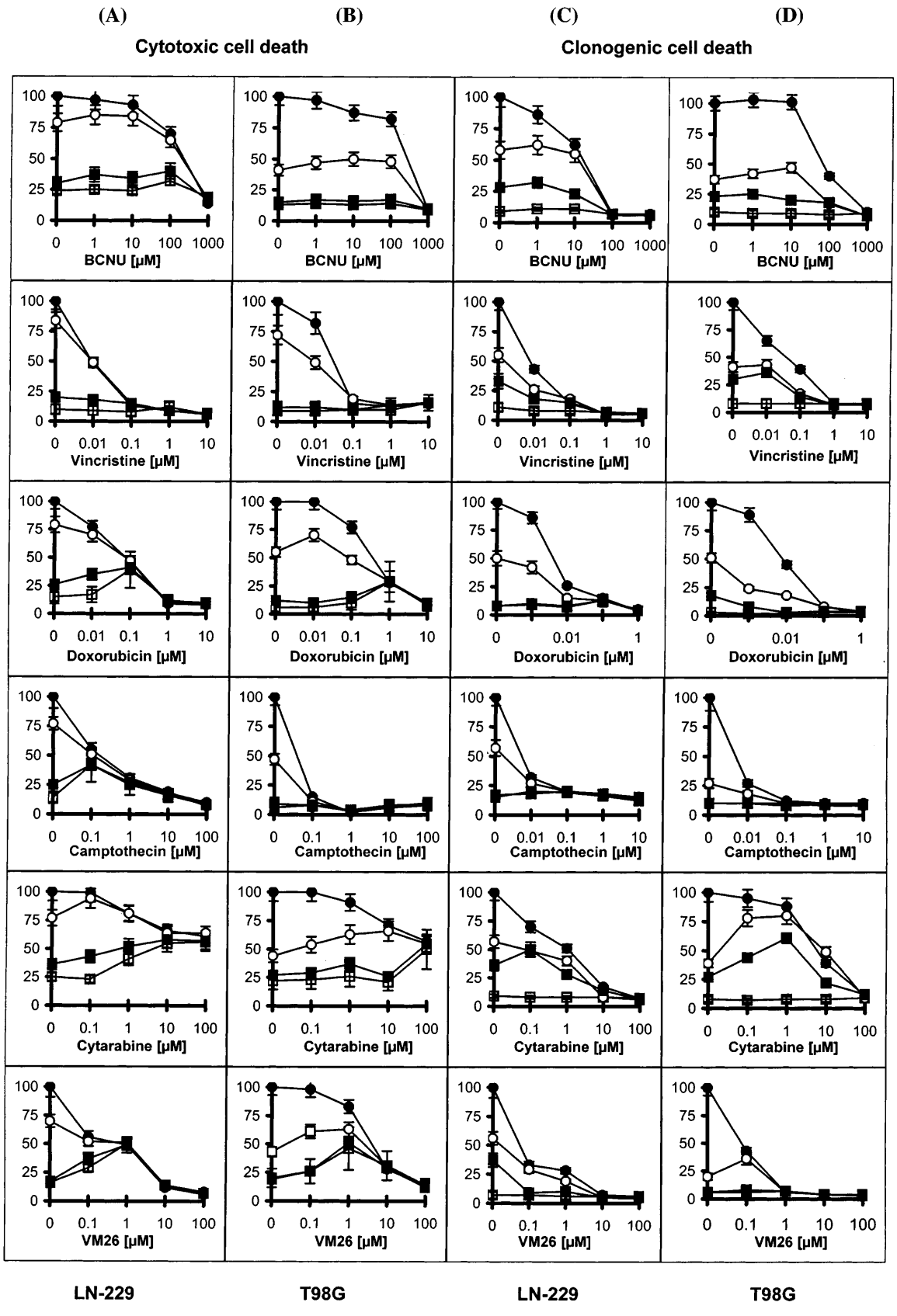
Statistical analysis

All viability and cell cycle data are from experiments that were performed at least three times with similar results. For the comparison of EC_{50} values of cell lines with wild-type versus mutant/deleted p53 genetic status or of cell lines with low versus high p53 protein expression, a t test for independent samples was used. Results were considered significant at a P value below 0.05. EC_{50} values of taxol cytotoxicity in hygro and p53-transfected cell lines were also compared by t test. Synergy was assessed by the fractional product method of Webb [41] and by isobologram analysis [2], as previously described [28, 29].

Results

Taxol-based combination chemotherapy of human malignant glioma cells results in antagonism rather than synergy

To study interactions between the cytotoxic and anticlonogenic effects of taxol and other cancer chemotherapy drugs in human malignant glioma cells, LN-229 or T98G cells were exposed to increasing concentrations of taxol and either BCNU, vincristine, doxorubicin, camptothecin, cytarabine, or VM26, either continuously for 72 h (cytotoxic cell death) or as a 24 h pulse with assessment of effects 5–10 generation times later (clonogenic cell death). In cytotoxic cell death assays, there was antagonism between taxol and either doxorubicin, cytarabine, and VM26 in both cell lines, and with taxol and camptothecin in LN-229 cells. No interactions between taxol and BCNU or vincristine became apparent.


(A)
(B)
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Cytotoxic cell death
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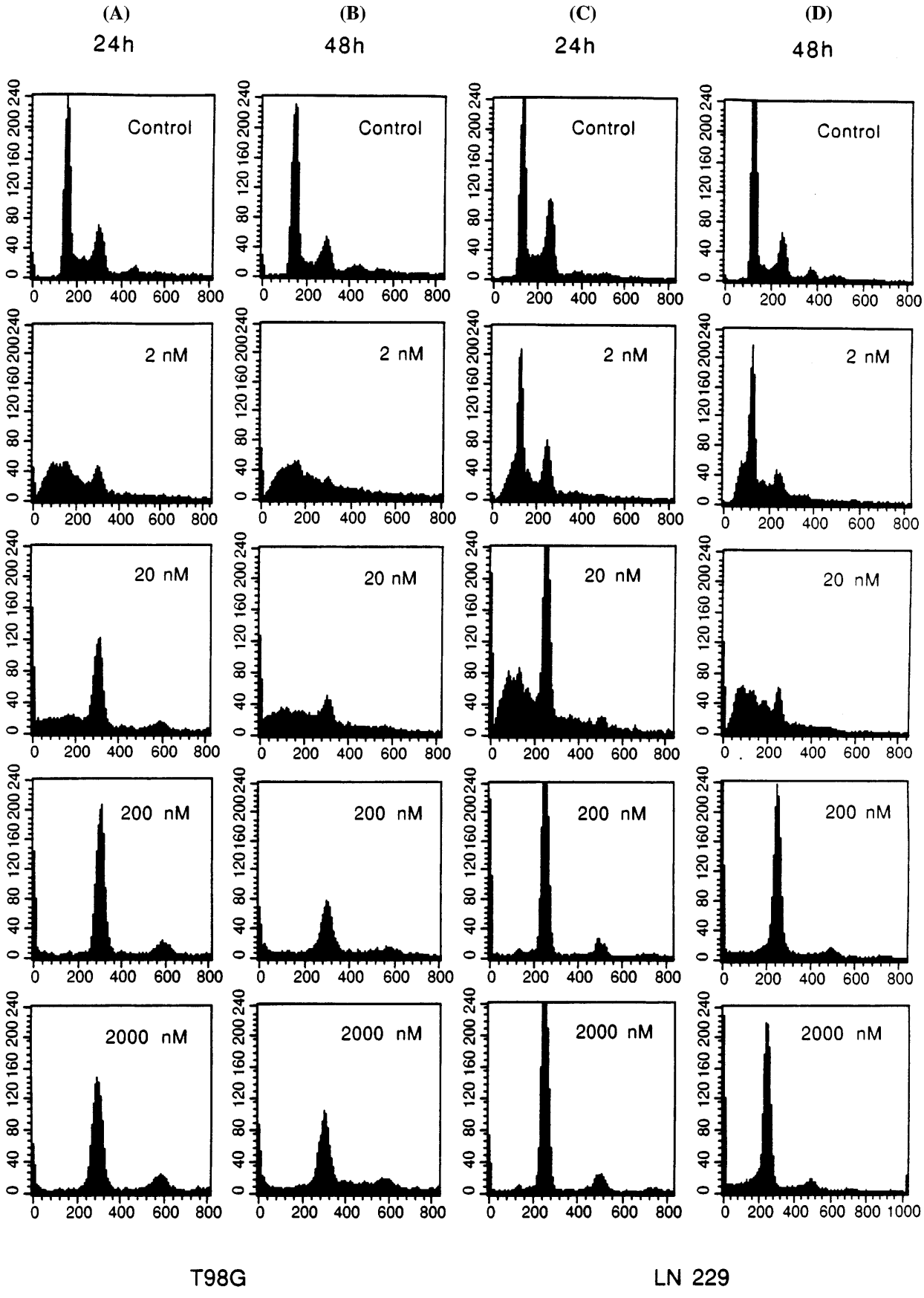
(A)
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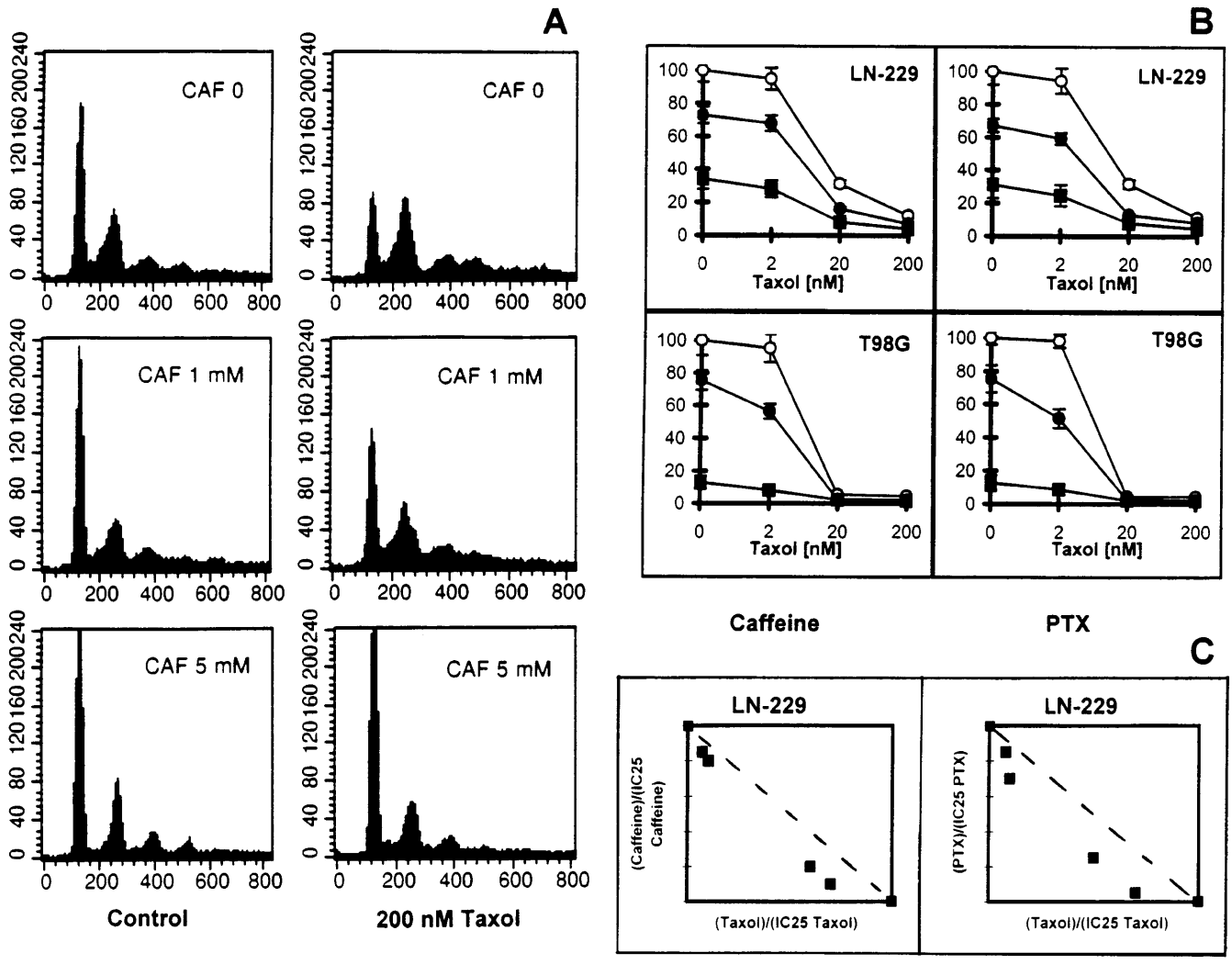

Fig. 3A–C Modulation of taxol-induced G_2/M arrest and cytotoxicity by caffeine or pentoxifylline (PTX). **A** LN-229 cells were untreated (*left*) or exposed to taxol (200 n*M*) for 24 h (*right*) in the absence (*top*) or presence of caffeine at 1 (*middle*) or 5 m*M* (*bottom*). Cell cycle analysis was performed as described in "Materials and methods." **B** LN-229 (*top*) or T98G (*bottom*) cells were exposed to taxol in the absence (*open circles*) or presence of caffeine (*left*) at 1 (*filled circles*) or 5 m*M* (*filled squares*) or PTX (*right*) at 1 (*filled circles*) or 5 m*M* (*filled squares*) for 72 h. **C** Isobologram analysis of interactions between taxol and caffeine (*left*) or PTX (*right*) at an IC_{25} (inhibitory concentration) level (for details, see [2, 29]). The line connecting the IC_{25} values represents independent effects, values below the line indicate synergy, and values above the line suggest antagonism. Thus, in the experiments presented, there is moderate synergy of the cotreatment with taxol and either caffeine or PTX in LN-229 cells

Of note, there was no combination of taxol and any drug at any concentrations that induced synergistic cytotoxicity in these assays, as assessed by the fractional product method [41] or isobologram analysis [2] (Fig. 1A, B). In clonogenic cell death assays, there was antagonism for the combination of taxol and cytarabine in both cell lines, and for taxol and VM26 in T98G cells (Fig. 1C, D). Again, no synergy became apparent.

Given the sequence specificity demonstrated for combination chemotherapy in many tumor cell lines with many drug combinations (see below), we also modified the assays to treat the glioma cells sequentially either first with taxol for 8 h and then for 48 h with VM26, vincristine, or cytarabine or vice versa. These data revealed clearly less antagonism than the coexposure assays, but still did not lead to the identification of a sequential treatment design that resulted in synergistic antiglioma activity (data not shown).

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Fig. 2A–D Taxol-induced cell cycle changes in human malignant glioma cells. **A, B** T98G or **C, D** LN-229 cells were untreated or exposed to taxol at 2, 20, 200 or 2000 n*M* for **a, c** 24 or **b, d** 48 h. Cell cycle analysis was performed by flow cytometry as described in "Materials and methods"

Antagonism of taxol-based combination chemotherapy: role of the taxol-induced G_2/M arrest

To monitor taxol-induced cell cycle changes, LN-229 and T98G cells were untreated or exposed to taxol at 2, 20,

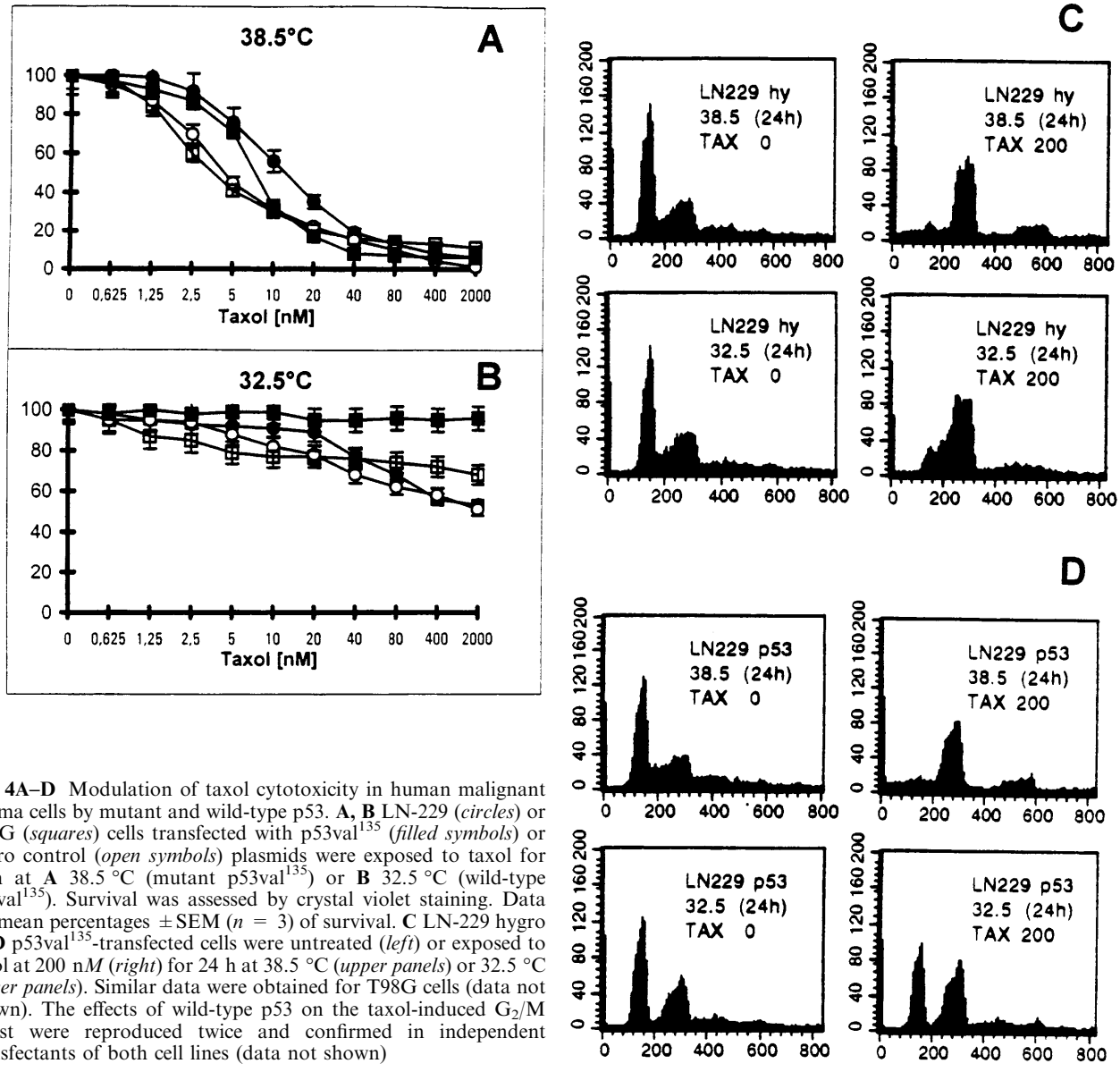


Fig. 4A–D Modulation of taxol cytotoxicity in human malignant glioma cells by mutant and wild-type p53. **A, B** LN-229 (*circles*) or T98G (*squares*) cells transfected with p53val^{135} (*filled symbols*) or hygro control (*open symbols*) plasmids were exposed to taxol for 72 h at **A** 38.5 °C (mutant p53val^{135}) or **B** 32.5 °C (wild-type p53val^{135}). Survival was assessed by crystal violet staining. Data are mean percentages ± SEM ($n = 3$) of survival. **C** LN-229 hygro or **D** p53val^{135}-transfected cells were untreated (*left*) or exposed to taxol at 200 n*M* (*right*) for 24 h at 38.5 °C (*upper panels*) or 32.5 °C (*lower panels*). Similar data were obtained for T98G cells (data not shown). The effects of wild-type p53 on the taxol-induced G_2/M arrest were reproduced twice and confirmed in independent transfectants of both cell lines (data not shown)

200, or 2000 n*M*, and cell cycle changes were assessed at 24 and 48 h. Taxol at 2 n*M* induced accumulation in S phase that was more prominent in T98G cells than in LN-229 cells. At 20 n*M* or more, there was a prominent G_2/M arrest in both cell lines. Further, tetraploid cells became frequent at 200 and 2000 n*M*, and G_2/M accumulation decreased from 24 to 48 h, presumably as a consequence of cell death from the G_2/M fraction. Overall, there was a correlation between the concentrations of taxol required to induce G_2/M arrest (Fig. 2) with the concentrations shown to antagonize the cytotoxic effects of other cancer chemotherapy drugs (Fig. 1).

Modulation of taxol cytotoxicity by caffeine and PTX

To examine the role of the induced G_2/M arrest in the cell's fate after taxol exposure, we exposed the glioma cells to taxol in the absence or presence of caffeine or PTX and monitored cell cycle redistribution and modulation of taxol cytotoxicity. The EC_{50} values for the intrinsic cytotoxicity during a 72-h exposure were 10 and 3.5 m*M* for caffeine and 7 and 4.5 m*M* for PTX in LN-229 and T98G cells, respectively. Flow cytometry confirmed that caffeine and PTX overcame the taxol-imposed G_2/M arrest in a concentration-dependent manner. Representative data for the effects of caffeine in LN-229 cells are shown in Fig. 3A. Importantly, completely subtoxic concentrations of caffeine or PTX also failed to modulate the taxol-induced G_2/M arrest.

Next, we examined whether coexposure to caffeine or PTX enhanced the cytotoxicity of taxol in human glioma cells. Nontoxic concentrations of caffeine or PTX did not sensitize either cell line for taxol cytotoxicity to a great extent. Cytotoxic concentrations of both agents killed the cells in moderate synergy with taxol (Fig. 3B),

Table 1 p53 genetic status, p53 protein expression, and EC_{50} values for taxol-induced cytotoxic cell death in 12 human glioma cell lines

	p53 genetic status	p53 protein expression	EC_{50} (n*M*)
LN-18	hetero, wt/mt^{238}	1	14 ± 5
U138MG	homo, mt^{213}	1	6 ± 2
U87MG	homo, wt	0	10 ± 3
LN-428	hetero, mt^{173}/mt^{282}	0	4 ± 2
D247MG	homo, wt	0	9 ± 3
T98G	homo, mt^{237}	1	4 ± 2
LN-319	homo, mt^{175}	1	400 ± 45
LN-229	hetero, wt/mt^{164}	0	12 ± 2
A172	hetero, wt/wt	0	10 ± 1
U251MG	homo, mut^{273}	1	8 ± 2
U373MG	?, mut^{273}	1	40 ± 6
LN-308	hetero, del/transloc	0	60 ± 8

p53 genetic status and p53 protein expression were previously reported [43]. EC_{50} values for taxol were determined as described in the "Material and methods" section and are expressed as mean ± SD of three independent experiments. Hetero, heterozygous; homo, homozygous; wt, wild-type; mt, mutant, the number indicating the position of the affected amino acid; del/transloc, p53 gene is deleted or translocated; 0, absent or weak p53 protein expression; 1, strong p53 protein expression

as determined by isobologram analysis [2] (Fig. 3C). Thus abrogation of taxol-induced G_2/M arrest by caffeine-like agents in glioma cells modestly enhances taxol cytotoxicity.

In order to assess whether caffeine might overcome the antagonism of taxol and other cancer chemotherapy drugs by promoting G_2/M transition, we then performed experiments similar to those shown in Fig. 1 in the absence or presence of PTX at 1 or 5 m*M*. Overall, these data were negative in that PTX did not overcome the antagonism reported in Fig. 1 (data not shown). Further, PTX also failed to synergistically enhance the cytotoxic effects of vincristine, VM26, camptothecin, cytarabine, cisplatin, or doxorubicin to a relevant extent (data not shown).

Taxol cytotoxicity of human malignant glioma cells: modulation by mutant and wild-type p53

LN-229 cells are heterozygous for p53 and retain wild-type p53 activity, as assessed by chloramphenicol-acetyltransferase (CAT) reporter assay and accumulation of p53 in response to DNA damage, whereas T98G cells are mutant for p53 [36, 43]. LN-229 cells are moderately more resistant to taxol than T98G cells [29]. To further examine the role of p53 for taxol cytotoxicity of human malignant glioma cells and a possible involvement in the antagonistic effects of taxol-based combination chemotherapy, we transfected LN-229 and T98G cells with the murine temperature-sensitive $p53val^{135}$, which assumes mutant conformation at 38.5 °C and wild-type conformation at 32.5 °C. At 38.5 °C, $p53val^{135}$ abrogates camptothecin-induced p21 expression in LN-229 cells and thus acts as a dominant-negative mutant over endogenous wild-type p53 [24]. At 32.5 °C, $p53val^{135}$ induces growth arrest in both LN-229 cells [35] and T98G cells (data not shown) and thus overcame endogenous mutant p53 function in T98G cells.

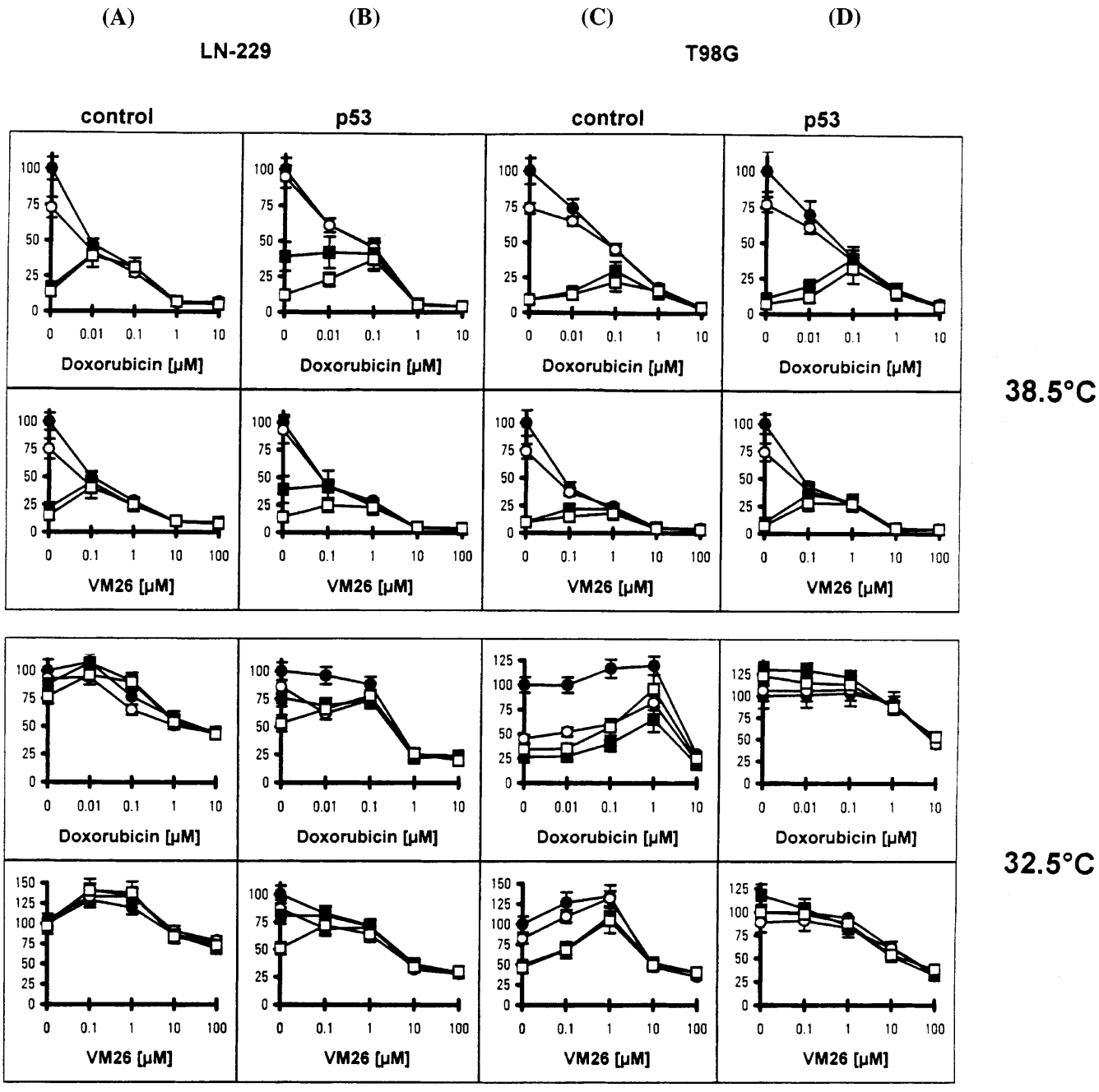
Accumulation of mutant $p53val^{135}$ attenuated taxol cytotoxicity of both cell lines significantly (Fig. 4A): the EC_{50} values were 4.5 and 13 n*M* for LN-229, and 4 and 8 n*M* for T98G, hygro control and p53-transfected cells, respectively ($n = 3$, $P < 0.05$, t test). Note that the EC_{50} values of hygro control cells in these experiments are slightly lower than those shown for LN-229 and T98G in Fig. 6 and in Table 1, reflecting enhanced cytotoxicity of taxol at a higher temperature (38.5 °C), probably due to increased metabolism. Similarly, "wild-type" $p53val^{135}$ (32.5 °C) virtually abrogated taxol cytotoxicity in T98G cells and conferred partial protection from taxol to LN-229 cells (Fig. 4B). Not unexpectedly, taxol cytotoxicity was reduced at lower temperatures, presumably reflecting decreased drug uptake or metabolism, and slowed cell cycle progression. Interestingly, cell cycle analysis of mock-transfected and $p53val^{135}$-transfected cells revealed that mutant $p53val^{135}$ had little effect on the taxol-induced changes in either cell line. In contrast, unexpectedly, wild-type $p53val^{135}$ *interfered* with the G_2/M arrest imposed by taxol in both cell lines. Figure 4C, D shows representative data for LN-229 cells. The results with the p53 transfectants from both cell lines were reproduced in pooled populations of independent transfectants (data not shown).

Altering the p53 status does not overcome antagonistic effects of taxol-based combination chemotherapy for malignant glioma

Next, we examined whether altering the p53 status affected the antagonism or lack of additivity or synergy of taxol-based combination chemotherapy. Some of the effects of $p53val^{135}$ gene transfer on drug cytotoxicity in LN-229 cells have been published [35]. Here, we were specifically interested in the effects of altering the p53 status on taxol-based combination chemotherapy. Expression of mutant p53 in either cell line did not overcome the antagonism of taxol and either doxorubicin, camptothecin, cytarabine, or VM26 and did not allow synergy to become apparent with BCNU or vincristine. Similarly, expression of wild-type $p53val^{135}$ had no specific effects on the effects of taxol-based combination chemotherapy. Representative data for doxorubicin and VM26 are summarized in Fig. 5.

p53 status does not predict sensitivity to taxol in a panel of glioma cell lines

Finally, we extended the analysis of a possible interrelation between the p53 status of the glioma cells and their sensitivity to taxol to a panel of 12 previously



characterized glioma cell lines [43]. EC_{50} values for these 12 cell lines were determined (Fig. 6, Table 1), and cell lines with genetic wild-type p53 status were compared with cell lines with mutant or deleted p53 genes (Table 1). No significant difference was apparent, regardless whether LN-229 cells which retain p53 wild-type activity but are genetically heterozygous for p53 were included in the group with wild-type p53 or in the group with mutant p53. In addition, the level of p53 protein expression, irrespective of genetic status, was determined by immunoblot analysis, and the six cell lines with weak p53 protein expression were compared with those with strong p53 protein expression (Table 1).

Fig. 5A–D Ectopic expression of mutant or wild-type p53val^{135} does not promote synergy of taxol-based combination chemotherapy. **A, C** Control or **B, D** p53val^{135}-transfected **A, B** LN-229 cells or **C, D** T98G cells were maintained at 38.5 °C (mutant p53, *upper panels*) or shifted to 32.5 °C (wild-type p53, *lower panels*) for 4 h and then treated with doxorubicin or VM26 in the absence or presence of taxol (*filled circles*, no taxol; *open circles*, 2 n*M*; *filled squares*, 20 n*M*; *open squares*, 200 n*M*). Survival was assessed by crystal violet assay. Data are expressed as mean percentages of survival ± SEM ($n = 3$)

Again, there was no significant difference in the EC_{50} values between these groups, confirming that sensitivity to taxol is independent of p53 in human malignant glioma cells.

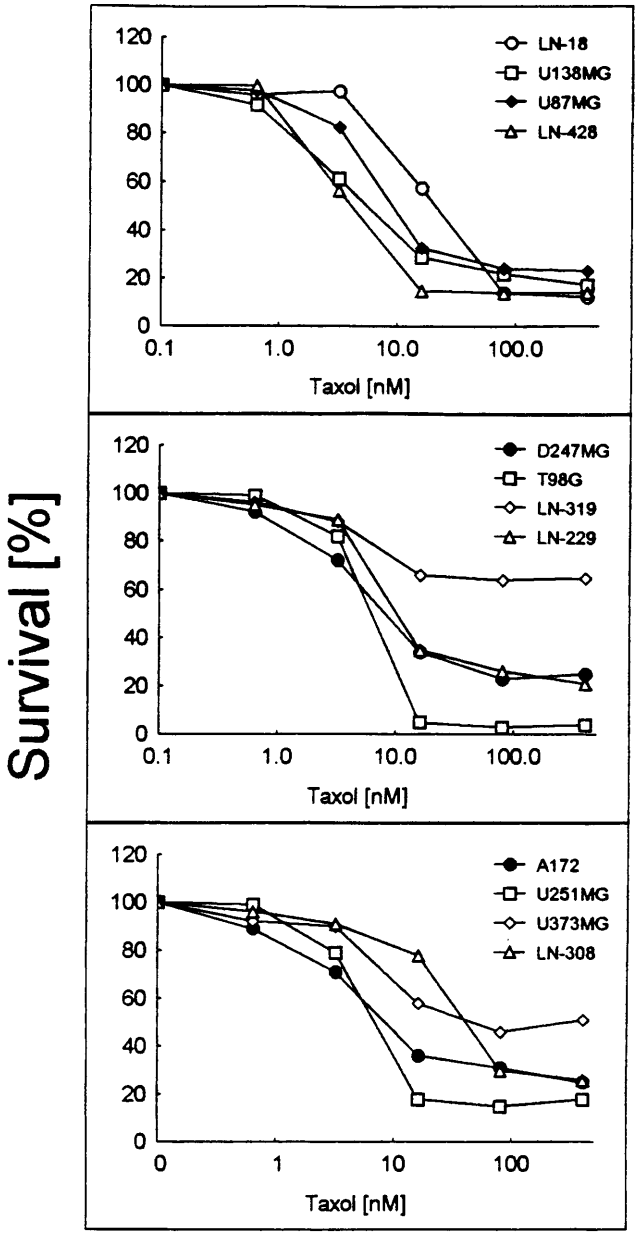


Fig. 6 p53 status has no influence on glioma cell sensitivity to taxol. Twelve human glioma cell lines with known p53 genetic status (*filled symbols*, wild-type p53; *open symbols*, mutant or deleted p53) were treated with increasing concentrations of taxol for 72 h; survival was assessed by crystal violet staining. Data are expressed as mean percentages of survival. One representative experiment of three independent experiments with similar results is shown

Discussion

Taxol has been one of the newer cancer chemotherapy drugs that was hoped to be more effective against human malignant gliomas than conventional chemotherapeutic drugs. However, clinical experience with taxol monotherapy has been disappointing [8, 26]. Therefore, in the present study, we examined whether taxol-based combination chemotherapy might be a promising approach to the management of these neoplasms. Prior cell culture studies had often revealed antagonistic rather than synergistic effects when taxol was combined with other antineoplastic agents. Especially the combination of the podophyllotoxin derivatives etoposide (VP16) and teniposide (VM26) with taxol often showed antagonistic effects when the drugs were incubated simultaneously [22]. This antagonism was commonly dependent on the schedule used [10, 13, 20]. However, several experimental clinical studies have evaluated taxol-based combination chemotherapy in cancers of the upper gastrointestinal tract, the urothelium, or the endometrium. For ovarian cancer, combination chemotherapy with taxol and cisplatin has been established as a first-line therapy. Similarly, taxol-based combination chemotherapy may be superior to other regimens in the management of head and neck squamous cell carcinoma, metastatic breast cancer, and non-small cell lung cancer.

In the present study, coexposure studies revealed significant antagonism of taxol and either doxorubicin, camptothecin, cytarabine, or VM26, with no significant interactions of taxol with BCNU or vincristine (Fig. 1). Antagonism was less prominent at lower concentrations of taxol and in clonogenic cell death assays than in cytotoxic cell death assays, suggesting that the taxol-induced G_2/M arrest (Fig. 2) was at least partially responsible for the antagonism of taxol-based combination chemotherapy. G_2/M checkpoint abrogators such as caffeine or PTX promoted moderate sensitization of the glioma cells to taxol (Fig. 3), but did not result in the antagonism of taxol-based combination chemotherapy being overcome. PTX has previously been shown to enhance the cytotoxicity of cisplatin in MCF-7 mammary carcinoma cells specifically when endogenous p53 function was disrupted by the ectopic expression of the HPV-16 E6 gene or a dominant-negative mutant p53 gene [12], and the same cells were sensitized to taxol by caffeine [31].

The interrelations between the p53 status and taxol sensitivity in tumor cells are complex. Even though taxol induces p53 accumulation in functional p53 wild-type LN-229 glioma cells [29], taxol showed p53-independent cytotoxicity in many tumor cell lines [30, 37], and neither p53 genetic status nor p53 protein expression correlated with sensitivity to taxol in an extended panel of 12 human glioma cell lines (Fig. 6, Table 1). Further, non-transformed human fibroblasts cells were sensitized to taxol when wild-type p53 was inactivated by the SV40 T antigen or HPV-16 E6 [17, 39]. Similar results were obtained in HPV E6-transfected ovarian cancer cells [38]. High activity of taxol against tumors lacking wild-type p53 function could be the result of the negative regulation of microtubule-stabilizing protein 4 (MAP4) by wild-type p53 [24]. MAP4 stabilizes microtubules, and down-regulation of MAP4 is associated with apoptosis [24]. Since taxol induces p53 expression in wild-type p53 cells, a subsequently decreased expression of MAP4 could antagonize microtubule stabilization by taxol. This association is supported by the observation

that increased expression of MAP4, as a consequence of p53 downregulation, increases the sensitivity to taxol in human fibroblasts [45].

Since LN-229 cells retain wild-type p53 activity, defined by transcriptional activity in a reporter assay and accumulation of p53 in response to genotoxic stress, whereas T98G are mutant for p53 [36], antagonism of taxol and other cancer chemotherapy drugs appeared not to be determined by the p53 status. To confirm this, we showed that ectopic expression of mutant or wild-type p53val^{135} did not promote glioma cell killing by combinations of taxol and any of the other drugs. Interestingly, however, accumulation of both mutant or wild-type p53val^{135} attenuated the cytotoxicity of taxol administered alone, consistent with a gain of function-type effect of p53 accumulation [42]. Wild-type p53val^{135} mediated protection from taxol, probably by overriding the taxol-induced G_2/M arrest. This observation corresponds to an enhanced G_2/M arrest of normal human fibroblasts exposed to DNA-damaging agents in the presence of p53 antisense oligonucleotides [7]. However, a p53-dependent overriding of an etoposide-induced G_2/M arrest enhanced rather than decreased the cytotoxicity of etoposide in M_1 myeloid leukemia cells [33], indicating that the consequences of p53-mediated abrogation of the G_2/M arrest may be both drug and cell type specific. In the case of glioma, however, taxol may not act in synergy with wild-type p53 gene therapy in vivo either.

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